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Towards Early Hemolysis Detection: a Smartphone based Approach

Universidade Fernando Pessoa
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Rumo à detecção precoce de hemólise: uma abordagem baseada em smartphone

Thesis presented to Universidade Fernando Pessoa as a requirement to obtain the doctoral degree in Ciências da Informação, under the supervision of Prof. Doutor Nuno Magalhães Ribeiro.

RESUMO

CHRIS CHIFOR: Rumo à detecção precoce de hemólise: uma abordagem baseada em smartphone
(Sob orientação do Prof. Doutor Nuno Magalhães Ribeiro)

Os especialistas em diagnóstico in vitro (IVDs) têm confiado maioritariamente na inspeção visual (ótica) manual e, em segundo lugar, em sensores óticos ou câmaras embutidas ou dispositivos médicos incorporados que suportam o exame da qualidade da amostra na fase pré-analítica. Com o aumento dos volumes de amostras para serem processadas e dos respetivos dados complexos gerados por esse processamento, aquelas técnicas tornaram-se cada vez mais difíceis de utilizar, ou os respetivos resultados não ficam imediatamente disponíveis. Para superar as complexidades impostas por tais técnicas tradicionais, o aumento do uso de dispositivos móveis e algoritmos de processamento de imagem no setor de saúde abriu caminho para a constituição de novos casos de uso baseados em análises móveis de amostras, pois fornecem uma interação simples e intuitiva com objetos gráficos familiares que são mostrados no ecrã dos *smartphones*. As interfaces gráficas e as técnicas de interação suportadas por dispositivos móveis podem pois proporcionar ao especialista em IVD uma série de vantagens e valor agregado devido à maior familiaridade com estes dispositivos e à grande acessibilidade que evidenciam atualmente, tendo o potencial de facilitar as análises de amostras. No entanto, o uso sistemático de dispositivos móveis no setor da saúde encontra-se ainda numa fase muito incipiente, em particular na área de IVD. Nesta tese, propõe-se conceber e discutir a arquitetura, a conceção e a implementação de um protótipo de uma aplicação móvel para *smartphone* (designada por "HemoDetect") que implementa um conjunto sugerido de algoritmos, interfaces e técnicas de interação que foram desenvolvidos com o objetivo de contribuir para a compreensão de técnicas mais eficientes para ajudar a detetar a hemólise, um processo que designa a rotura de glóbulos vermelhos (eritrócitos) e libertação do respetivo conteúdo (citoplasma) para o fluído circundante (por exemplo, plasma sanguíneo), complementando-as com estatísticas e medições de laboratório, mostrando a utilização de um protótipo durante experiências, permitindo assim chegar-se a um conceito viável que permita apoiar eficazmente a deteção precoce de hemólise.

ABSTRACT

CHRIS CHIFOR: Towards Early Hemolysis Detection: a Smartphone based Approach
(Under the supervision of Prof. Doutor Nuno Magalhães Ribeiro)

In Vitro Diagnostics (IVDs) specialists have been firstly relying on manual visual (optical) inspection and, secondly, on optical sensors or cameras embedded or built-in medical devices which support the examination of sample quality in pre-analytical phase. With increasing sample processing volumes and their generated complex data, these techniques have become increasingly difficult or results are not readily available. In order to overcome the complexities posed by these traditional techniques, the increased usage of mobile devices and algorithms in the healthcare industry paves the way into shaping new use cases and discovery of mobile analysis of samples, as they provide a user-friendly and familiar interaction with objects displayed on their screens. The interfaces and interaction techniques rendered by mobile devices, bring, to the IVD specialist, a number of advantages and added value due to increased familiarity with the devices or their accessibility, which is made easier. However, they are at the beginning of their journey in the healthcare industry, in particular in the IVD and point-of-care areas. In this thesis, the proposal is to discover and discuss the architecture, design and implementation of a *smartphone* prototype app (called "HemoDetect") with its algorithms, interfaces and interaction techniques which was developed to help detect hemolysis which represents the rupture of red blood cells (erythrocytes) and release of their contents (cytoplasm) into surrounding fluid (e.g. blood plasma), and complementing it with from-the-lab statistics and measurements showing its utilization during experiments, which ultimately may be a feasible concept that could support early hemolysis detection.

RÉSUMÉ

CHRIS CHIFOR: Rumo à detecção precoce de hemólise: uma abordagem baseada em smartphone

(Sous la supervision du Prof. Dr. Nuno Magalhães Ribeiro)

Les spécialistes du diagnostic in vitro (DIV) se sont d'abord appuyés sur l'inspection visuelle (optique) manuelle et, ensuite, sur des capteurs optiques ou des caméras intégrées ou intégrées à des dispositifs médicaux qui facilitent l'examen de la qualité des échantillons en phase pré-analytique. Avec l'augmentation des volumes de traitement des échantillons et des données complexes générées, ces techniques sont devenues de plus en plus difficiles ou les résultats ne sont pas facilement disponibles. Afin de surmonter les complexités posées par ces techniques traditionnelles, l'utilisation croissante des appareils mobiles et des algorithmes dans le secteur de la santé ouvre la voie à la définition de nouveaux cas d'utilisation et à la découverte d'analyses d'échantillons mobiles, car ils fournissent une interaction conviviale et familière. avec des objets affichés sur leurs écrans. Les interfaces et les techniques d'interaction rendues par les appareils mobiles apportent au spécialiste des dispositifs de DIV un certain nombre d'avantages et de valeur ajoutée en raison d'une familiarisation accrue avec les appareils ou de leur accessibilité, ce qui est facilité. Cependant, ils sont au début de leur parcours dans le secteur de la santé, en particulier dans le domains des DIV et point-of-care. Dans cette thèse, la proposition est de découvrir et de discuter de l'architecture, de la conception et de la mise en oeuvre d'une application pour *smartphone* (appelée «HemoDetect») avec ses algorithmes, interfaces et techniques d'interaction, qui a été développée pour aider à détecter l'hémolyse qui représente une rupture des globules rouges (érythrocytes) et la libération de leur contenu (cytoplasme) dans le liquide environnant (par exemple, le plasma sanguin), en le complétant par des statistiques de laboratoire et des mesures montrant son utilisation au cours des expériences, ce qui pourrait finalement être un concept réalisable qui pourrait permettre une détection précoce de l'hémolyse.

DEDICATION

I would like to dedicate this thesis to my family and thank them for their support (my wife, Dr. Vera Chifor, and my son, Aaron Chifor).

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I would like to convey my special thanks to my supervisor, Prof. Doutor Nuno Magalhães Ribeiro, for his valuable guidance and support.

Also, I would like to thank to my current client and employer, Roche Diagnostics (Schweiz/Switzerland) AG for encouraging an open research culture and providing full access to a wealth of high-quality digital and non-digital research articles and books as well as professionally equipped laboratories.

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LIST OF ABBREVIATIONS

3D – Three Dimensional

AI – Artificial Intelligence

API – Application Programming Interface

AR – Augmented Reality

BLM – Bulk Loader Module

CI – Color Index

CIE - Commission Internationale de l'Éclairage (in French); International Commission on Illumination

CMYK – Cyan Magenta Yellow and Key (Black)

DAO – Data Access Object

ED – Emergency Department (in Hospital)

ESR – Erythrocyte Sedimentation Rate

HCP – Healthcare Professional

HI – Hemolysis Index

IDE – Integrated Development Environment

LED – Light-Emitting Diode

LIS – Laboratory Information System

MDL – Medical Decision Level

NFC – Near-Field Communication

NIR – Near Infrared

OS – Operating System

PCB – Printed Circuit Board

POC – Point-of-Care

QR – Quick Response

RBCs – Red Blood Cells

RGB – Red Green Blue

TAT – Turn Around Time

UI – User Interface

UX – User Experience

VR – Virtual Reality

CHAPTER 1 – INTRODUCTION

1.1 The problem of hemolysis detection

Hemolysis (also known as haemolysis) may occur within the body (in-vivo) and during or after blood collection (in-vitro). Hemoglobin released from red blood cells (RBCs) in the blood plasma is referred to as free hemoglobin. Hemolysis is a pathological process (Plebani et al., n.d.) characterized by the breakdown of RBCs or erythrocytes, with the resulting release of hemoglobin and other intracellular components into the surrounding fluid (e.g. blood plasma) leading to various degrees of life-threatening conditions (e.g., anemia, when the concentration of hemoglobin declines very rapidly and/or falls below 6 mg/dL). Hemolyzed blood samples are frequently received in clinical laboratories, comprising as much as 3.3% of all routine samples and accounting for up to 40%-70% of all unsuitable samples identified – nearly five times higher than other causes, such as insufficient, incorrect, and clotted samples (Lippi et al., 2008). Hemolysis, most often, results from damage to red blood cells during blood collection. Several causes have been traditionally associated with an increased burden of in vitro hemolysis, including difficult venipunctures, use of inappropriate devices, under filling of blood tubes, exposure to extreme temperatures and physical forces during sample transportation via pneumatic systems and centrifugation at a too high speed of partially coagulated samples. In addition, even an excessive shaking or mixing of blood after collection (e.g., for times longer than recommended or with great force) is also usually acknowledged as a leading source of RBC injury (Plebani et al., n.d.). Pre-analytic processes (both technique and equipment) play a significant role in hemolysis rates (Shaw et al., 2012). Hospital emergency departments (EDs) have been identified as a source of hemolyzed samples, with observed percentages of affected samples ranging from 6.8% to 19.8%, and some as high as 30%; these levels are markedly elevated compared with other hospital departments (Shaw et al., 2012). The American Society for Clinical Pathology established a benchmark of 2% or lower for hemolysis rates among laboratory blood samples, and most blood collection sites outside the EDs achieve this level or better (Lowe et al., 2008). Therefore, hemolysis, represents

the most frequent error of sample rejection in point-of-care or healthcare facilities and clinical laboratories (Plebani et al., n.d.). In this thesis, the objective is to propose a potential solution for the hemolysis problem based on the modern smartphone device and a custom-developed prototype app acting together as a hemolysis detection instrument in point-of-care.

The use of mobile devices in the healthcare industry, in particular in the point-of-care (POC) diagnostics, has seen a significant increase in the past decade and has transformed the aspects of clinical practice (Ventola, 2014). Diagnostics companies constantly increase their efforts and invest considerable amounts of funding into research and development of portable (highly mobile and easy to connect), small footprint, user-friendly, inexpensive, fast and reliable diagnostic POC solutions (which combine hardware, software and cloud-based technologies to achieve a seamless ecosystem in which the patient data is secured and utilized to produce improved patient outcomes). Smartphones alone, or in combination with add-on devices have demonstrated capabilities of data collection, analysis, display, and transmission, making them popular in POC diagnostics (Xu et al., 2015). This naturally led to the rapid growth of the development of medical software applications (apps or mHealth apps) which run on increasingly powerful mobile devices (each year exciting smartphone developments are released, especially in the camera feature set). These mHealth apps are now available to support healthcare professionals (HCPs) with many important clinical tasks, such as, information and time management; health record maintenance and access; communications and consulting; reference and information gathering; patient management and monitoring; clinical decision-making; and medical education and training (Ventola, 2014). However, digitalization of healthcare is only at the start of its exciting and adventurous journey. There are still many unanswered questions or topics, which still offer tremendous research opportunities and open frontiers to further exploration. *Hemolysis detection* is one of them.

Therefore, this thesis proposes to showcase the development of the "HemoDetect" custom-built smartphone app, from the conceptual ideation to its design, software development and testing during the experiment, trying to accomplish one key objective: **hemolysis detection** in real blood samples or artificially prepared samples - and thus, potentially, make a

positive contribution and future impact in improving the quality of healthcare and patient outcomes.

1.2 Motivation

1.2.1. Challenges with traditional approaches

The turnaround time (TAT) represents the average amount of time, usually measured in days, that it takes a laboratory to complete a work assignment, or sample order (or simply known as "orders"). The TAT is measured as the period of time between the creation of the work assignment or sample order and its completion (Collins & Collins, 2018). Table 1 summarizes the average TAT considering the characteristics for both samples and patients in a study performed by Goswami et al. (2010).

Sample Type	Patient Type	Avg TAT
Clinical biochemistry samples (routine)	Inpatient (hospitalized)	5.5 h
	Outpatient (non-hospitalized)	24 h
STAT samples (emergency)		1 h

Table 1. Average TAT evaluated for one year (Goswami et al., 2010)

The TAT is critical in life-threatening situations. The shorter the TAT, the better. Patient results would be released faster, allowing for timely treatment. The steps below illustrate a typical workflow (or sample journey) that samples follow in an automated laboratory:

1. Blood samples are received and get registered in the laboratory IT system (LIS) – typically by scanning the barcode labels;
2. The closed samples are directly loaded into a Bulk Loader Module (BLM) which takes care of sorting the samples (typically positioning the samples correctly);
3. Depending on the ordered test(s), samples can be processed from the BLM to the centrifuge (high G-forces for coagulation, auto-balance, start by timer, and optional barcode scanner) or
4. Forwarded to the pre-analytical systems (sorting, decapping and optional aliquoting, recapping, quality and liquid level assessment);

5. In case the pre-analytical system is linked to an automated transportation system, the samples could be passed directly onto analyzers (e.g., clinical chemistry, immunochemistry, serology) by being transported in sample racks or in single sample carriers;
6. The test results from the analyzers and the precise location of the sample (both remaining and aliquots) are then forwarded and stored in the LIS;
7. The remaining samples (including their aliquots) are then either forwarded to the post-analytical units for archiving and storage for a predefined period of time (in refrigerated storage solutions) or disposed (biological waste).

If the blood sample is hemolyzed, the patient test results are jeopardized and possibly erroneous. The sample workflow is performed and completed in vain, time and energy is wasted, and the cost of the order still needs to be billed and paid (which finally results in increasing global healthcare costs). As an alternative to laboratory testing, simply performing and relying on visual inspection to detect hemolysis in the blood sample is *time consuming, highly subjective and not standardized* (Simundic et al., 2009). Therefore, a simple and automatic detection system is needed which could support in deciding quickly and upfront if a blood sample is eligible to start its workflow for further testing in the point-of-care or an automated laboratory. The personal motivation to build a solution such as this, originates in the deep passion for healthcare combined with an engineer's strong urge to build a working prototype app.

1.2.2. The plea for a smartphone-based approach

The "HomeDetect" app prototype proposed in this thesis provides a TAT of up to 1.10 hours and a cost of 199 CHF (Swiss Franc) or 198.78 USD (US Dollar) which represents the cost of the Android smartphone used in this experiment (with the assumption that in a future production version of the app, the app cost is included in the contract with a healthcare vendor which usually brings a complete offer of services, such as maintenance, support, connectivity, and security). In case of point-of-care facilities with already existing mobile infrastructures, the app could be installed on mobile devices which already exist or are in use in the POC facility (to help keep costs as low as possible). Medical apps (from

various healthcare vendors or apps from the same vendor which need to remain as individual apps) may co-exist on the same mobile device (installed on the same device) – thus having the advantage of reducing cost by sharing the same hardware resources.
Besides the TAT, and the cost factor, the easiness and convenience of use of mobile devices is equally important. Medical staff usually have their mobile device within their immediate reach and the procedure of taking a picture of the blood sample and then getting the result of hemolysis level would seem convenient, straightforward and uncomplicated.

1.2.3. Disadvantages of using smartphone apps in healthcare

Despite the increasing trend in usage of mHealth apps in healthcare facilities, there are, however, some HCPs who remain reluctant to adopt the usage of mobile apps in their medical daily practice. And they have some legitimate concerns about this aspect. Although they provide tremendous benefits, the overall (mHealth app) package needs to be completed so that the whole end-to-end experience is at its highest possible quality standard ensuring customer satisfaction and value added. There are several major areas which need to be clarified or improved in order to secure higher and faster adoption rates of mobile apps in healthcare. The list below provides a summary of these areas of concern.

1. Verification and validation standards (which need to be put in place by the corresponding regulatory bodies) that need to be fulfilled or complied to; the intended uses need to be established and followed by the HCPs; regulations need to be in place in order to avoid unintended consequences (Wallace, Clark, & White, 2012) such as fines for irregularities, erroneous or inaccurate patient results and patient data privacy risks.
2. Quality standards need to be in place and respected; while developing a healthcare mobile app, a product development process is strongly recommended be followed, which includes proper product documentation (e.g., user manual, service manual, installation instructions), regular quality check gates and traceability (for audit purposes).

3. Discussions, interviews with HCPs (as future end users) would need to be conducted to be able to better understand their needs and to steer the development efforts in the right direction. In a new development project, it is highly recommended to include the user right from the start, who can participate and bring feedback on requirements or real-life use cases. This concept puts the user at the center of the mHealth app.
4. Better usability, interaction techniques, and overall user experience. The most well designed app is brings no value added or usefulness to the HCPs if the content is inaccurate, erroneous, outdated, or too difficult handle and to navigate (Moodley, Mangino, & Goff, 2013).
5. Security, data distribution and storage, connectivity, user manuals, interface specifications (e.g. APIs) and other related aspects need to be clarified through official high quality documentation processes which must be compliant with the regulations in place (hence the importance of a vendor's reputation and credibility on the market).
6. Awareness regarding potential risks with some apps and the type of access they require (which may be downloaded from the official Apple or Google Play app stores); for example, if a mHealth app is in the catalogue of an official app store, that does not necessarily mean that the app is to be considered credible. In these cases, due diligence is highly recommended, hence the recommendation to the healthcare facility management to search for the benefit and advantage of having a partnership with an established and certified healthcare vendor.
7. Another concern that patients might have is the fact that a biological sample needs to be collected and analyzed (e.g. human blood for early hemolysis detection). There is limited understanding and lack of consultation from the patients about the biological sample collection process. A clear collection protocol with rules well explained and clear data collection procedures would certainly facilitate and gain patients' trust and endorsement related to collecting biological samples.

1.2.4. Exploiting smartphone apps to increase healthcare quality

Mobile devices have become commonplace in healthcare (Ventola, 2014) partially driven by their successful personal adoption in the modern everyday lifestyle of individuals. Users are exposed to new devices which get fast upgrade cycles on a regular basis (in most cases, yearly, but lately with a clear tendency of multiple releases over a year for the same model range). At the same time, manufacturers fast track their timelines to implement the next level of innovation as quickly as possible (e.g., introduction of foldable screens such as Samsung Galaxy Fold which had a challenging early market release; increased number of cameras on the device or improvements on the existing cameras by extending the supported number of mega pixels, wide range or lenses; addition of artificial intelligence algorithms, AI, to specific modules such as the camera module; improvements on the battery lifetime and recharging time; development of embedded software voice and text personal assistants). These innovations keep coming at the fingertips of the users (including the healthcare workforce), who get the new features and developments in a fairly short span of time, being distributed through the updated mobile devices presented to the worldwide market. But the hardware aspect is just one piece of the puzzle. Popular mobile operating systems (such as iOS and Android) are continuously developed and improved by tech giants with enormous financial resources, for example, Apple, and, respectively, Google, providing robust development frameworks for creating and maintaining apps. App developers or vendors follow this trend and take advantage of the latest hardware and platform developments, working hard to keep their apps up-to-date, rich and relevant for the target user groups. Feedback from users returns very quickly as well (e.g. a direct channel being the user reviews in app stores) and this important user feedback helps the app product managers taking the most fruitful decisions in regards to the next feature or function from the development backlog. And thus, the communication and development loop between app creators and end users remains active and healthy. This ecosystem is encouraging more innovation and more development of apps in many domain areas, healthcare included, as never happened before.

The “HemoDetect” app prototype proposed in this thesis positions itself and is trying to provide a helpful contribution to the “Clinical decision-making” (no. 6) category which is

one of the categories in the list below, by providing an aid in the diagnosis process (Ventola, 2014).

1. Information management;
2. Time management;
3. Health record management and access;
4. Communications and consulting;
5. Reference and information gathering;
6. Clinical decision-making;
7. Patient monitoring;
8. Medical education and training.

Furthermore, the list below shows the subcategories of "Clinical decision-making". These subcategories represent examples or use cases which can be found under the above mentioned category (Ventola, 2014).

a. Clinical decision support systems;
b. Clinical treatment guidelines;
c. Disease diagnosis aids;
d. Differential diagnosis aids;
e. Medical calculators;
f. Laboratory test ordering;
g. Laboratory test interpretation;
h. Medical exams.

Understandably, mobile apps are at the beginning of their journey in the medical space, and, as they mature and grow, the value added for patients will increase and be of relevance more and more. We already see a shift from apps which track user vital signs to more complex opportunities and use cases. In the diagnostics area, the long term ambition is to

develop the connected core laboratory, which communicates directly with the POC devices which would act as nodes in the connected ecosystem. Due to efficient integrations between mobile and immobile or bulky devices (e.g. analyzers), faster and better results will certainly help to increase the diagnosis quality and ultimately the care and wellbeing of patients.

1.3 Hypothesis

The use of a mobile app in hemolysis detection, such as "HemoDetect", would have the potential to improve the early detection of hemolysis in blood samples ahead of lab routine processing, with implications on significantly reducing the full turn-around-time (TAT) taken for sample processing by detecting the sample quality in advance, and thus improving the overall process and providing better patient care.

1.4 Research objectives

1.4.1. Benefits and goals of a smartphone based approach to detect hemolysis

Mobile devices and apps provide many benefits or advantages for healthcare professionals (HCPs), perhaps most significantly, increased access to (physically smaller) point-of-care tools or devices, which have been shown to support better clinical decision-making and improved patient outcomes (e.g., a better response time to delivering a precise diagnostic, especially when life-threatening, or a faster patient reaction to a prescribed treatment). The ultimate goal of a mobile medical app, also referred as an mHealth app or healthcare app is to democratize the detection and prevention (as early as possible) of medical conditions (such as blood hemolysis) which could be life-threatening in a short span of time.

The objective of the research work proposed in this thesis is to detect hemolysis in blood samples by using a custom-built smartphone app which employs the camera to photograph a whole blood sample or artificially prepared samples which replicate exactly the hemolysis levels, and algorithmically process the resulting bitmap picture, using image processing and analysis techniques, in order to detect the hemolysis level by considering both the

luminance (photometric measure of the luminous intensity per unit area of light travelling in a given direction) and the color of the highest density pixels (a pixel is the smallest unit of a digital image or graphic that can be displayed and represented on a digital display device, the word is coming from "pix" which means "picture" and "el" which stands for "element").

The following research objectives, listed below, support the main objective mentioned above.

1. To raise awareness of the hemolysis problem;
2. To design, develop and test a custom-built smartphone app prototype (called "HemoDetect");
3. To conduct an empirical study which consists of an experiment on a predefined number of sample tubes, in order to determine the effectiveness of the hemolysis detection approach suggested in this thesis;
4. To evaluate, compare and discuss the experiment results;
5. To present and discuss future research ideas or concepts based upon the results obtained with the work described in this thesis.

1.4.2. Raising awareness of the hemolysis problem

As mentioned at the beginning of this thesis, hemolysis still poses a health risk to patients' safety and wellbeing. Hemolysis manifests clinically as anemia, fatigue, jaundice, hematuria, and kidney failure, among many other symptoms (Shapira et al., 2001). In addition to renal damage, mechanical hemolysis can cause hyper coagulation, bleeding, thromboembolism and neurologic dysfunction, such as a stroke and an altered mental status (Olia, Maul, Antaki, & Kameneva, 2016). Even low levels of hemolysis have been shown to drastically increase RBCs aggregation at low shear conditions (Seiyama, Suzuki, & Maeda, 1993). Additionally, the release of hemoglobin from the overstretched RBCs into the blood plasma may have a toxic effect on the cardiovascular system due to its ability to bind nitric oxide, an endothelium-derived relaxing factor, leading to vasoconstriction,

hypertension, renal damage, and platelet activation (Minneci et al., 2005). By the end of the 19th century, it was already recognized that hemolysis promotes intravascular thrombosis (Olia et al., 2016). Experiments using stroma-free RBCs lysate revealed that a hemoglobin concentration as low as 30 mg/dL induced spontaneous platelet aggregation, which increased in direct proportion to the concentration of lysate (Wurzinger, Blasberg, & Schmid-Schönbein, 2017). All these findings or facts show the need of hemolysis detection as early as possible, providing early warning and clarity in the diagnosis process.

1.4.3. Design, architecture and development of the prototype mobile app

The design of the prototype mobile app is simple, user-friendly, and the result of the hemolysis level is interpreted and displayed to the user in an easy to understand manner. The prototype mobile app (software component), which is installed on a Huawei nova 3i smartphone (hardware component) running Android [version 8.1.0 (Oreo)] mobile operating system (complete smartphone specifications available in **Appendix 1**) is developed using the Android Studio IDE (Integrated Development Environment). The app code repository is available on GitHub (the code repository link is listed in **Appendix 2**).

1.4.4. Planning the experimental environment

The app will be tested on a set of 70 samples (with various levels of hemolysis) – recommended for best performance. It was determined that the number of samples tested (70) and the repeated test cycles would yield sufficient experiment results. Testing more samples than 70 would not add any particular benefit or finding. The result of each test will be reported to the user as hemolysis free (≤5 mg/dL), low hemolysis (5 – 30 mg/dL), medium hemolysis (30 – 60 mg/dL), high hemolysis (60 – 300 mg/dL), or very high hemolysis (≥300 mg/dL). The measurements are recorded in a database for storage purposes and further data analysis.

1.4.5. Conducting the experiment or empirical study

The experiment unfolds using the following procedural steps as listed below.

1. Testing each sample by manually picking up the sample (with medical rubber gloves for safety and touchscreen capacitive test purposes, for both artificial and real samples);
2. Capture an image of the upper part of the sample tube using the "HemoDetect" prototype app (on some cases using camera zoom depending on the size of the area which needs to be photographed);
3. Confirm the image captured by tapping a checkmark button;
4. Read the hemolysis result level that the app displays and record it into the database.

1.4.6. Evaluating the results

Evaluation of results (data aggregation, data analysis, graphs) is performed using Tableau and SPSS software which is connected to the results database.

1.4.7. Discuss future work

Hemolysis detection is a topic that will certainly continue to preoccupy the scientific community. In regards to the continuation of the research presented in this particular thesis, the course of future research is excitingly oriented mostly in the direction of using 3D mobile technologies which support augmented reality, AR (e.g. HoloLens 2 from Microsoft). As an illustrative image, the human operator (enhanced with a VR kit) who picks up a sample tube and by simply looking at it and activating the VR kit control will immediately see the hemolysis level, which is then selected to be transmitted to the LIS. Also, robotics, connectivity and security would represent additional important topics for future research work.

1.5 Research methodology

1.5.1. Setting boundaries of the problem space

Firstly, a hemolysis related literature review was performed with a strong focus on specific topics related to causes, detection and its widely-accepted reference measurement levels. The problems with current detection approaches were carefully identified and noted; also related work literature was attentively read. Other literature, which focused on Android app development, mobile user experience, modern laboratory infrastructure and sample workflow, was also consulted with the objective of establishing a framework of key concepts and technologies which served as valuable input for the design and development of the "HemoDetect" prototype app that intends to address the issue of early hemolysis detection predominantly in point-of-care.

1.5.2. Prototype app design

The second step in the methodology consisted of the design of a prototype app ("HemoDetect") which detects hemolysis of sample tubes. Following the design, the development of the app was performed and key findings were identified while testing (e.g. calibration and refinements). These elements provided evidence that the prototype app would work as expected and return accurate experiment results accordingly.

1.5.3. Planning and prototyping the experimental environment

In order to demonstrate the applicability of the approach and to be able to provide a concrete environment for evaluation, the prototype app was used during experiments in laboratory. The experimental environment was planned based on the preliminary testing needs identified during app development and testing. It was based on more than 70 tube samples which were tested with the "HemoDetect" app, monitoring in particular the behavior of the app, its usability, the returned hemolysis level results and the occurrence of events and types of levels and timings.

A database for recording the experiment results was also created to support while performing data analysis and concept evaluation.

1.5.4. Concept evaluation

The empirical study has the objective of testing how effective the hemolysis detection is using the "HemoDetect" app. Also, during the experiment, it collected users' input and recommendations which were annotated for future consideration. The experiment included more than 70 sample tubes prepared in the laboratory. These sample tubes were prepared in advance and labelled with barcode labels accordingly. Each sample was then photographed with the "HemoDetect" app and the hemolysis level was recorded in the database. Also, the time between the sample being picked up for hemolysis detection and the result being displayed on the smartphone screen was also recorded. The estimated additional time needed for real blood samples for gravitational sedimentation was also considered and factored in the final timings.

1.5.5. Analysis and implications for design

The last step in the methodology included the statistical analysis of the data gathered during the experiment that was undertaken to assess the effectiveness of hemolysis detection with the "HemoDetect" prototype app.

The implication of the experimental results was also determined for the design of future versions of the app. Aspects deserving further research were also identified.

The experiment results presented in this thesis indicate that hemolysis detection using a smartphone app provided a real benefit for fast hemolysis detection, especially because it provided a fast TAT and there was no need of additional hardware except the smartphone. The results also show that hemolysis detection using a smartphone app contributes to a safe and cost-effective diagnosis.

This research methodology is followed linearly throughout the present thesis.

1.6 Summary

This chapter introduced the hemolysis detection problem with a summary of challenges faced while using traditional detection methods and a proposition of a modern smartphone-based approach to address the hemolysis detection problem and achieve fast and accurate hemolysis level results. A summary of the areas of concerns of mHealth apps was also presented.

Then, this chapter continued with the presentation of the research objectives of this thesis with a strong focus on benefits and goals of using a smartphone app to address the problem of hemolysis detection while raising awareness of the impact of hemolysis in human healthcare.

A thesis work plan with steps and research methodology was also introduced, with an emphasis on the design, architecture and development of the prototype app. The testing plan in the envisaged experiment was also summarized, followed by information on the discussion and evaluation of results, and possible future research work and concept ideas.

In conclusion, to achieve better patient outcomes, problems with current diagnostic approaches need to be resolved. Hemolysis detection is one key area where HCPs would like to experiment and use better, cheaper and faster methods in routine operations. Performing a simple visual inspection is far from optimal and certainly not safe.

One way to address these problems is to use smartphone technologies to support hemolysis detection. Since the healthcare universe relies more and more on mobile devices and technologies, it makes sense to employ them for obtaining better quality of healthcare. This way, hemolysis is detected and potentially treated, or cured. This thesis proposes the exploration of a very small part of the greater area of analysis of human body fluids.

CHAPTER 2 – HEMOLYSIS DETECTION: A REVIEW OF RELATED RESEARCH

2.1 Overview

Although hemolysis is a frequent and expensive problem with a negative impact on patient outcomes, there is lack of consistency in hemolysis detection and reporting between different research studies or methods. However, researchers are tirelessly working and scouting for different approaches and striving for standardization to detect hemolysis. Improving consistency would support generation of benchmark data (or standardized data) used to create best practices to monitor and reduce this leading cause of pre-analytical laboratory or point-of-care error (McCaughey et al., 2016). While researching on hemolysis detection and writing this thesis, articles, books or studies were consulted on prominent research platforms such as PUBMED, Nature, Google Scholar, Mendeley, ScienceDirect, Scopus, Frontiers, or ResearchGate. They provided an immense wealth of knowledge and strengthened understanding of hemolysis detection approaches.

2.2 Research on general hemolysis detection methods

In December 2016, a review article was published in the "The Clinical Biochemist Reviews" on PUBMED, called *"Current Methods of Haemolysis Detection and Reporting as a Source of Risk to Patient Safety: a Narrative Review"* (McCaughey et al., 2016). In their research, the authors aimed to investigate current methods of hemolysis detection and reporting, knowing with certitude that hemolysis detection is still an immature field of research due to a relatively small amount of published material and studies related to this subject (McCaughey et al., 2016).

From a total amount of 50 studies that met the criteria set by the authors, 20 detected hemolysis using the Hemolysis Index (HI), 19 by visual inspection and 13 by undefined methods. Sixteen studies reported the analyte of interest (substance whose chemical constituents were being measured), with only three studies reporting a hemoglobin level at which the sample tube would be rejected.

Considering the amount of studies where the visual inspection is used to detect hemolysis in blood samples, it is certain that this traditional method is still being widely used, rising up to 38% of the total studies which were analyzed.

When detecting hemolysis by visual inspection, only four studies reported the plasma free hemoglobin level equivalent to the color change used to detect a hemolyzed test sample. In these four studies, the mean plasma free hemoglobin level used to detect hemolysis by visual inspection was 850±436 mg/L (McCaughey et al., 2016).

Like in any other research field, the reference values or the benchmark values are important for comparisons and standardization. Reaching consensus about reference values is not a trivial task especially when the field of research is still immature. One of the advantages of the standardization is the fact that based on conclusions drawn from comparisons, the performance of the hemolysis detection executed by certain point-of-care or wards could be improved (the areas of improvement are identified or pinpointed with more confidence). Inconsistencies, if present, are easier to identify while, for example, executing comparisons between two data sets. Based on the accumulated know-how and taking advantage of a standardized approach, certain best practices could be issued with the scope of improving the effectiveness of the hemolysis detection. But, at the base of any standardization process, lays the quality of the data (the highest the quality of the data the more robust the developed standard would be).

To be able to reach a satisfactory level of accuracy while collecting data, the point-of-care facilities would need to respect and fulfil certain protocols for hemolysis detection and reporting. Without rules in place (as also explained in subchapter 1.2.3), the result of the standardization would be superficial. It is important that from the start, certain rules to be followed about how to collect data, how to record it, which parameters to measure and how to build the report. There were many attempts so far to consolidate knowledge and reach a consensus about which samples should be categorized as hemolytic. For example, a group of scientists agreed that hemolysis should be defined as “any samples where one or more tests were not performed or one or more results were rejected or not reported due to hemolysis” (McCaughey et al., 2016). Certainly, there are different individual opinions or

voices which might not entirely agree with this statement. And probably it is expected to be the case. Nevertheless, one thing is absolutely clear: there is a small degree of commonality or standardization between healthcare entities which measure hemolysis and report it. This comes only to strengthen the need for greater standardization and consistency especially when traditional techniques for hemolysis detection are still in use (visual inspection).

Blood sample tubes, are still being inspected visually to detect hemolysis as mentioned above. This is not a good practice and it should not be used today, due to unreliable results. Before the commercial availability of automated pre-analytical systems, equipped with both hardware (cameras) and software, visual inspection was used even to a greater extent. Today, in some insufficiently equipped laboratories or point-of-care facilities, this practice continues to be used. Due to lack of affordable alternatives, the professionals working in these environments believe that visual inspection can be still be used and trusted. Efforts are considered to increase awareness about the disadvantages of the visual inspection method joined by funding initiatives to better access to modern or mobile laboratory equipment.

In a modern laboratory, one of the measurements performed on the sample tubes is hemolysis level or hemolysis index which is a serum indicator that represents the amount of free hemoglobin in the blood plasma or serum. The hemolysis index or hemolysis level is considered to be the standard to be measured in most of the laboratories. Modern pre-analytical systems can identify and report with a clear distinction between low hemolysis or medium hemolysis, for example, but in some cases they are not able to distinguish between high or very high. The precision of the measurement allows this differentiation to be possible, whilst for the human eye, during a simple visual inspection, this could have been absolutely unidentifiable. This is why the visual inspection is to be avoided, especially when technology and machinery can do a much better job, providing reliable results. Certainly, there is the cost factor, which is high, since such pre-analytical systems are expensive to buy or lease and also to clean, maintain, service or repair. This situation shows once again the need for a more affordable, smaller, but equally or even more efficient (more hemolysis levels which are reported) hemolysis detection solution, like the one which will be proposed in this thesis, based entirely on the smartphone and its capabilities.

But, before going into the core elements of this thesis, it is imperative to describe further the current state of the hemolysis detection methods and reporting aspects. Understanding the current methods used in the field and how healthcare professionals document measurements or findings was essential while writing this thesis. Previous researchers thought that discrepancies between how a laboratory detects hemolysis and reports it, compared with another laboratory are worthwhile to be investigated. A research paper in a form of a narrative review was most appropriate to identify and document the hemolysis detection methods (McCaughey et al., 2016). The authors researched thoroughly on noteworthy platforms such as PUBMED, Embase, Medline and CINAHL databases in January 2015 to find studies that investigated pre-analytical errors, rejected sample tubes or in vitro hemolysis. Studies which were subjected to rigorous peer review, being published between January 2000 and December 2014 were also included in the search, with the condition if they reported on primary data from general population, or provided an overall rate of hemolysis or provided sufficient information from which to calculate an overall rate (McCaughey et al., 2016). Articles that reported on specific patient conditions such as hemolysis rates for patients with diabetes were excluded by the authors from their research due to the fact that they were offered specific information about hemolysis levels in the context of specific diseases which may have a strong influence on the quality of blood properties. The search protocols implemented and respected by the authors are carefully chosen and very impressive.

At the start of the research of this thesis, while consulting general articles about hemolysis detection, it was becoming more and more clear that there are massive gaps in hemolysis detection and reporting. For example, a healthcare facility which relies entirely on visual inspection for detecting hemolysis would record large variations in their measurements. On top of this, even if two separate healthcare facilities would employ the visual inspection technique and would record the results, the variations would still be present (probably due to the fact of lack of inconsistency on what is actually measured or how it is interpreted or the scale or calibration curve used). The same is also applicable for laboratory pre-analytical systems, due to inconsistent calibrations or different rules for system's teaching which could lead to the results with considerable variations. When considering

inconsistency in hemolysis detection, it is important to note that the effect of hemolysis interference on test results depends on the analyte being tested (on what actually gets tested or measured) with sample tubes that are considered as being hemolyzed for a specific analyte, and potentially demonstrating a level of hemolysis that does not have an impact on other analytes (McCaughey et al., 2016).

Reaching greater consistency in hemolysis detection and reporting is something that many renown scientists are calling for. Their wish is to see progress and tangible improvements on this front; certainly, mindful of the fact that in the process of improving these elements, specific aspects must be considered as shown below.

- The level of quality of the pre-analytical devices which are used
- The discrepancy between the different manufacturers of these devices when it comes to reference values, hemolysis levels or measurement choices
- Also, worthwhile to mention is the opacity of the image processing algorithms or methods (since these devices have a commercial aspect, patents, implementations and formulas, are not shared publicly, not even with the final customer who purchased or leased the device); the majority of these devices use multi-wavelength spectrophotometric measure technologies subjected to Intellectual Property Management from a legal perspective (to protect the interest of the issuing company and keep its technology secret)
- The sample tube collection on which the tests are observed or carried out; the level of training of the staff extracting the blood from the patients, the materials which are used

Compared to the chemical methods of detecting hemolysis, the photometric methods for plasma free hemoglobin have been shown to be safer, easier and more accurate with satisfactory consistency of the hemolysis level observed amongst different analytical platforms (McCaughey et al., 2016). In addition, the photometric method is conveniently cheaper than the chemical one (e.g. no need of reagents or third-party substances which are used).

Further studies will be needed to be able to deepen research and learn about external factors and their impact on the hemolysis levels. Perhaps that future studies, which will have the purpose of establishing standards in hemolysis detection and reporting would rely on past research, and hopefully, consider even this present thesis and its findings. Creating standardization and improving consistency, demands, undoubtedly, as many articles and as many studies or experiments as possible since lessons learnt, data, figures, and statistical findings sit at the base of a sustainable standardization for hemolysis detection and reporting.

The quality of data that is generated out of hemolysis studies, is directly proportional with how well the hemolysis detection methods, which are researched, are documented, explained, and rendered as easy-reproducible by opening access with a full disclosure mindset. By applying this rigorous approach, the quality of the data could be improved and made valuable. This is one of the key mission statements which will be repeatedly acknowledged during the writing of this present thesis.

Unfortunately, one particular commonality found in the literature about hemolysis detection, which was consulted while writing this thesis, was related to the fact-based aspect and overall quality aspects with several elements enumerated in the list below.

- Research objective too broad or not exactly clear
- Figures or formulas were ambiguous; not precise enough
- The materials which were used in the experiment(s) were not well documented which turns the experiment(s) partially or completely irreproducible by another researcher
- Statistical population composed of insufficient number of sample tubes or little variation in the sample tubes which were tested
- Statistical analysis completely omitted or not clear
- Pictures or images not convincing enough or completely missing

With these points in mind, a selection of hemolysis detection methods was performed, with the purpose to present and comment on only the relevant and well written articles or papers. Certainly, a potential minor level of subjectivity or bias might have also altered the selection (ultimate objectivity is not claimed here at all). Caution and carefulness was applied where possible when interpreting the results from individual studies or citing specific data (considering various circumstances and judging them in the whole context of the hemolysis detection research subject). Despite the large variability in the hemolysis detection methods and the hemolysis level or hemolysis index which was reported, the selection was performed considering that the field of research is still in its inception phase or beginning and relatively immature. Rules on which the acceptance or rejection of a sample tube was made were also considered in each study context. In general, a set of characteristics were traced or observed while making the selection. These characteristics, which are not exhaustive, and presented in the list below, confer to the respective research the authority and validity and, potentially, in the future, contribute to the federated effort of improving the consistency of hemolysis detection and reporting.

- State exactly which hemolysis method it is used (e.g. conduct a spectrophotometric assessment of the plasma free hemoglobin to detect hemolysis)
- Indicate a clear specification of the units of measure (e.g., mg/L or mg/dl)
- Identify and specify the analyte of interest (in clinical laboratory or clinical chemistry, an analyte is a biologically important substance from the human body fluids that is undergoing analysis, often referred to as a "test"). For example, an analyte might be the hemoglobin, or bilirubin, or potassium; in general ions, salts, minerals, small organic molecules or large macromolecules (primarily proteins).
- Record the measured values and how they compare to reference values or intervals or a medical decision level (MDL) to provide diagnostic and clinical meaning for the measured values
- Perform an analyte specific assessment for determination of significance and provide a clear statement of the plasma free hemoglobin level at which a sample tube would be accepted or rejected (McCaughey et al., 2016)

As mentioned above, there is no definitive claim on the exhaustiveness of these characteristics, but they are considered of being of great importance, which have an impact on the research findings and conclusions. These characteristics could also be seen as a future roadmap to improve the consistency of hemolysis detection and reporting between point-of-care facilities, clinical laboratories or other healthcare entities which perform sample testing. By respecting these characteristics, it will definitively help in obtaining reliable data of hemolysis levels. Then, this data could be considered as the benchmark data which could be used to support the design and testing of quality practices that monitor and reduce hemolysis, improving the safety and efficiency of laboratory processes and patient care (McCaughey et al., 2016).

In conclusion, despite hemolysis being a frequent, recurrent and high-cost problem for national healthcare systems, which are constantly fighting to preserve the health and wellbeing of populations, it is fair to state that there is poor consistency in hemolysis detection and reporting between the existing studies at the moment.

Improved consistency would facilitate the creation and implementation of best practices with the purpose to monitor and reduce this leading cause of pre-analytical error and achieve greater results in patient early diagnosis and patient care.

2.2.1. Optofluidic sensor for hemolysis detection

Recent research in hemolysis detection shows the need of having a better integrated approach into PoC systems which is essential in fulfilling the mission statement of detecting hemolysis *as-early-as-possible* and *as-closest-as-possible* to the patient.

This need is very well observed by research scientists and there are a number of research studies which strive to make hemolysis detection systems as compact as possible to be able to ready for possible integration in PoC systems upon going through the typical product development process, design control and commercialization phases (analysis, feasibility, development, implementation, manufacturing and sales).

For example, in this research paper, a PoC hemolysis sensor combining evanescent (quickly fading) absorption detection and local plasma separation without the need of any previous sample preparation was researched and developed by Zhou et al. (2018).

The authors of this research highlighted that current or traditional methods entail centrifugation for cell-plasma separation, which is complex, time consuming, and not easy to integrate into point-of-care (PoC) systems (Zhou et al., 2018). This created a window of opportunity for the group of researchers and they came up with a proposed system to detect hemolysis which does not require centrifugation of the sample tube with whole blood.

They have embarked on a mission to demonstrate an optofluidic sensor composed of nanofilters on an optical waveguide, which enables evanescent-wave absorption measurement of hemoglobin in plasma with the capability of real-time inline detection on whole blood without extra sample preparation (such as centrifugation). Long-term testing with inline integration in a modified, commercial blood gas analyzer shows high reliability and repeatability of the measurements even with the presence of interference from the yellowish substance or analyte in the blood, called bilirubin (Zhou et al., 2018).

The authors think that their work has large potential in improving diagnosis quality by enabling PoC hemolysis detection in blood gas analyzers and can also lend unique sensing capabilities to other applications dealing with complex turbid media, which represents the media in which light scattering by constituent or generated irregular (randomly distributed) optical non-uniformities is of significant intensity. Light scattering in turbid or thick medium leads to a change in the initial direction of the light irradiating the respective medium.

The plasma separation is achieved with an integrated nanofilter comprising an array of nanowells. Separation of blood plasma and RBCs is achieved by either active methods, such as magnetic, dielectrophoretic, and acoustic, or passive methods, such as hydrodynamic, sedimentation, and filtration.

The optofluidic strategy is based on an innovative dual function of nanofilters that act as a component of the waveguide as well as a functional filter for RBCs. The nanofilters enable light propagation without diffraction and scattering while effectively blocking cells or

platelets (small blood cells that help blood clotting) with short diffusion length and time. Grating couplers are integrated to couple broadband LED light into – and spectrally disperse light out of – the waveguide sensor, enabling spectral analysis without using an extra spectrometer or monochromator. The wavelength range of the LED light source is adjusted to the highest optical absorption of hemoglobin. The absorbance measured with the optofluidic sensor is compared with photospectrometer reference data. The sensor was integrated into a modified, commercial blood gas analyzer (ABL90 FLEX, Radiometer Medical ApS, Denmark), to assess its repeatability, reliability, and durability.

Through a rigorous measurement routine process, the authors demonstrated an optofluidic waveguide sensor for in-line hemolysis detection on whole blood with unique, integrated cell or plasma separation ability. The arrangement of nanofilters on top of the waveguide enables local blocking out of the evanescent field and allows independent choice of light wavelength and filtration size.

The fabrication process can be easily scaled up for industrial production with low cost and high production yield. The sensor can be used as a flexible, standalone device or can be easily integrated into existing PoC platforms. It is also demonstrated that a spectrometer-less analysis of spectral extinction of molecules can be successfully achieved by employing outcoupling gratings as a dispersive element and a line camera for readout. The portable optical setup is capable of being integrated into a commercial blood gas analyzer. Long-term tests up to 7 weeks were also carried out to demonstrate the high repeatability and reliability of the hemolysis sensor subject to whole blood samples even with the presence of strong interference from bilirubin. The authors are convinced of the large potential for the sensor to be used in-line in blood gas analyzers for hemolysis detection as well as in other fields related to turbid media measurements (Zhou et al., 2018).

Although the research results and conclusions are promising, being definitively a good step into tapping into the PoC system universe, the sensor's materials and its potential systems' integration in blood gas analyzers (which, in most of the cases, are fairly bulky) could drive up the overall cost of the proposed solution. The footprint is also another aspect which is comparably larger than one of a smartphone.

2.2.2. A simple method to monitor hemolysis in real time

Most of the research articles put the blood sample tube at the center of the research; a static whole blood sample tube extracted from the patient, or an equivalent solution simulating the blood and with its different hemolysis levels. This is because most of the diagnostic testing is performed based on a "sample" taken from the patient at a given point in time, a sample which represents a "snapshot" with a date stamp (and in some cases, even with a full timestamp) of that particular human body fluid or other human testing material (e.g. tissue). Then, the sample tube can begin its testing journey, finishing either in a cold storage unit (for later retrieval for retesting or just temporary storage) or in the biological waste bin. If the test would need to be repeated after a certain period of time, a new fresh blood sample would need to be extracted (to check if the blood sample tube is still hemolyzed or not, and, if yes, to which degree).

In this research article, the team of authors goes beyond the static aspect, to a more dynamic one, and proposes a simple technique to monitor changes in hemolysis levels continuously and in real time (Van Buren, Arwatz, & Smits, 2020). That means there is no sample tube, there is no snapshot, but an ingenious circuit made of a magnetic stirrer with an open reservoir, a peristaltic pump which pumps the blood and a 3D printed channel with a conductive floor and ceiling in the test section.

While the RBCs (red blood cells) rupture, the overall electrical conductivity of the blood increases. The authors use porcine blood in their experiment and demonstrate that small changes in this blood can be detected through a simple resistance measurement.

The application of the proposed solution by the authors could be imagined for a patient which undergoes a dialysis process and in clinical procedures where real-time and continuity are two important requirements. For example, during medical procedures where the blood is temporarily or definitively removed from the human body or inserted from and into a human body, with the intention of either cleaning it or replace it, such an application would make sense (real-time and continuous). Or, for example, if the dialysis machine is faulty, and the patient's blood is running through it, then it makes perfect sense to have a hemolysis detection mechanism which is working real-time and continuously.

In majority of the product development processes for medical devices or materials, there are a number of product risk assessments and product constraints which must be carefully assessed and documented by the project development teams supported by the regulatory affairs and quality experts. One of the product design constraints for certain medical products, such as hypodermic needles used in phlebotomy process (making a puncture in a vein, usually in the arm, with a needle, for the purpose of drawing blood) is to prevent hemolysis. The same could be applicable to certain medical pumps or prosthetic organs (Van Buren et al., 2020). In other words, the materials or devices that are used to work with the human blood should not damage or alter it in any way.

The cytoplasm of red blood cells (RBCs) is rich in hemoglobin, a biomolecule that contains iron, can also bind oxygen, and is responsible for the red color of the cells.

As red blood cells rupture, they release their hemoglobin into the plasma, which is the largest part of the blood. It makes up more than half (about 55%) of its overall content. When separated from the rest of the blood, plasma is a light yellowish liquid. Plasma is composed on mostly water, salts and enzymes. Once the hemoglobin is released into the plasma, the plasma starts to have different color variations of red.

With the help of a spectrophotometer, which measures how much light of a given wavelength is absorbed by the sample tube, the hemolysis level can be measured by separating the plasma from the red blood cells and analyzing the amount of cell-free hemoglobin.

A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed, after it passes through the sample tube solution. With the spectrophotometer, the amount of a known chemical substance (concentration) can also be determined by measuring the intensity of light detected. Depending on the range of wavelength of light source, it can be classified into two different types as shown below.

- Ultraviolet-visible spectrophotometer: uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum

- Infrared spectrophotometer: uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum

Today, spectrophotometry is considered the most accurate method for measuring hemolysis. There are also a number of other possible methods that do not require extracting red blood cells or using chemical analysis but the authors wanted to research a method which did not require any prior specialty sample preparation. The method they proposed will allow clinicians to monitor hemolysis during a procedure and provide immediate information on the level of blood damage.

The authors had the challenge to continuously monitor the possible changes in the hemolysis level of a patient. To be able to reach a continuous real-time requirement, the authors considered measuring the electrical resistance of the blood. Human blood conducts electricity. The healthier the blood cells the smaller the overall conductivity due to the protection layer of the red blood cells. When hemolysis occurs, the hemoglobin is dispersed and the electrical conductivity of the blood increases. By observing and measuring these conductivity fluctuations, the authors concluded that blood electrical conductivity could be correlated with the level of hemolysis and determine what exactly is the hemolysis level based on these measurements.

The technique does not demand any external sources of light, or an obscure environment, or the waiting time for sedimentation of the red blood cells from the blood plasma. Moreover, the proposed technique does not require any prior specialty sample preparation or any specific chemical analysis or detection. The authors imagined their solution as a continuous blood flow where hemolysis is detected and measured as soon as something happens (e.g. as soon as the red blood cells start to rupture).

The materials used by the authors in their experiment are listed below.

- A test cell that consists of a small converging or diverging channel equipped with top and bottom electrodes
- A laminar flow loop driven by a peristaltic pump
- A high-quality inductance-capacitance-resistance meter for continuous sampling

- A conventional conductivity probe for periodic sampling

The researchers started by testing the system using a potassium chloride or potassium salt based solution to achieve specific levels of salinity in deionized water. This would simulate different level of blood hemolysis in a real-life scenario. The saline resistivity was then measured continuously using the inductance-capacitance-resistance meter for continuous sampling and periodically using the conductivity probe for periodic sampling. The measurements were then compared with the corresponding theoretical values.

After completing the tests with the saline solution, the authors continued their experiments using porcine blood (chosen due to the similarities it shares with the human blood in terms of blood cell sizes and hemoglobin concentration). They have started to inject 500 mL of non-hemolyzed porcine blood into the system and then, every 10 minutes, they added an additional volume of 50 mL of the mechanically damaged blood into the stirring reservoir, to be able to observe the gradual increase of the hemolysis levels.

Each addition of damaged blood causes a step change in the blood electrical resistance showing a direct correlation between the change in blood conductivity level and the total hemolysis percentage. The authors wanted to compared the results obtained with measurements on the same samples performed by a spectrophotometer. For this purpose, they have extracted small samples of 1.5 mL for each simulated hemolysis level, which were then measured by the spectrophotometer. The comparison shows that the results are similar and very close.

These experiments show that electrical conductivity of blood cells can help in detecting changes in hemolysis of blood. The measurement is continuous, in real-time, and with a high potential to be easily implemented in the healthcare practice. At the moment, the technology is limited to execute measurements of the relative changes in the blood for a specific patient. It is not to be used to measure quantitative blood damage because the electrical properties of blood samples may vary from one patient to another.

There are, however, situations where this method could be used. For example, as mentioned at the beginning of this research review, the solution could be used to detect dialysis

machine malfunctions that may damage otherwise the non-hemolyzed blood by comparing blood conductivity on the machine input and output.

The authors acknowledge that future research work would be needed to perfect the proposed solution, and to explore further the impact on possible patient variables (such as hemoglobin or hematocrit concentration, blood conditions that may impact conductivity) and flow variables (such as laminar or turbulent, flow rate, temperature).

In any case, it is encouraging to observe the efforts which are being made in blood hemolysis continuous detection in a real-time situation for the patient.

2.2.3. Point-of-care hemolysis detection in blood gas specimens directly at the emergency department

The objective of this study was to assess the proportion of hemolytic blood gas samples performed at the emergency department and calculate the predictive values of a novel point-of-care solution for hemolysis detection (Duhalde, Skogö, & Karlsson, 2019).

In clinical decision support systems, the blood gas analysis represents an important and frequent topic. At the emergency departments, blood hemolysis is a common problem which impacts the accuracy of the analysis results. Blood gas analyzers are not equipped with technology to detect blood hemolysis. If a hemolyzed sample is analyzed by the blood gas analyzer, the final result and its interpretation is highly compromised or erroneous.

The authors of this research observed a valid need of implementing a hemolysis detection method at the point-of-care. They have embarked to test blood samples with a device called Helge H10 s-system, commercially available, from HemCheck[1] company. The tests have been performed at the emergency department and the results were recorded.

In terms of establishing reference values, the researchers decided to determine them based on a traditional analyzer.

This proposed method identified hemolytic samples with a sensitivity of 80%, specificity of 99%, positive and negative predictive values were of about 89% and 98% respectively.

[1] Consulted online (last checked on 11/02/2020): https://hemcheck.com/

Traditionally, the biomarkers included and measured by the blood gas analyzers were limited to just a few, such as acidity of the blood, the oxygen gas or carbon dioxide levels in the blood, or oxygen saturation of the blood. In the recent years, a number of technological advancements and research, materialized in adding more biomarkers to be measured, for a more targeted and precise diagnosis. For example, the following new biomarkers were added to the test list: potassium, chloride, ionized calcium, magnesium, creatinine and bilirubin, as well as, hemoglobin.

In emergency departments, the use of blood gas instruments is high. Point-of-care analysis instruments help decreasing the turn-around-time (TAT) of the sample test order, and help patients by providing immediate results.

In the past, point-of-care measurements were considered as not reliable enough. That is why the medical staff decided to send the sample tube to a specialized laboratory. In recent years, the quality of the point-of-care instruments was considerably increased, partially due to new technologies available and continuous innovation efforts. Learnings from the field were also playing a key factor.

Medical guidelines state that blood gas specimens affected by hemolysis should not be analyzed. Emergency departments could benefit from a reliable method for detecting hemolysis directly at the point-of-care and could increase the quality of the results provided by blood gas analyzers and other point-of-care tests.

The authors were convinced by the fact that a point-of-care hemolysis detector could improve the clinical processes starting in the emergency department. In their study, they have set two main research objectives as shown in the list below.

- Investigation of the prevalence of hemolysis in arterial and venous blood gas samples at an emergency department in Sweden
- Perform a method comparison between a novel hemolysis detection system for use at the point-of-care and routine hemolysis detection in the central hospital laboratory used as the reference method

The materials used in their study are listed below.

- Samples from patients from the emergency department which were collected for blood gas analysis
- Helge device from HemCheck company. The product consists of two different disposable tests, v-Test for vacuum tubes and s-Test for blood gas syringes, both of which can be used in the same reader. With a simple push of a button, the reader quickly shows if the blood sample is hemolyzed or not. The aim is to identify hemolyzed blood samples at the time of sampling (point-of-care) and with the same quality as a laboratory. The Hemcheck test has been under development for several years and has originated out of need, innovation and a user-friendliness consideration. Its CE-marked and patented. The single-use test is used by adding a small amount of blood to the disposable test device, where the plasma and serum are then separated. The disposable test is then placed in the digital reader and within a few seconds the blood is analyzed for hemolysis. Helge device signals if the sample is hemolyzed or not. The Helge device comprises two tests and a common reader mechanism as shown in the list below.

1. v-Test – disposable test for vacuum tubes

   Single-use tests for vacuum tubes are possible using a cylinder-shaped plastic housing with reading windows adapted for the digital reader. The cap on the sample test tube does not need to be opened and is instead activated by penetrating a needle into the blood sample cap. A needle guard protects the user.

2. s-Test – disposable test for blood gas syringes

   Single-use tests for blood gas syringes consist of a plastic connector (s-Test) with a reading window adapted for the digital reader. The blood gas syringe is placed in an opening on the top of the s-Test and a small amount of blood is dispensed into the test and then placed in the reader.

3. A common reader

   For easy and a user-friendly reading of the disposable v- and s-Tests, there is a common reader that, with a simple push of a button, signals if the blood sample is hemolyzed or

not. The reader is activated with the pressing a button, starting the evaluation of the disposable test. A photometric analysis of plasma or serum is performed. The answer is communicated through a red or a green lamp. The reader is mobile, easy to clean and can easily sit on a medical trolley or next to a blood gas analysis instrument. Helge performs the analysis in a matter of seconds, opening up new possibilities for assessing blood sample quality.

During the study, the group of researchers tested unidentifiable blood samples on the Helge H10 s-system, which uses a photometric technology for hemolysis detection at the point-of-care. The system analyzes free hemoglobin in plasma quantitatively in two steps.

From a syringe, the user dispenses whole blood into a disposable test where plasma is instantly separated from whole blood by vertical and lateral flow filtration. The user inserts the disposable test into the reader and initiates a measurement. A sensor measures photometrically the color of the hemoglobin. The camera sensor is made up of a matrix of light sensors. Each light sensor has a red, green or blue (RGB) color filter, thus the RGB model is used. When used as a camera, the signal from each red, green and blue light sensor in the matrix is used to create a color picture. In the present application, no spatial information was desired and all the RGB values from the sample area are combined to one red, one green and one blue value which is correlated to hemoglobin concentration.

The camera technology, and the use of RGB color model indicate that color models or color spaces could be used to photometrically analyze the image. Further analysis of the image processing algorithms was not possible during the review of this article, due to the commercial characteristic of the Helge system (the technology is protected).

During measurements, the hemoglobin concentration was translated to a hemolytic index (a mapping according to specific concentration rules). The user can define which values should be considered positive (the cut-off limit is adjustable using software settings in the Helge system).

The authors performed whole blood measurement of hemolysis in this study, with high specificity (99%) and sensitivity (80%) using the point-of-care method compared to the laboratory’s routine method. The technical progress shown in this study provides a new

opportunity to increase patient safety and overall pre-analytical quality by detecting and possibly preventing analysis of hemolytic blood gas samples.

Among analytes of the blood sample, the ionized calcium has also been identified as sensitive to hemolytic interference, and when present, a negative bias is introduced. This analyte is of particular interest to measure in patients who have sustained blood loss, as severe bleeding increases the risk of developing hypocalcemia, which is associated with increased mortality, hypotension and inhibition of clot formation (Duhalde et al., 2019).

Additionally, if these patients receive even small volumes of blood transfusions, the hypocalcemia is severely made worse. Immediate diagnosis and treatment are needed to minimize adverse effects. Recent research data also suggests that ionized calcium is superior to total calcium, albumin-corrected or not, when abnormal total calcium values should be followed up or in patients with known hypoalbuminemia (Duhalde et al., 2019).

The evaluated device, called Helge H10 s-system, provides a new possibility for healthcare providers to identify hemolyzed samples, a pre-analytical error that previously has been hard to detect in blood gas testing. It opens up even more the point-of-care testing subject and how it can be improved.

A strength presented by the authors in this study was that hemolysis measurement was performed with the point-of-care method directly after blood gas analysis, and instantly followed by centrifugation of residual whole blood and aliquoting to sample tubes, while all tasks took place in the emergency department. In terms of blood extraction, almost 95% of the samples were venous which represents a large quantity of venous blood gas samples that were collected in this study. The authors considered as a drawback in the data collection, that the blood collection material, other than syringe model, was not safe enough. Evidence suggests that blood collection material (e.g., peripheral intravenous catheter, straight needle) may create spurious hemolysis. This factor could be valuable to assess in the setting described by the authors.

Another drawback with this study is the subject matter. Defining and grading hemolysis is not a standardized topic. In this case, the authors have compared a novel point-of-care instrument with a hemolysis detection method that is evaluated and deemed good enough

for practical purposes. However, there is no established or ultimate standard for defining in vitro hemolyzed samples (Duhalde et al., 2019). The authors acknowledged as well the general lack of consistency and standardization when it comes to measuring hemolysis and reporting it.

Finally, as a summary, hemolysis is common in blood samples collected for blood gas analysis at the emergency department and can be detected directly at the point-of-care using a novel technology for hemolysis detection provided by the Helge H10 s-system from the HemCheck company.

2.2.4. Hemolysis detection in sub-microliter volumes of blood plasma

The authors set as objective and scope for this study the development of a simple, sensitive, and cost-effective method for hemolysis detection in the presence of interferents, such as bilirubin, and lipids in sub-microliter volumes of blood plasma (Azhar et al., 2020).

This method might have a particular application for point-of-care diagnostic instruments which use low sample volumes. Not all patients or donors can give the same high quantities of blood, generally, for performing laboratory analysis where aliquoting the sample tubes is a common practice.

For example, elderly people, newborn babies or patients who suffered massive blood losses, could have only micro-quantities of blood taken for its analysis. Compared to a healthy adult, these situations are rather special but it is important to acknowledge this aspect.

In the point-of-care, capillary blood which is extracted from a fingertip, for example, with a quick sting, has a particular importance, since there is no need for specialized medical staff (e.g., nurse, or assistant) to drawn blood from a vein (which in itself poses a higher risk of blood hemolysis). However, the risk of blood hemolysis is not mitigated fully, not even in the case of capillary blood collection.

Taking blood from a capillary vessel is less painful for the patient, it is quicker, and it has a lower cost especially being beneficial for home users (the user does it himself or herself) or

in poorly equipped medical facilities. Comparisons between venous and capillary blood and their analysis variations or applications depending on the case are still researched by the research community. In this study, the authors wanted to use as less blood as possible, especially trying to address the situations where certain patients have a special characteristic (e.g. newborn babies). For a baby, it is enough just a low volume of blood (in some test cases, just a drop). That is why, the authors believe that the method they proposed is significant and relevant in the current IVD and point-of-care market.

The test samples used in the experiment were prepared as a mix of hemoglobin, bilirubin and lipids of different concentrations.

The detection sensitivity was of more than 90% accuracy and approximatively 10% coefficient of variation across 27 unknown samples. The spectral interference due to overlapping absorption spectrum of bilirubin and scattering spectrum of lipids was resolved using linear matrix algebra algorithms.

Due to the sub-microliter detection volume and high sensitivity, the authors believe the system has realistic potential to be implemented in point-of-care medical devices that demand relatively low volumes of test sample material.

The team of authors have established an experimental multispectral optical setup for the detection of hemolysis. The wish was to develop a system which does not require expensive components such as filters, or moving mechanical parts (which can be expensive to replace if they break) or microscopic objectives.

The optical setup had the following main hardware components as shown in the list below.

- the multi-wavelength light source
- a sample tray
- imaging module
- opto-mechanical components and electronics

The images were captured with a computer while the LEDs were manually and sequentially turned on or off.

The light source mentioned above, was used as a multispectral light source, with emission at multiple visible wavelengths and with relatively low spectral linewidths. The optical components needed in the experiment were simplified due to the small area on which the LED sources were mounted. The specific central wavelengths were provided by the red, green, and blue LEDs. In order to get a stronger absorption peak of hemoglobin, a 4^{th} LED needed to be installed (violet). Then a spectrometer was used to capture the spectrum of all the four LEDs. A beam combiner was used to combine the light from the LED panels and a diffuser was used to homogenize the beam intensity spatially and to remove the influence of the small spatial separation of the RGB LEDs. Additional lens were used to direct the beam through the sample plane into the imaging module. This is another example of research where the RGB color model is playing an important role.

The sample tray consisted of two standard microscope glass slides, with two parafilm spacers sandwiched on either side.

The authors stated that when the sample is loaded on the glass slide, the capillary flow might be affected by various elements as shown in the list below.

- Impurities which might be collected
- Non-uniform surface properties
- Air bubbles
- Partially filled sample in the microfluidic chamber

In order to mitigate these risks, the researchers performed a deep cleaning of the glass slides. If the issues would still persists, the samples were then discarded.

The detector sensor used was a monochrome 8-bit camera. It is surprising that a monochrome camera was used, but that was for a good reason. Monochrome cameras provide higher contrast than color cameras due to the absence of Bayer filter (a color filter array for arranging RGB colors on a square grid).

The higher contrast translates to a higher dynamic range of the reported object. Therefore, the output captured images by the sensor detector are in grayscale.

In terms of risk mitigations using software, the authors thought about excluding the air bubbles which might potentially “survive” by using image processing techniques which are capable of identifying and excluding such air bubbles by analyzing sharp gradients in the specific field of view.

The opto-mechanics and electronics used in the setup are shown in the list below.

- A diffuser, to homogenize the light coming from the various LEDs
- A beam combiner which was used to combine light from the ultraviolet LED with the RGB light
- Achromatic lenses
- Optical rails and rail carriers
- Filter holder
- Basic cage components
- Resolution target
- A rotary switch to change the LED wavelength manually
- Resistors

The test samples were prepared to be able to test the functionality and verify the optical setup. Blood plasma which was separated from the whole blood was used as a base solution in which predefined concentrations of hemoglobin, bilirubin and lipids were added. The plasma base was prepared out of blood which was collected from healthy donors.

In order to obtain a clear plasma fraction on top of the sample preparation tube, the tube was centrifuged, and the separating gel (from the sample tube) which creates two layers would keep the resulting cellular sedimentation at the bottom of the tube. Then a pipette was used to carefully pipette out the plasma fraction without destroying the separating gel layer. The plasma was then stored in a cooled unit for future use. It is important that prior to begin any testing on the plasma, to bring it back to the room temperature (not to be used in cold state).

In general, clear plasma which was obtained through other methods (e.g. sedimentation), or anticoagulant coated blood collection tubes which are available on the market, will yield equivalent results compared to the plasma fraction prepared above, according to the authors.

The group of researchers also paid a high importance to the general laboratory safety processes by using protective equipment and sterilization while manipulating the samples or syringes.

The hemoglobin preparation performed by the authors entailed sonication of the sample tubes using a probe sonicator to agitate the particles in the sample tube. The hemolysate which was obtained after the rupture of the red blood cells, was then centrifuged in order to remove any cell residue, and then a dilution was performed with the blood plasma to obtain the different concentrations of hemolysate solution for the study, according to dosage rules which were predefined.

The bilirubin preparation was performed in an obscure environment or with minimal light exposure, due to the sensitiveness of bilirubin to light. The researchers prepared several solutions of bilirubin, too.

The lipid preparation consisted of preparation of the intralipid solution, which is a solution widely accepted to simulate lipids. This solution was then diluted in filtered plasma to obtain the required concentrations.

All the prepared samples were then diluted in saline solution and measurements were performed using a spectrophotometer. The samples which remained undiluted were used to calibrate the optical setup of the experiment.

The calibration data set was prepared by adding the prepared hemoglobin, bilirubin and lipid preparations to the blood plasma. The blood plasma, with no additions was considered as the hemolysis free sample. In case of any interference in the filtered or non-filtered plasma, or if any anticoagulant residue is mixed in the blood plasma, this should be constant across all samples and can be treated as hemolysis free samples.

The range of sample concentrations used in the calibration data set for characterization of the optical set up was broken down per test samples defined in the list below.

- Calibration data points for hemoglobin
- Calibration data points for bilirubin
- Calibration data points for lipids

To be able to measure and collect information during the calibration process, the authors used a spectrophotometer. This measurement process had the objective to verify the sample preparation methods of the samples. Once the objective was successfully met, the samples prepared were then used to calibrate the experimental set up for optical density against concentration for each analyte. The calibration process was constantly performed with various sets of test samples containing different hemoglobin, bilirubin and lipid concentrations. These samples were considered calibration or training samples.

In a similar way, samples were then prepared with the three analytes (hemoglobin, bilirubin and lipid) in different concentration combinations. These samples were used to compare and verify the linear matrix algorithm for interference determination and optical setup.

Each sample was photographed under the illumination from the LEDs. The optical density values were measured and then plotted to the corresponding concentrations.

The algorithm for interference determination developed by the authors calculates the optical density using a mathematical expression.

The principal reference instrument in the study was a spectrophotometer as chosen by the authors. The optical setup was constantly compared with the reference instrument.

While comparing the optical setup measurements of hemoglobin and bilirubin with the measurements of the spectrophotometer for the same analytes, the authors concluded that the measurements in both systems show a close correlation in terms of sample concentrations.

While performing the same comparison of measurements on lipids, a deviation for the sample concentration was identified between the two instruments. The researchers believed

that this was due to scattering property of the anisotropic lipid molecules (they change their properties), and they are subjected to a wide range of physical processes where moving particles or radiation of some form (e.g. light), is forced to deviate from a straight trajectory by localized non-uniformities (including particles and radiation) in the solution through which they circulate.

The samples which were used while performing the calibration of the optical setup, were chosen to be able to represent the clinically relevant range values.

The verification of the experimental setup was performed after successful calibration. In order to change the LED illumination, the rotary switch was used with a manual operation. The images or photographs of the samples were captured using a commercially available camera and its proprietary software application.

The optical densities of the images were then processed and calculated using the algorithm for interference determination. The concentration of each analyte was determined based on the algorithm calculations. The concentrations of hemoglobin and bilirubin, on average, were deemed to be conform with the expected values. The concentrations of lipids registered deviations due to scattering. But this did not pose a problem, since the study was focused primarily on hemolysis detection, according to the authors.

The solution proposed by the authors is designed to detect hemolysis in small volumes of blood in test samples (low or sub-microliter volume). The experiment shows and opens up opportunities in point-of-care where there is potential to implement testing of hemolysis in low volumes of blood (taken from the finger, for example).

A mathematical algorithm was used to process the images taken in gray scale at different wavelengths. The experimental setup was tested using reference or testing samples previously prepared according to predefined concentrations or specifications. The researchers avoided to integrate complex and costly optical filters or any other hardware parts in the optical setup in order to keep the overall cost of the solution as low as possible.

The group of authors reported a hemolysis detection with an accuracy level of more than 90%. The proposed solution has strong potential to be integrated in medical devices that are

used in point-of-care facilities, especially for use cases where low volumes of sample material is extracted from patients.

2.2.5. Plasma automatic hemolysis identification on aligned dual-lighting images of cultured blood agar plates

In recent years, the resolution of the industrial cameras used in medical devices increased exponentially. Similarly to the cameras of the smartphones, in fact. This offers the opportunity for performing advanced image analysis on images captured in medical setups, including analysis of cultures present on medical plates.

The authors of this research, propose a solution to detect hemolysis on blood agar plates. If hemolysis is detected, this could be very important and helpful in assessing the critically levels of existing pathogens and signaling the presence of specific bacteria (Savardi, Ferrari, & Signoroni, 2018). The authors rely on analysis of images under certain illumination scenarios. The same plate is photographed twice (using top-light and back-light) and then the obtained images are jointly aligned with high accuracy so that the data becomes spatially available for measurement and analysis. Segments of the images with bacterial presence are further processed by the designed system and then the hemolysis results are displayed to the user.

The accuracy of the method proposed is approximatively of 88.3% and it was measured using different clinical scenarios. The authors believe the system is robust enough, providing good results even in particularly hard conditions such as low contrasting or changes in the lighting.

Bacteria culturing on agar plates is a very common practice in modern diagnosis. This is used in detection of infections which is particularly important due to the increasing criticality of the infections in the last decades and the alarmingly bacteria’s resistance to antibiotics. Discovering the type of infection and prescribing the most efficient and suitable treatment is essential for patient care.

Bacteria culturing is providing valuable information about which types of pathogens are present in the sample and their virulence levels. In this research, the authors focused on

detection of hemolysis which is associated with bacteria colonies cultured on blood agar plates (Savardi et al., 2018).

Until recently, the traditional method used by microbiologists or skilled medical staff to detect hemolysis, upon sample preparation (which was manually executed), was the visual inspection of the plate with the culture which is back-lit. This method is considered biased and not reliable. The hemolysis produced by bacteria needs time to form, so it is very important that this is recognized as early as possible, in order to speed up the diagnosis process. There is a need for an automated solution to perform the analysis as fast as possible and with an acceptable error rate.

In modern laboratories today, computer vision techniques and sophisticated Laboratory Information Systems are used to perform these tasks. The amount of processing power and digital material of the samples is incredibly high. This is one of the reasons the authors have chosen a fully automated system to be used in their experiment, which is commercially available from the Copan company.

The system takes care of the incubation and imaging of the agar plates. The work in the digital bacteriology is made easier for the user by the automated tasks which are performed by the system. Before and during the incubation, the computerized vision system acquires images that will be available in digital high-quality format to the laboratory technician to perform the reading phase. The user is interfacing with the system through a web-based GUI which can be used to read and manage the plates through simple commands. This is particularly helpful for the safety of the medical staff in the laboratory since there is no need to move and pick up the samples manually from the incubator. The reduction of the hands-on time on the re-work steps is enabled by the robotic plate management system, smart incubators and state-of-the-art image acquisition technology. Since this is a commercial system, protected by patents, the source code of its software is not publicly available and therefore cannot be analyzed further.

A blood agar plate goes through several incubation times, and the system is able to capture images at different points in time. Also, different lighting conditions are able to be performed by the system (front-light or back-light). The high-quality digital image which is

produced, appears very natural to the user which makes the reading easier. In order to detect hemolysis as early as possible, high spatial resolution images are used.

The group of researchers have designed a keypoint based solution to achieve the robust, fast and accurate alignment of images acquired under widely different lighting conditions and displaying critical aspects.

Based on this alignment the researchers developed a joint dual-image classification of bacterial colony segments for the detection and classification of even the most subtle effects produced by blood hemolysis. The results which were obtained were fully satisfactory in different clinical scenarios.

The bacteria culture is photographed several times, under various lighting conditions. The information which is critical to detect the effects of blood hemolysis is scattered across these images. To be able to organize and centralize the information into one place, the images are aligned in a spatial mode. Typically, the images which are back-light provide the information about the hemolysis effects. The authors use machine learning algorithms to detect and classify the hemolysis type and they are confident that their proposed solution is backed up by results which demonstrate that it is feasible, reliable and has a high performance rate.

To be able to fully test their solution, the authors made sure to have a satisfactory representation of hemolytic and non-hemolytic bacteria colonies. It is important to avoid as much as possible errors in reading the hemolytic ones. The database of tested plates was amounting to 235 plates, produced based on sheep blood.

The images were taken by the laboratory automation system, in a linear approach with two illumination modes for each plate: top-lit and back-lit (with the specification that the image was captured from the top).

Upon digitization of the plates, the authors extracted the hemolysis types. A GUI was built to facilitate and help with the task of labeling in the laboratory. Also the GUI was equipped with verification and validation mechanism to help avoid or reduce errors. Where applicable, uncertain cases were discarded. The authors selected segments which contained

only one type of hemolysis and categorized them accordingly (based on hemolysis type, for example).

During the experiments, the authors were confronted with a recurring problem. The alignment of images was not accurate enough for the requirements of the experiment. This was due partially to trepidations or vibrations from the conveyor belt which was transporting the plates. Also, the movement of the plate during the scanning intervals was not to perfection, altering to a small degree the position of the plate.

To solve the alignment problem the researchers considered information-theoretical techniques but this proved to be too slow and not precise enough. Then, they have considered the feature-based method, where the image alignment is performed by identifying distinctive keypoints in images. Keypoint detection and description method creates the possibility of detecting common feature points which are then used to identify the changes in the substance, making the estimation of the image alignment transformation reliable enough.

For example, two images of the same blood agar plate are considered to be aligned. They have different illuminations (top-lit and back-lit). A minimum number of regions of interest is selected for the keypoint extraction, matching and filtering. At the end of the process, the authors obtain 30 keypoint pairs which can be further processed to achieve the most accurate estimate of the correct transformation.

In order to perform feature extraction and description, the authors considered and evaluated techniques which were readily available. They have carefully analyzed their performance and their features by performing quick tests or prototyping to check their feasibility to meet their experiment requirements.

The authors concluded that they needed a feature extraction and a description technique which would allow the analysis of a differential multiscale representation of the image by performing targeted or localized search of the regions of interest (blob detection) from the aligned image and real-valued (image gradient based) methods which are known to find good matching inside bacterial colonies (Savardi et al., 2018).

The two methods, selected by the authors based on their performance for their particular experiment are listed below.

1. The scale-invariant feature(s) transform that represents a feature detection algorithm in computer vision to detect and describe local features in images
2. The KAZE feature(s) which have been developed by detecting and describing image features in a nonlinear scale space through the application of nonlinear diffusion filters. KAZE is a Japanese word that means “wind”.

These two techniques are then alternatively used in the experiment to determine the keypoints of the images. Then the matching system would test all the possible scenarios and identify the unambiguous matchings.

The matches that were selected, were then used to estimate a mapping function that was applied to the measured image and comparing it to the reference image. One of the findings that the authors observed was the processing time of each plate. Despite using a laboratory automation system, the processing time may grow into a critical factor. Especially if, in a real-life scenario, the laboratory would have to process a three or four digit number of samples over a time period of 24 hours. The researchers thought about solutions on how the processing time could be reduced. One of the key areas of the transformation process of the images was the colony segmentation mask. Its components could be considered regions of interest to be able to localize the keypoint extraction. So, instead of processing the whole image, the authors started to extract features from a smaller and randomly selected number of regions across the colonies.

During experiments, the hemolysis measurements should not be affected by the background color of the agar. Similarly to sample tubes, the agar plates may come from different manufacturing companies, with different material properties. In order to prevent this issue, the authors normalized the values in relation with the background mean and variance values of the reference images of the agar plates.

Moreover, in some cases, the agar plate has some inscriptions on its bottom, which are stamped there by the manufacturing company. This is similar to a label or manufacturer

watermark which is applied on a sample test tube. These inscriptions, with their shadow(s) may affect the readings (bacteria growing in that specific area, for example) and subsequently the very interpretation of the measured hemolysis levels.

The authors identified this issue and the countermeasure that they applied was to simply exclude these regions with inscriptions from the detection and interpretation process, especially for the back-lit images where these inscriptions and their shadow(s) would interfere most.

In general, inscriptions on materials or recipients are imposing a risk when it comes to computer vision techniques applied in sample testing.

The team of authors have used Python programming language or optimized existing or customized C++ libraries to develop the fine image alignment software, which is responsible for the end-to-end alignment process.

The images of the plate, which are top-lit and back-lit, and segmentation mask serve as an input for the alignment software. The software then returns the aligned version of the image. Also, the software has an integrated debug functionality to be able to detect if any issue occurred while aligning process was running.

Then, the feature extraction and dimensionality reduction is performed for the classifier. A script is responsible to load the dataset and metadata, to be able to train the classifier (by using machine learning techniques). Then the software returns the hemolysis type and level of the measured plate images.

The reference images of the plates were prepared by a specialist in the laboratory, carefully aligned in a manual mode. During automatic measurements, the authors observed that despite the proposed co-registration method’s robustness, approximatively 50% of the errors were due to residual misalignments. In particular, for smaller colonies, this influenced the final result, yielding an incorrect hemolysis classification.

As a summary, this research had the focus to automate hemolysis analysis by observing the interaction between growing pathogens and blood agar. Illumination of plates is considered

in the solution proposed, and acknowledged as an important factor which influences the image analysis while detecting and classifying hemolysis levels.

The authors believe, that the proposed system brings accurate results which are highly satisfactory considering the speed of processing which is in line with the automated laboratory system workflow.

In future research works, the group of scientists envisages to investigate the root cause and origin of errors observed during the experiment and to develop specific solutions to mitigate them.

The proposed technical solution for hemolysis detection and classification on blood agar plates is something novel, especially in association with analysis of images acquired by a performant laboratory automated system.

The main achievements of this research were the robust dual-light image alignment and the hemolysis classification modules which treat the difference in lighting with good results in terms of time processing performance and reliability.

Each colony segment was classified with the corresponding hemolysis level with a precision of 88%. In terms of errors, the root causes investigated so far were especially related to alignment of images. Also, the inscriptions of the agar plates might interfere in the image analysis.

The promising results obtained during the study, and the proof that usage of the segment-based information of hemolysis to suggest hemolytic plates open up new future opportunities in image analysis for early detection of hemolysis in the microbiology field.

2.2.6. Disposable hemolysis detector

The patented disposable hemolysis detector[2] has the purpose to detect hemolysis from a sample of a patient's blood. The method is described by the inventors as a sealed chamber with a fixed volume in which the sample of blood is inserted.

[2] Consulted online (last checked on 11/09/2020): https://patents.google.com/patent/WO1996023223A1/en

This chamber has a predefined internal air pressure. A specific volume of fluid that includes the sample of blood is received into the sealed chamber. As a result, the internal pressure of the sealed chamber raises to an increased level of internal air pressure.

This causes blood plasma section of the blood sample in the chamber to permeate a membrane disk that covers (partially) the bottom of the chamber or a side of the chamber. A test volume of the plasma portion of the sample is received by a hemolysis detection method (e.g. indicator paper disk) after the test volume of the plasma portion has permeated the membrane, and a hemolysis condition is detected in accordance with a *hue* (one of the main properties of a color, also known as color appearance parameters) associated with the test volume received into the hemolysis detection means.

According to its inventors (Kyu H. Lee and John A. Taylor), the proposed solution is mainly destined to be used in non-laboratory environments. The invention could be used by doctors in point-of-care facilities for example, to be able to detect hemolysis.

The objective of the invention is to propose a system for separating a blood sample into its plasma and cellular elements which is cost-effective, and which does not require external instruments or mechanisms for controlling the velocity and flow of blood at a membrane surface.

A determination of whether the constituent of interest is present in the whole blood sample is then made by observing the hue of the indicator paper.

The patented hemolysis detector invention is composed of the following elements, as shown in the list below.

1. A sealable chamber having a sample injection port for receiving a sample of blood into the sealed chamber. The sealed chamber looks like a syringe, a cylinder and is built from a clear rigid plastic (e.g., acrylic, polyvinyl chloride, polycarbonate or polysulfone) and it has a fixed internal volume. The chamber is mounted on a chamber base built from the same material as the chamber.
2. The sample injection port is built out of latex rubber or other elastic material (to prevent introduction or exiting of air in and from the chamber).

3. The chamber base on which the sealable chamber is mounted; the chamber base is also built from the same material as the sealable chamber.
4. A microporous membrane disk is provided and it is placed at the bottom end of sealable chamber. It is only permeable to the plasma portion of a whole blood sample and non-permeable to the cellular portion of the sample.
5. A channeling structure is positioned immediately below the microporous membrane disk. It has the purpose to collect the plasma portion of the whole blood sample as the plasma portion permeates through the membrane disk and then channels it to a clear capillary tube. The hemolysis detector is preferably assembled by positioning the channeling means within the chamber base, and then ultrasonically welding sealable chamber to chamber base with the membrane disk located in between. Ultrasonic welding represents an industrial process whereby high-frequency ultrasonic acoustic vibrations are locally applied to work pieces being held together under pressure to create a solid state weld. It is commonly used for plastics and metals, and especially for joining dissimilar materials.

The process steps performed to detect hemolysis as explained by the inventors are summarized in the steps below.

1. Injection of a wetting solution such as saline into the sealable chamber with the help of a syringe that has been inserted through the sample injection port
2. The hemolysis detector is inverted causing the wetting solution to wet the membrane disk positioned at the bottom of sealable chamber. This wetting step causes the membrane disk (which was previously dry and therefore permeable to air) to become impervious to air, therefore transforming the sealable chamber into a sealed chamber having a fixed volume of air inside.
3. A sample of a patient's whole blood is injected into the sealed chamber through the sample injection port. Since the sample injection port is made out of latex or rubber, no air escapes are possible from the sealed chamber during injection of the whole blood sample into the chamber.

Since fluid is added to the sealed chamber but no air is allowed to escape from the chamber, the internal pressure in the sealable chamber increases as a result of the injection of the whole blood sample into the sealed chamber during this step. The inventors issued an equation, which is used to determine the change in pressure that results from the injection of a whole blood sample into the sealed chamber. The result is obtained by solving the below equation for the quantity P_2.

$$p. * v. = P_2 * V_2 \ (1)$$

Where the equation terms are the listed below.

- p. = the pressure of the air in the sealed chamber prior to the injection of the whole blood sample into the chamber (this will typically be the ambient air pressure)
- v. = the volume of air in the sealed chamber prior to the injection of the whole blood sample into the sealed chamber
- P_2 = the air pressure within the sealed chamber after the whole blood sample has been injected into the chamber
- V_2 = v. – (minus) the volume of the whole blood sample injected into the sealed chamber during step 3 as mentioned above

The membrane disk is itself used as a mean for transforming the sealable chamber into a sealed airtight chamber because the membrane disk (which originally was in a dry state and pervious to air) becomes impervious to air when it is wetted.

4. Hemolysis detector component is shaken to mix the whole blood sample injected into the sealed chamber during step 3 with any wetting solution remaining in the sealed chamber from step 1.
5. Next, the hemolysis detector is inverted, and the plasma portion of the whole blood sample previously injected into the chamber permeates through the membrane disk. The increased internal pressure generated by the injection of the whole blood sample into the sealed chamber in step 3 functions during step 5 with a force to push

the plasma portion of the whole blood sample through the membrane disk. This force is not too high in order to not cause damage to the blood sample. As the plasma portion of the whole blood sample permeates the membrane disk, it is then collected by the channeling element and then channeled into a clear capillary tube.

6. The hemolysis detector is inverted again and the hue of the plasma in the capillary tube is observed either with the naked eye (visual inspection) or with a column magnifier or lens. If the hue of the plasma is amber, this indicates that the whole blood sample was normal. Alternatively, if the hue of the plasma is pink or has a reddish tone, this indicates that hemolysis has occurred. In this specific variation of the invention, the hemolysis detection is performed by analyzing the clear capillary tube and by observing the hue of plasma in the tube. Basically this is performed by visual inspection or with the help of special lenses. Visual inspection is considered to be a biased method, subjective and prone to human interpretation errors. Traditionally, the visual inspection is performed on a normal sample tube with regular dimensions. In this specific case, the inventors propose a clear capillary tube, to be able to improve the visual inspection result (taking advantage of the small diameter of the capillary tube which is about 1.5mm). It is true that a clear capillary tube provides better "visibility" in order to determine if the sample is hemolyzed of not, compared to a typical test tube. Perhaps analyzing capillary tubes by visual inspection will produce better results if compared with visual inspection of bigger sample tubes; but, visual inspection of capillary tubes, remains, however, biased and prone to human errors (depending strongly on how the human eye reacts to the hue of the plasma in capillary tube).

The inventors propose a series of solutions variations on the same theme. For example, in other variations, the hemolysis detector could be tipped sideways and agitated in order to cause the wetting solution injected to wet the membrane disk. In another variation, they propose to shake the hemolysis detector to mix the whole blood sample injected into the sealed chamber with any remaining wetting solution which may have been left in the sealed chamber. In other variations of the invention, the inventors propose as hemolysis detection

means, an indicator paper, such as guaiac paper, which may be used to detect hemolysis from the plasma after it has permeated through the membrane disk.

Since the variations of the solutions proposed have many commonalities, in this review, only the differentiations are highlighted, for comparison purposes.

Other variations of the sample material input methods are listed below.

- One or more drops of a whole blood sample are placed within the perimeter of the first side of the membrane disk. If the membrane disk is formed of a hydrophobic or slightly hydrophilic paper, it should be pre-wetted with a wetting solution such as an aqueous solution of 5-20% isopropyl alcohol or ethyl alcohol prior to dropping the whole blood sample onto the membrane disk. Other organic solvents can also be used as a wetting agents, as long as these solvents do not leave a residue in the membrane disk that could interfere with the blood test. The purpose of this pre-wetting step is to hydrophilize the membrane disk. The pre-wetting step can be accomplished by simply dropping one or more drops of the wetting solution onto the membrane disk. After one or more drops of a whole blood sample are placed within the perimeter on the first side of the membrane disk, the plasma portion of the whole blood sample will wet the membrane disk as well as the indicator paper disk lying beneath the membrane disk. If the wetting of the indicator paper disk propagates beyond the perimeter, a drop of developer solution is applied to the wetted portion of the indicator paper disk lying outside of the perimeter.

In order to detect hemolysis, in the final step, the inventors propose other solution besides the observation of the clear capillary tube as summarized below.

- Using an indicator paper disk or strip. This is typically porous paper impregnated with guaiac resin. Such paper displays a blue color in the presence of hemoglobin when treated with a hydrogen peroxide solution. This solution is better than visual inspection but it relies on external chemical agents which increase the cost of the solution.

In some variations, specific skills are required from the user who performs the hemolysis analysis as summarized below.

- Skills required related to working with microporous membranes, including hydrophobic membranes that require a pre-wetting step
- Skills required related to membranes which may have other shapes than the disk shape and could also be used instead of the membrane disk
- Skills required related to indicator paper which may have other shapes than the disk shape and could also be used instead of the disk
- Skills required related to an optical sensor system (formed of a light transmitting source and a light sensor) that may alternatively be used to analyze the plasma from the sample tube in order to determine whether hemolysis has occurred or not

The proposed innovation brings a new perspective on using the air pressure to separate blood from the blood plasma and employs visual inspection techniques or indicator paper techniques to detect hemolysis.

However, it is observed that besides the subjectivity of the visual inspection, in some variations of the proposed invention, specific operator skills are necessary to handle the membranes, the indicator papers or the laboratory optical sensor system.

The inventors highlighted that their invention could be further developed to be able to work out other variations or solutions while guarding the original and essential principles of the invention.

2.2.7. Arrangement for detection of hemolysis

The following invention, which is named as arrangement for detection of hemolysis[3] by its inventor (Mathias Karlsson), consists of a device for visual detection of hemolysis in a whole blood sample from a pierceable container, a surface arranged to engage with the stoppered container, at least one visible detection compartment and a transfer passage (e.g.

[3] Consulted online (last checked on 11/09/2020): https://patents.google.com/patent/WO2013085462A1/en

needle) with a separation filter which is connected to the visible detection compartment. The separation filter has typically a porous structure which provides a capillary action for efficient plasma separation.

A stoppered container or tube represents an airtight container made out of glass or plastic. At one end, the stoppered container is equipped with a rubber surface or a material which provides the capability to be pierced (e.g. with a needle) but without any air leakages.

The detection compartment and separation device are arranged within a housing (a cylinder), while the transfer passage is linked with the help of a needle from the stoppered container to the housing and positioned adjacent to the separation filter.

The plasma is then extracted from the stoppered container through the transfer passage into the detection compartment. Before the plasma reaches the detection compartment, it goes through a separation device for separating plasma from blood cells.

The inventor stated that the invention could be used in a treatment room, without the need of performing tests in a traditional laboratory. The detection of the hemolysis is meant to happen in the immediate vicinity and sequence of the collection of blood sample in approximatively 30 seconds to one minute's time.

The quantity of sample material required by the proposed solution is small.

This invention has similarities with the disposable hemolysis detector invention. For example, the detection is performed by visually inspecting the *hue* of the serum or plasma (from a pink to a red gradient scale).

The visual inspection of the serum or plasma is performed by observing the hue of the plasma portion which has been absorbed by the visible detection filter inside the detection compartment. The visual inspection might be biased and subjected to human interpretation errors.

The inventor has highlighted a series of advantages of the proposed innovation summarized in the list below.

- There is the possibility of attaching the device to the blood draw equipment and observe the hemolysis level before continuing the draw of blood. The idea is to prevent removing the equipment to collect blood from the arm of the patient and a new blood sample could be extracted without the need for another puncture in case the first sample shows hemolysis.
- The risk of bubble appearance is significantly reduced by using the filter (e.g. separation filter and detection filter) which is made out of porous material in the detection compartment
- The detection filter has usually a white color which helps in the visual examination of hemolysis; to even further help in detection of plasma hue, this detection filter could be of a light blue color in order to intensify the color differences
- The risk of blood clogging is eliminated by having a larger cross sectional filter area compared to the cross sectional area of the transfer passage
- The inventor highlights the possibility of having chemical reactions in the detection compartment in order to increase the confidence level of the hemolysis detection. Although this is an advantage and could possibly be used, it is yet another possibility which privileges the chemical analysis, which is a traditional method, demanding time and more cost (e.g. the price of chemical compounds, reagents or filters impregnated with these respective chemical substances could be high).

The solution proposed is relying on visual inspection of the hue of the serum or blood plasma after the red blood cells are filtered or separated. As this method is error prone, subjective and unreliable, the invention proposed is not an ideal standalone solution. Perhaps combined with chemical analysis, or other hemolysis detection methods the solution could be feasible to be integrated and used in the point-of-care, especially while the phlebotomy process is also performed (drawing blood from a patient).

2.2.8. Blood hemolysis analyzer

The inventor (Michael Tarasev) proposes a blood hemolysis analyzer[4] which is able to determine the concentration of hemoglobin derivatives in the blood sample. This is accomplished through absorption of electromagnetic radiation by cellular hemoglobin (within the red blood cells) and cell-free hemoglobin at two wavelengths (or more).

Most hemoglobin derivatives from blood are present in the red blood cells. Compared to them, the blood plasma might contain a lower concentration of hemoglobin derivatives. This distribution has an impact on the increase or decrease of the light absorption by the two mediums. The hemoglobin of the red blood cells absorbs less light than the cell-free hemoglobin present in blood plasma.

The proposed system exploits the differences in magnitude of the absorption spectrums of cellular hemoglobin derivatives and cell-free hemoglobin derivatives to quantify low concentrations of the latter compared to the much larger amounts of hemoglobin in the red blood cells.

The proposed system consists of the following elements, as summarized in the list below.

- A light source (including its power source) which emits light in the ultraviolet and visible ranges of the electromagnetic spectrum
- A focusing mirror which partially surrounds the above light source and focuses the light so that an incident beam of light is directed to the collimating optics system
- The optics system which has the role to direct the rays of light so that the rays are parallel to each other, and are directed towards the filters
- The filters which have the main purpose to block the light in specific spectral regions
- Other materials: beam splitter(s), mirrors, diverse slits for exit and entry. These components direct the light beam into two separate, parallel paths as it passes into the sample block.

[4] Consulted online (last checked on 11/09/2020): https://patents.google.com/patent/US7790464B2/en

- The sample block that includes one or more quartz cuvettes or blood transfusion tubes
- A bracket that holds the cuvettes or transfusion tubes securely in the path of the light beam
- The detection block includes entry and exit slits coupled with mirrors, and together focus the light beam on the turning mirror which directs the light beam to a dispersion element (e.g. defraction grating) that disperses light of different wavelengths over a defined area
- The detectors generate a signal in response to the received light and transmit the signal further to the signal processing block
- The signal processing block includes signal amplifiers, and appropriate logic circuits for signal correction to produce and record signal. Multi-wavelength analysis algorithms are used to process signal amplitudes. In other variations of the invention, the inventor proposes a dedicated programmed processor or an external computer using specific software programs to analyze the signals.

Spectrophotometric methods of hemolysis detection rely on the characteristic of hemoglobin to absorb electromagnetic radiation in the visible and near ultraviolet portions of the spectrum. This property of hemoglobin enables it to be detectable and quantified.

The inventor had the idea of creating a system which would have different applications to cater to other important aspects of the blood as the listed below.

- Blood transfusions. While the blood is stored in cold units, the membrane of the red blood cells loses some of its elasticity characteristic. The inventor thought about the idea to detect the membrane's elasticity state before the actual blood transfusion, in order to validate the state of the blood, which represents a benefit in the context of patient safety.
- Separation of blood plasma. The inventor considered to be useful to have a system that measures hemolysis level without requiring separation of red blood cells from

the plasma and other cellular components as a pre-requisite. Moreover, this would be help avoiding any alternative chemical or enzymatic analysis or reactions.

In terms on how the measurements are executed, the process steps are summarized below, as described by the inventor for measuring the concentration of cell-free hemoglobin derivatives in a biological sample that contains red blood cells.

- The light source (e.g. a deuterium arc lamp) emits light which passes through the blood sample. The sample block allows the light to pass through to be able to reach the blood sample.
- Some light is absorbed by the cell-free hemoglobin derivatives. The rest of the light is absorbed by the hemoglobin derivatives from the red blood cells.
- The absorption detector(s), that have the responsibility to detect the light absorption by the blood sample, generate a signal in response to the received light and transmit the signal further to the signal processing block
- The processing block is performing comparisons and calculations between the changes of light absorption at two wavelengths (or more). In this way, the concentration of cell-free hemoglobin derivatives is calculated.

In a similar manner, in another variation of the invention, the inventor proposes the measurement of concentration of cell-free hemoglobin in a biological sample including red blood cells. The light is passed through the blood sample. The light absorption by the blood sample is detected. The absorption spectrum of the blood sample at two wavelengths (or more) is then compared to obtain the cell-free hemoglobin derivatives in the blood sample.

In order to measure the fragility level of the membrane of the red blood cells in a blood sample, the proposed invention uses a light source as in the previous use cases. Then a device is configured to apply a predefined stress to the membranes of the red blood cells as the sample is transported through the device. The sample block is configured to allow light with a specific wavelength range to pass through the blood sample. The cell-free hemoglobin derivatives will absorb some of the light while the rest of the light will be absorbed by the hemoglobin derivatives which are present inside the red blood cells. The

measurement of the concentration of the cell-free hemoglobin derivatives and the level of fragility of the red blood cells membranes is facilitated, as in the previous use cases, by the absorption detectors and the signal component. The fragility of the red blood cell's membranes is determined based on the comparison between the absorption spectrum of cell-free hemoglobin derivatives in the blood sample to a flattened absorption spectrum of the hemoglobin derivatives contained within the red blood cells.

As an application for this invention, the inventor proposes a situation wherein a patient's blood is diverted to a flow cell for analysis, while undergoing a procedure requiring external manipulation of the blood.

For this experiment, the blood sample is placed in a quartz cuvette or a flow through sample cell and positioned in the light beam.

As the light beam passes through the sample, a portion of the electromagnetic energy of the light beam is absorbed by the hemoglobin derivatives in the blood sample. The light beam is then directed to an array of detectors that detect electromagnetic energy in the specific wavelength range.

In another variation of the invention, the light beam is split by a beam splitter, and the light beam is passed through two cells, one of which (sample cell), contains the sample to be analyzed, and the other (reference cell) contains water, or any other appropriate substance that does not absorb light in the same specific wavelength range.

In another variation of the invention, the reference cell is used to correct for light scattering and/or the flattened absorbance due to hemoglobin derivatives contained in the red blood cells. In this case, the reference cell would contain unstressed red blood cells and the sample cell would contain stressed red blood cells.

In another variation of the invention, the reference cell would contain a plasma free sample, in which the volume of plasma has been replaced by an optically clear solution that preserves the red blood cells intact. The detector(s) compare the portion of the light beam that passed through the sample with the portion that passed through the reference material, and detects the reduced electromagnetic energy in the specific range that was absorbed by

the sample. The above comparison is made by the detector(s) at two or more wavelengths in the specific portion of the spectrum.

In another variation of the invention, the difference in absorption is determined at the wavelength at which the peak absorbance occurs, at one wavelength that is longer than the wavelength of peak absorption, and at one wavelength that is shorter than the wavelength of peak absorption. By comparing the change in absorption at these three points, the steepness of the absorption curve could be assessed. A steep absorption curve is a characteristic of cell-free hemoglobin derivatives, while a broad, flattened curve with no single absorbance maximum, is a characteristic of hemoglobin contained within red blood cells. The concentration of the cell-free hemoglobin derivatives could, thus, be determined.

In another variation of the invention, the concentrations of the hemoglobin derivatives are calculated using multi-wavelength analysis and an absorbance formula.

In a following step, the stressed red blood cells are placed in the path of the light beam, and the amount of absorption in the specific region of the electromagnetic spectrum is determined. Finally, the absorption spectrum of the cell-free hemoglobin derivatives released from the red blood cells is compared to the flattened spectrum of the hemoglobin derivatives in the intact red blood cells.

In another variation of the invention, the spectrum obtained from the sample before the stressor is applied is compared to the spectrum of the stressed sample, and the increase in the amount of cell-free hemoglobin derivatives is determined.

The invention relies on the spectrophotometric method which is one of the most reliable methods used in hemolysis detection. Due to the patented aspect of the invention and limitations in the information publicly available, the review was kept to a general level without assessing the details of the invention (e.g., software or algorithms).

2.2.9. Hemolysis sensor

A group of researchers from the University of South Florida has developed an optical spectroscopy probe[5] that is capable of detecting hemolysis levels in whole human blood samples.

The research idea started with the objective of developing a hemolysis detection solution which would not require plasma separation but be reliable enough, yielding correct results and, thus, reducing the processing time of a sample.

With this in mind, the researchers embarked on the development of a novel optical spectroscopy probe with a distal tip and a microfluidic filtering chamber that allows influx of fluids only (no particles will go through).

The proposed device is developed with a broader perspective in mind, that is, to analyze liquid samples for specific purposes or applications. Hence, the device is capable to analyze blood samples in order to detect hemolysis, but without blood plasma separation.

The researchers stated that the device is able to detect a specific concentration of a drug in a liquid sample. No reagents are necessary for this purpose, thus reducing costs.

The proposed solution is returning quantitative results in a short time which could be useful in a point-of-care setting.

To demonstrate the feasibility of the proposed solution, the researchers posted a graph which shows the cobalamin concentration in a sample, proving that the presented concept is able to detect an analyte or a constituent of interest (in this case, cobalamin) in a sample.

At the time of writing this review, this research and solution was in progress of getting patented. Therefore, the software code or materials used in the concept could not be analyzed in-depth as they were not publicly available.

[5] Consulted online (last checked on 11/09/2020): https://www.research.usf.edu/dpl/content/data/PDF/12A091.pdf

2.3 Research on mobiles devices in hemolysis detection and related fields

2.3.1. Intelligent NFC potassium measurement strip with hemolysis check in capillary blood

Another relevant example of research which aims to render hemolysis detection as early and as mobile as possible at the PoC is the intelligent NFC potassium measurement strip with hemolysis check in capillary blood developed by a group of researchers from Austria. The background and motivation of their research is to ultimately help patients through their innovation.

130 million people in Western Europe and the USA suffer from chronic cardiac degeneration or kidney insufficiency (Kollegger et al., 2018). The medical treatment requires a routine medical examination accompanied by laboratory blood analysis. This is cost-intensive for the healthcare system and also time consuming for the patients.

The authors presented an economical, wireless sensor node solution in terms of an intelligent near field communication (NFC) potassium measurement strip usable in a home environment. The strip comprises of two different electrochemical sensors for the acquisition of two parameters in capillary blood and an autonomous sense and identification grain for the electrical analysis, communication, and data transfer. The latter is equipped with an integrated amperometric and potentiostatic functionality, radio frequency identification interface for high frequency and has been designed using a standard process. It facilitates the implementation of two different measurement techniques, that are necessary for the accurate determination of the potassium concentration in capillary blood, whereby the occurrence of hemolysis can be detected. The advantage of the strip is that it could be integrated at large scale and is exclusively powered by the NFC reader device (Kollegger et al., 2018).

The strip prototype which has the dimensions of 74 mm × 15 mm is basically a thin polyethylene terephthalate foil sheet which serves as a carrier for the sensor technology. The analog circuitry including the integrated antenna is fabricated on a flexible PCB foil, which is glued to the carrier. The chip has been wire-bonded to the flexible PCB and an

additional epoxy glob top serves as a protector for the fragile bond wires. Additionally a ground plane is positioned around the sensitive data acquisition area to reduce outside interference. The antenna coil is implemented with six windings and is tuned to the resonant frequency using a resonant capacitor. The analogue circuitry is connected to the hemolysis sensor (Kollegger et al., 2018). The amperometric sensor interface can be used for carrying out the voltammetry analytical method, which is the representation of the current as a function of the applied potential.

The test sequence for the usage of the intelligent strip is straightforward and easy to manipulate. The aim of the researchers was to obtain a quantitative determination of potassium in human blood by screening capillary blood. Initially, the patient pricks a finger using a lancing device to obtain a capillary blood sample. The first blood drop must not be used since it may contain skin particles or cells and could potentially influence the result. While taking the blood drop, a smooth finger massage from back to tip which should be performed with minimum pressure.

Further, its placement separated from the sensors makes the manipulation easier for the patient or healthcare professional, because the NFC reader device can be placed directly on the antenna without coming into contact with the blood sample.

The blood sample is applied to the plasma separation membrane. This filter captures the cellular components of the blood (red cells, white cells, and platelets) without lysis, while the plasma flows to the membrane. Subsequently, the plasma sample is drawn via a micro-channel to the potassium and hemolysis sensor for consecutive analysis. Finally, the measurement process is performed and the results are displayed on an Android smartphone.

To measure hemolysis level, a defined hemoglobin test solution of 500 mg/l was used to demonstrate the behavior of the hemolysis sensor and the amperometric sensor interface. Initially, the strip is powered, thus triggering the sensor chemistry so it can react in the background. After 30 seconds the configurable voltage is turned off because the sensor needs some equilibration time; 30 seconds later the voltage is switched on again and after a sensor settling time of about 30 seconds a constant current can be observed. The threshold

current for the hemolysis detection must be set using empiric measurements in the course of a sensor calibration process.

Undoubtedly, the intelligent, disposable blood analysis strip is a brilliant idea which was implemented by the authors. They presented a single-chip solution merged with a dual purpose electrochemical sensor for a blood potassium and hemolysis determination for use in a domestic or PoC environment. The device is suitable for mass production, because of the simple setup and minimal number of components. Patient risks from potassium lapses can be reduced significantly by means of the simple and effective regular potassium and hemolysis levels check that can be provided by this means. Once again the mobile phone could be used as a near-field communication (NFC) reader. The measurement itself is triggered by the respective NFC device (mobile phone) and an Android application. The potentiostatic and amperometric sensors are evaluated separately for each potassium acquisition. The result of the potassium measurement is only displayed, however, when the hemolysis check is passed.

The disadvantage of this proposed solution is the fact that the mobile phone and its app needs to be working in combination with the intelligent strip and not standalone, which might increase the overall cost of the solution due to the additional manufacturing cost of the strip. However, one of the advantages, is the fact that a small amount of capillary blood is extracted from the patient.

2.3.2. Hemolysis detector

Besides groups of researchers who are affiliated typically to public or private universities, research centers or institutions, or companies from the private business sector seek also to seize the opportunity and thrust themselves, supported by their internal researchers, into innovation efforts to create solutions or products for early hemolysis detection.

A relevant example is the solution proposed by the Ultimaker company, from The Netherlands.

The objective of their project was to develop a method for detecting high levels of free hemoglobin in blood samples in a hand-held testing device that is relatively cheap and easy

to use. The client for this project was Instrumentation Laboratories of Bedford, MA (which is an integral part of the Werfen company since 1991).

Ultimaker is proposing a hemolysis detector[6] device which consists of a blood plasma separator and an optical detector. The plasma separator uses a microfluidic system to isolate plasma from red blood cells. Free hemoglobin remains in the plasma, permitting efficient detection using a photo resistor and LED to determine the concentration of free hemoglobin.

An option for optical analysis for a mobile phone is also provided, where a phone analysis unit and instructions for the ImageJ analysis software are included (the camera of the phone photographs the sample and the image is loaded onto the ImageJ desktop software for further image processing).

From the information available in the user manual, it was deducted that the ImageJ software application that was installed on a desktop computer was making use of image processing algorithms which probably analyze the colors from the bitmap area of interest.

Since the project was developed for a private business sector client, active in the hemostasis diagnostics, the source code of the ImageJ software, was, understandably, not made accessible. Therefore an in-depth analysis of the source code was, unfortunately, not possible.

The disadvantage of this approach is the fact that many actors are involved in the proposed solution (e.g., computer, phone, desktop software, phone filter attachment) which could lead to potential communication errors or malfunctions. Also, the filter attachment will have to be designed and manufactured individually for any relevant mobile phone form factor used in the point-of-care facilities. However, it is highly laudable that companies from the private business sector are investing their considerable research funds into innovation projects, such as hemolysis detectors.

[6] Consulted online (last checked on 01/01/2020):
https://ultimaker.com/download/67409/BloodHemolysis (outdated due to website re-design)
New link ("hemolysis detector" section) consulted online (last checked on 11/23/2020):
https://ultimaker.com/learn/3d-printing-with-umaine-bioengineering-students

2.3.3. Spectral analysis methods based on background subtraction and curvature calculation used in the detection or quantification of hemolysis and icterus in blood-derived clinical samples

The research project developed by Huynh et al. (2017) proposes the detection of hemolysis at the collection site, without a spectrophotometer, by photographing the plasma fractions of centrifuged collection devices using a simple point-and-shoot camera (or a cell phone camera) with the light path that includes a hybrid filter, which is composed of a narrow-band pass region and a wide-band pass region (e.g. 5-nm and 65-nm wide filters for hemolysis measurements).

The authors aimed to find new methods to detect and quantify hemolysis and icterus that may cause assay biases. These methods need to determine each of these interferents in the presence of various other interferents. They also need to have less stringent requirements in the development and implementation compared to those of conventional analyzers which currently must be satisfied (Huynh et al., 2017).

The authors developed two spectral analysis methods that obtain absorption signals of interest by background subtraction or by calculating the spectral curvatures near the peaks of interest. They have optimized and tested the performance of these methods using a plasma sample set with permutations of the levels of hemolysis, icterus, and lipemia (using 510 samples in total).

The processed signals correlated well with concentrations of hemoglobin and bilirubin, indicators of hemolysis and icterus, respectively. Through iterations of randomly splitting the samples for calibration and testing, the two new methods performed as well as those used on conventional analyzers.

The research team demonstrated that the two methods can reduce the application requirements or pre-requisites as summarized below.

1) Prior knowledge of the absorption spectra of individual interferents;
2) Calibration over a wide concentration range for each interferent;

3) The need for full-range spectrophotometers spanning most of the ultraviolet or visible spectrum.

A hardware setup to detect and quantify hemolysis or icterus with a camera and two optical filters was also proposed.

This work indicates that new methods of spectral analysis can reduce practical constraints in the development of interference screening systems. These methods could also benefit other assays that rely on reading spectral signals.

The two methods described herein have three major practical advantages versus interference correction methods that are based on absorbance values at multiple wavelengths across the ultraviolet or visible range, such as those used for hemolysis and icterus detection on many commercial analyzers.

Firstly, the methods described herein are less susceptible to the presence of unknown interferents. The choice of wavelengths in the traditional methods depends on the absorption wavelengths of known interferents, while both curvature calculation and background subtraction are mostly agnostic of the interferents and only depend on the absorption of the substance of interest. Even though the optimized hemolysis and icterus signals slightly deviated from the peaks (415 nm for hemolysis), the signals at the peaks would still provide good performance, with values distinguishing the two lowest interference levels (of hemolysis or icterus).

Secondly, the traditional methods require calibration using samples with wide ranges of interference levels, while the methods using background subtracted signals or curvatures do not require it. The research team performed an example of an analysis to demonstrate this notion.

Traditional methods gave large biases and poor correlations, while those using background subtracted signals and curvatures gave good results. As expected, the maximum level of interference used for calibration had to be increased for the performance of traditional methods to improve. In contrast, the methods using background subtracted signals or curvatures performed very well, even when a maximum level is used for calibration. The

independence of the methods involving background subtracted signals and curvatures allows the calibration to be done even with samples of limited interference levels (e.g., those naturally collected instead of those made via a comprehensive procedure like the samples used in the research work presented by the authors).

The new methods, as described by the authors in their paper, are based on background subtraction and curvature calculation, and provide the ability to quantify and detect hemolysis and icterus with several practical advantages as shown in the list below.

1) Better robustness in terms of eliminating signals from unwanted substances, some of which may not be known beforehand
2) Smaller sets of samples used for calibration with fewer levels of interference
3) Simpler instruments (spectrophotometers with smaller detectors or short wavelength ranges or cameras equipped with pairs of filters)

These new methods do not have advantages over traditional methods with respect to the number of discrete wavelengths required. A camera-based implementation would require further hardware engineering, and the implementation of these new methods, in general, may involve other methods of performing background subtraction (e.g. those with other blurring methods) or curvature calculation (e.g. those using methods other than circle fitting). Such implementation could benefit cases of sample collection in settings with limited resources. For example, in remote sites, where samples are collected and sent to centralized laboratories, the ability to detect hemolysis interference at the point of blood collection would allow for immediate re-drawing of blood. Furthermore, if the hardware is adapted to work with small volume samples (e.g. those collected from finger), it would be possible to integrate the methods described herein with point-of-care diagnostic instruments and contribute to the effort of bringing diagnostics to developing countries or other under-served settings. Overall, these new data analysis methods can enable new practical possibilities in the development of interference screening methods.

The authors indicated the possibility of integrating the method described in their research with a point-of-care diagnostic instrument (Huynh et al., 2017) that might increase the

footprint of the solution. On top of that, the centrifugation time is added to the overall TAT, which will reflect as an increase.

2.3.4. A mobile-phone approach to hemolysis detection

The group of researchers developed a mobile-based platform which can quickly measure the level of hemolysis by analyzing the color of the blood plasma (Archibong, Konnaiyan, Kaplan, & Pyayt, 2017).

In their tests, the scientists privileged small volumes of blood, collected in clear capillary tubes or microtubes. The solution proposed consists of several custom hardware and software elements, summarized in the list below.

- A smartphone equipped with a camera and a LED flashlight;
- A custom developed software app installed on it which will perform the image processing;
- A 3D printed sample holder which is custom-manufactured based on the smartphone specifications (the holder has a ring slot and a lid and is attached to the back of the smartphone). The purpose of the lid is to cover the compartment where the sample tube is placed in order to keep the lighting conditions at a certain constant level. The ring slot has the task to hold the capillary tube or microtube with the blood in front of the camera. The sample holder will have different dimensions based on the dimensions of the smartphones on the market (for each smartphone model a specific sample holder must be built). The authors stated that the sample holder cost would be significantly low.

The method to separate red blood cells from the blood plasma is sedimentation (due to gravitation, the RBCs fall at the bottom of the tube). The blood plasma will then remain in the upper part of the sample tube.

With the help of the smartphone, the tube is then photographed and the image is analyzed based on the color components of the blood plasma. The smartphone software app converts

the color values to a concentration of free hemoglobin and it yields the hemolysis results on the screen of the smartphone.

It is important to mention that the authors have performed pre-calibrations in order to determine the calibration curve and its reference values. The proposed solution provides the hemolysis level measurements in approximatively 10 minutes, and the total cost includes the cost of the sample holder and smartphone. The software app is not estimated in terms of cost.

While photographing the sample tube, the app collects 12 images and identifies the blood plasma portion of each image. Then the 10x10 pixels region of interest (ROI) is defined for further analysis. The RGB color model values are extracted for each pixel and then converted to CIE L*a*b* values. The authors concluded that this color space, also written and known as CIELAB or CIELab color space is ideal to characterize samples that contain hemoglobin and bilirubin (Archibong et al., 2017). While researching on color spaces in image analysis methods for the present thesis and solution, this particular research work proved very useful in providing some insights into this proposed color space. The idea of using CIELAB color space seems promising and results would seem to have a satisfactory success rate.

Further on their research, the authors calculated, based on the CIE L*a*b* values, a color index that is compared to the reference values determined previously a through calibration process which took into account the clinical or widely accepted relevant values found in specialized medical literature. The algorithm of the app converts the color index to a concentration of free hemoglobin and displays the hemolysis level on the smartphone screen.

During the experiment, the authors ѕolutions prepared based on purchased plasma and hemoglobin with various concentrations of hemoglobin. The calibration curve determined for the experiment (which is incorporated into the image processing software), was used to relate these color indices of these solutions to known or reference values of plasma hemoglobin concentrations.

The research results are definitively promising based on the accuracy of the measurements. One disadvantage that stands out is the fact that the holder attachment needs to be individually designed and 3D-printed for each mobile phone form factor. Given the plethora of market smartphones form factors that are currently used in laboratories or point-of-care wards and the rapid pace at which the manufacturers release new smartphones, this solution may be time consuming and difficult to manage in terms of infrastructure. Moreover, the solution was developed only to support capillary tubes and microtubes, which represent a very small fraction of the sample tube traffic in a typical laboratory or point-of-care. Nevertheless, this research remains yet another important milestone in the hemolysis detection field, which is performed using mobile devices.

2.3.5. Sickle cell detection using a smartphone

The authors of this research work, believed that systematic testing procedures in parts of the world with poor medical capabilities, supported by simple, fast and accurate testing platforms would need to be developed or enhanced in order to be able to diagnose the sickle cell disease (Knowlton et al., 2015). Sickle cell results in an abnormality in the oxygen-carrying protein hemoglobin found in RBCs and, if left undiagnosed, can cause life threatening “silent” strokes and lifelong damage (Knowlton et al., 2015).

Sickle cell disease is a group of blood disorders typically inherited from a patient’s parents, therefore it is hereditary. The most common type is known as sickle cell anemia.

The authors propose a testing platform that is supposed to use just a small volume of blood sample. The idea of the research is based on the higher density of sickle red blood cells under deoxygenated conditions.

The materials used by the researchers in their study are summarized in the list below.

- A lightweight and compact 3D-printed attachment which is installed on a smartphone. It includes a LED to propagate light to the blood sample, an optical lens to be able to magnify the image and two permanent magnets for magnetic levitation of red blood cells.

- A smartphone
- A paramagnetic medium with sodium metabisulfite
- A micro-capillary tube which is inserted between the two permanent magnets

While executing the experiments, the researchers compared the levitation patterns of sickle cells versus red blood cells which levitate in the magnetic field.

In a point-of-care facility, the traditional techniques to detect sickle cell disease are summarized below.

- Usage of solubility tests method
- Non-electrolyte hemolysis method
- Filter paper method

A more modern method is considered to be the density-based diagnostic testing that provides a higher sensitivity to different types of sickle cell disease. This method could employ the alternative of the separation of blood cells (by centrifugation of blood samples) or magnetic levitation alternative.

The magnetic levitation method is used in general to detect the density differences in cells. This is possible due to two characteristics of red blood cells, that is that they experience temporary or permanent changes in their magnetic and density signatures.

By using a magnetic field, the authors were able to differentiate between the high density RBCs and the low density RBCs. By using this technique, the authors avoid using centrifugation of samples and their experiment require low volumes of blood. Also, there is no need to observe the sickle shape using a microscope because this is performed by the imaging processing that is performed on the smartphone at a lower image resolution.

The magnetic levitation platform is a 3D-printed tool and in combination with the smartphone, they are responsible for the process steps as listed below.

- Restrict the red blood cells in the micro-capillary tube by using magnetic levitation

- Photograph the red blood cells which are levitating the magnetic field (with the smartphone camera and an optical lens)
- Perform automatic analysis of the red blood cells distribution using the Android app installed on the smartphone

The components of the smartphone attachment (or 3D-printed holder) are summarized in the list below.

- An aspheric lens to magnify the image which will be captured by the camera of the smartphone, 3D-printed lens frame, diffuser and LED illumination that are used to enhance imaging
- A battery and on/off switch to power the LED
- Two permanent magnets with the same poles facing each other

The sample preparation was performed via pipetting in a laboratory setup. The levitation of the RBCs requires a 10 minutes period of time.

The images are captured using the smartphone camera. Then they are processed by the Android app running on the smartphone. The image pixels are scanned in the x-direction and two arrays are saved as show in the list below.

- The average of the pixel intensities
- The gradients of pixel intensity

Based on the above arrays, the app recognizes the RBC confinement and evaluates a Gaussian fit to the pixel intensities in that region of interest. The image analysis performs firstly an average of the pixel intensities in the x-direction and creates an array of x-axis averages indexed by the y-axis location. The next step performed by the app algorithm is the measurement of the change in pixel intensity from pixel to pixel in the x-direction and it creates an array of x-axis gradient data, also indexed by the y-axis location. The region of micro object confinement is then identified as the peak in both the density and the gradient arrays. A Gaussian distribution fit is then calculated for the pixel intensity array and

displayed in the user interface. The mean and four times the standard deviation of the Gaussian distribution (in terms of y-axis pixels) is then calculated and reported to the user.

According to its creators, the proposed smartphone based magnetic levitation platform, combined with the smartphone app, may be valuable for early screening of sickle cell disease with potential to be extended to other blood diseases.

The proposed solution comprising of a smartphone attachment which is composed of a 3D-printed holder, optical components (aspheric lens and holder), LED illumination, and two permanent magnets with the same poles facing each other and the Android app that was custom-developed to perform the image analysis is in many ways similar to other proposed solutions which use a 3D-printed holder attached to a smartphone. It is definitively encouraging to notice similar phone-based detection approaches in other fields related to blood analysis (e.g. sickle cell detection). However the smartphone (with its camera and flashlight capabilities) is not the unique and central piece of hardware used in the solution. It needed to be enhanced with other components to be able to perform the measurements with a satisfactory degree of accuracy. All the additional components either need to be molded and manufactured according to the smartphone specifications or they need to be purchased off the shelf, thus increasing the cost of the solution. Where the smartphone excels, and it is definitively a strong point which is exploited in many works, is the camera capabilities, image processing power, and excellent hardware for running user-friendly software apps for image analysis.

2.4 Research on use of color spaces to determine blood hemolysis levels

2.4.1. Use of the color spaces in determining the level of hemolysis in blood under storage

In this full conference paper text, the authors describe the development and experiment of an optical non-invasive method to determine the free hemoglobin level by measuring the absorption of transmitted monochromatic lights by using color spaces such as CIE XYZ or CIELAB (Can, Ülgen, & Akın, 2015).

The specimens used in the experiment are transfusion blood bags which are stored in cold facilities. During processing and storage, the red blood cells (RBCs) inside the blood in the transfusion blood bags may be affected in their internal chemical and structural composition producing the so-called blood lesions. Hemolysis is one of these lesions and considered as a quality criteria for transfusion blood products, therefore, for patient safety, blood bags need to be hemolysis free and in good condition.

The authors state very strongly, in at least two instances, that the visual assessment of blood hemolysis of the transfusion blood bags is inaccurate and represents a subjective method; in return, by using a set of tristimulus values or color coordinates in a color space to measure the level of hemolysis is a more accurate method (Can et al., 2015). During the experiment, the researchers recorded the measured values of several colorimetric parameters, such as tristimulus X from the CIE XYZ color space, CIE a* which is one of the three coordinates of CIELAB color space that represents the position or coordinate of the color between red and green (a*, where negative values indicate green and positive values indicate red) or the CIELAB *chroma* which represents the colorfulness of an area judged as a proportion of the brightness of a similarly illuminated area that appears white or highly transmitting as defined by the Commission Internationale de l'Éclairage, in French, or, in English, the International Commission on Illumination (in short, CIE). Often times, chroma is associated or confounded with the *saturation* which represents the colorfulness of an area judged in proportion to its brightness as defined by CIE. The *colorfulness* is the attribute of a visual perception according to which the perceived color of an area appears to be more or less chromatic as defined, also, by the CIE.

At the end of the experiment the authors concluded that the most accurate method to measure the color of a sample is by specifying the visual stimulus reflected from the sample, in a three dimensional color system or color space instead of comparing with colored charts (Can et al., 2015).

The objective of this research was to measure the hemolysis level with a non-invasive color measurement method (using color spaces) and the authors concluded, based on their

experiment results, that this is possible, safe and could be applied in the future to improve the quality of blood transfusions.

Using color spaces while trying to determine with precision the color of an object or substance, especially when luminance factor is considered, is a widely accepted and validated method due to its precision and uniqueness (the color coordinates or tristimulus values and luminance are present in three dimensional systems and yield with high accuracy their precise values). Therefore, it is a tested and acknowledged method, accepted by the scientific research community.

This is one of the strong arguments as to why, in this present thesis, the method of measuring color coordinates from color spaces was chosen.

2.4.2. Point- of- care testing of plasma free hemoglobin and hematocrit for mechanical circulatory support

In this research work, the authors developed a simple and rapid point-of-care device with the objective of measuring the plasma free hemoglobin (PFHb) and hematocrit (Hct), based on colorimetry (Shin, Lee, Shin, Cho, & Kim, 2021), which is the science and technology used to quantify and describe physically the human color perception (similar to spectrophotometry).

The point-of-care device is composed of several elements as summarized in the list below.

- A camera module (to take images)
- A small centrifuge system
- A custom software application (that includes the motor control algorithm for the centrifuge system and the image processing algorithm for measuring the color components of blood from the images taken by the camera)

The image analysis process begins with the segmentation of the region of interest (ROI) in the acquired image after centrifugation of the specimen. The image analysis is then performed simultaneously with the color space analysis of PFHb, Hct, and Hb. The analyzed result is printed on the screen of the device.

The region of interest (ROI) at the initial step of image processing is segmented to cover the entire channel of the cartridge. The authors developed a customized channel cartridge and holder design. The channel cartridge was developed specifically for the system, and was fabricated out of acrylic material using a laser cutting machine. The cartridge consists of three parts, the lower, middle, and upper part, and all parts have the same thickness of 2 mm. The holes on both sides of the blood chamber are used to fix the cartridge at a given position in the holder. The hole in the upper part is the blood chamber, and after the blood is dropped into the chamber, it is covered with a black cap to prevent the blood from flowing outside during the centrifugation process.

The color of the cap serves as a marker for the chroma difference (ΔC) when analyzing images. The formal definition of chroma is based on the idea that when a chromatic light-reflecting object is increasingly strongly illuminated, the colorfulness of its appearance increases, but the brightness of a similarly illuminated white object increases proportionately, so its intrinsic strength of color or chroma can be defined as the colorfulness judged relative to this brightness.

The image processing algorithm developed by the researchers performs a set of defined procedural steps as summarized in the list below.

1. For each pixel, red, green, and blue (RGB) values are extracted
2. The channel is divided into the region of red blood cells (RBCs) and plasma based on the specific threshold of red color
3. In order to measure the PFHb level, a second ROI with the size of 20×100 pixels is segmented from the previously distinguished plasma region. RGB values are then extracted from it, and then converted to CIELAB values.
4. The color components are represented by L*, a*, b*, where, L* is the brightness, a* is the degree of red and green, and b* is the degree of yellow and blue. CIELAB values were extracted from the region of black marker and the plasma in the second ROI.

5. The chroma difference (ΔC) was calculated using the a* and b* values of the second ROI, and then converted to the PFHb levels by the predefined calibration curve where, am and bm are the CIELAB components of the region of black markers, and, as and bs are the CIELAB components of the region of the plasma in the second ROI
6. To exclude the change of the color components caused by the brightness, the difference between the average brightness value (L) of the black marker obtained from the calibration images and the brightness value of the black marker obtained from each sample image was added as an offset value to the chroma difference (ΔC)

During experiment results analysis, the authors identified which are the next steps in their future research work, and they are summarized in the list below.

1. In their study, the validation was performed on a limited range of plasma free hemoglobin, hematocrit and hemoglobin levels
2. To further refine their proposed solution, the researchers envisage a possible plan to employ both colorimetric and spectrophotometry in order to raise the accuracy of the measurement resolution and possibly to measure more hematological analytes (the substance of interest from the blood which would need to be measured)
3. The authors also plan to develop a network system using mobile software that would enable measured results and records to be checked or retrieved, even when users are in special equipped laboratories or medical wards.

This promising research shows that using color spaces (e.g. CIELAB) and performing image analysis by starting with extraction of RGB values for each pixel from the image or ROI is a solid and verified scientific approach.

Additionally, the authors identified a method to exclude the change in color caused by the lightness or brightness parameter. When performing image analysis the surrounding light in which the photographed sample is located influences the colors of the subject, therefore it is important to take this parameter in consideration while executing experiments.

Considering the fact that the authors plan to develop a network system using mobile software, paves a good and promising path towards the rationale of employing mobile devices in hemolysis detection for better connectivity. One of the arguments as to why a mobile smartphone has been chosen as the hardware device in this present thesis, is the fact that modern mobile technology, not only provides exceptional cameras to capture images in detail and with high resolution for more precise image analysis, but it also provides many advantages when it comes to be connected in a network or ecosystem, where mobility and connectivity are essential while working with patient analysis results and the time aspect is a critical factor.

2.4.3. Methods for hemolysis interference study in laboratory medicine – a critical review

In this review, the author aims to provide insights into the methods available for studying hemolysis interference in clinical laboratories (Marques-Garcia, 2020). One of the methods described in the review, is the spectrophotometric method used to study hemolysis interference.

Hemolysis may occur in vivo and in vitro. In vivo hemolysis is a result of a number of circumstances and diseases (inherited from parents or acquired hemolytic anemias), whereas in vitro is triggered by improper or wrongful procedures during sample blood collection (e.g. phlebotomy procedures).

Traditionally, both types of hemolysis, in vitro and in vivo, could be assessed by visual inspection, comparing the serum or plasma with a color scale after centrifuging the blood sample. The author stresses the fact that visual inspection has important disadvantages since it is a time-consuming method, with a high workload for laboratories and it is not very viable (Marques-Garcia, 2020). Visual inspection result is also influenced by both the observer (who is biased) and his or her level of completed professional training, which is hard to standardize in order to avoid differences between laboratories.

The author also emphasizes that the assessment of hemolysis by visual inspection can be hindered by the presence of other interferers such as bilirubin, that influences the color of the sample preventing an accurate reading.

One of the methods used to study the hemolysis interference is the spectrophotometric method. The author brings an important observation related to the wavelength which is used to measure the analytes. The wavelength which is used in measurements needs to avoid overlapping with the hemoglobin absorption spectrum.

Depending on the hemolysis degree, the assays (oxyhemoglobin or deoxyhemoglobin) of the analytes will be prone to interferences. The author states the importance of considering these factors while developing spectrophotometric devices, in order to avoid overestimations or underestimations of measurements.

Besides advocating for additional attention paid to detection of hemoglobin interferences, the author expresses the need to continue making progress in the development of intelligent algorithms, which does not stop at only analyzing the hemolysis level, which can also be capable of analyzing or differentiating the hemolysis type: in vivo from in vitro and vice versa. The author believes, that, by tackling these challenges in future clinical practice, jointly coupled with harmonization or standardization in hemolysis detection, would certainly increase the quality of prevention practices and patient safety.

2.5 Summary

The possibility that mobile devices (in particular smartphones) can support hemolysis detection is a conclusion that stems from previous research. This chapter has highlighted some of the most important research efforts using mobile devices or custom devices to detect hemolysis or similar medical conditions. They play an increasing and important role in the healthcare sector, standalone or in combination with other devices.

In previous research works reviewed in this chapter, the use of color spaces (e.g., CIELAB, CIE XYZ), and color models (e.g. RGB) has been chosen due to several validated reasons and arguments. This was one of the key findings in support of a smartphone-based

approach for hemolysis detection. The summary below presents the main arguments and reflections pointed out in the literature reveals.

1. Color is very important and relevant to human eye. While analyzing hemolytic sample tubes, the color factor is in fact the parameter that is studied (from yellow plasma color and hemolysis free to very red color of the blood plasma when blood hemolysis is present in the patient's sample); the color in general is an ubiquitous attribute when it comes to analyzing objects and recognize information by humans.
2. Color is widely used as principal filter criteria in object recognition and tracking. This is why, traditionally, visual inspection was the departure point (in absence of alternative methods) in hemolysis detection which later proved to be subjective and inaccurate. While performing hemolysis detection without any reactive chemical reagent, compounds, additions or reactions, which is in fact the objective of the present thesis (to provide a non-intrusive method that does not alter the blood or the plasma's chemical composition), the most promising parameter which remains to be analyzed is in fact the color change of the sample tube, more specifically, in the plasma section of the sample tube.
3. The RGB model is undisputedly the most widely used color model in computer graphics today. Many studies, research works and real-life applications use this color model as a base for color filtering, segmentation and analysis in image processing.
4. The international CIE organization is the body which issued the first color spaces and it is a widely known and valued consortium formed by valued scientists, experts and enthusiasts, which has the objective of preparing and publishing standards, reports and other publications concerned with all matters relating to science and technology in the fields of light and lighting embracing fundamental subjects as vision, photometry and colorimetry). It is important to note that since 1999 onwards, also the optical, visual and metrological aspects of the communication, processing and reproduction of images, using all types of analogous and digital imaging devices, storage media and imaging media are also covered by the CIE.

5. In color spaces, the physical aspect of the light is considered. The color space describes with high accuracy how all the colors can be formed on a plane with a given illumination level.
6. The CIE XYZ color space is calculated by using the light wavelength from the physical representation of the color. When judging the relative luminance (brightness) of different colors in well-lit situations, humans tend to perceive light within the green parts of the spectrum as brighter than red or blue light of equal power. This color space considers this fact by setting Y as luminance, Z is quasi-equal to blue, and X is a mix of response curves chosen to be non-negative. Setting Y as luminance has the useful result that for any given Y value, the XZ plane will contain all possible chromacities at that respective luminance.
7. CIELAB is indirectly obtained from the primary CIE XYZ color space. The CIELAB color space is formed by a lightness or brightness (L) component and two color components (a and b). This color model can express a wider color range or gamut than the RGB model, for example.

In conclusion, color spaces seem a viable choice when image color analysis is desired. This thesis focuses on the smartphone as the standalone hardware device, equipped with a modern integrated camera, which acts as a platform for running a custom-built software app which employs image processing and analysis algorithms for analyzing the sample tube image considering the surrounding light conditions and color identification using color spaces as proven methods in image analysis, and quickly rendering an accurate and reliable hemolysis level result on the smartphone’s screen.

CHAPTER 3 – KEY DESIGN CONCEPTS, MATERIALS AND METHODS

3.1 Overview

Having reviewed related research and collected a set of useful results concerning hemolysis detection, it is now important to introduce a set of design concepts that supports the development of a smartphone-based approach to detects hemolysis that is proposed in this thesis.

The working prototype of such approach consists of an Android smartphone (equipped with an integrated camera) and an Android app which is installed on it. In terms of testing material, the population of specimens used in the experiment is comprised of filled and capped sample tubes (artificial or real blood sample tubes). The method used to carry on the evaluation, is processing each sample tube with the help of the prototype app in order to measure the hemolysis result.

In this context, this chapter begins by describing the key concepts related to the design of the prototype app's software functionalities that will be incorporated in the smartphone-based approach suggested in this thesis as well as the color spaces that serve as the basis for the detection functionalities implemented in the prototype app. The prototype app will serve to test these design decisions as well as to evaluate the overall performance of the detection mechanisms suggested. The chapter then moves to the description of the experimental environment that will be setup to perform the experiments in order to evaluate the prototype app.

3.2 Software design

3.2.1. Software architecture diagram

While architecting the prototype app that will detect hemolysis, a few fundamental architectural principles were considered, such as the **separation of concerns** (the code written for activities or fragments contains the logic that handles the UI and the OS interactions) and **driving the UI from a model** (components that are responsible for

handling the data, independent from the View objects and other app components, being beneficial, for example, to not lose the data if the OS destroys the app to free up resources). Due to a high amount of images stored on the memory of the smartphone, the possibility of deleting the test images in case the app is uninstalled was left active while executing the experiment and measurements.

In order to be able to test these principles, a solid architecture was constructed based on model classes with well-defined responsibilities of managing the data, in order to have a prototype app which is testable and consistent.Figure 1 illustrates how the architecture components relate to one another while designing the prototype app.

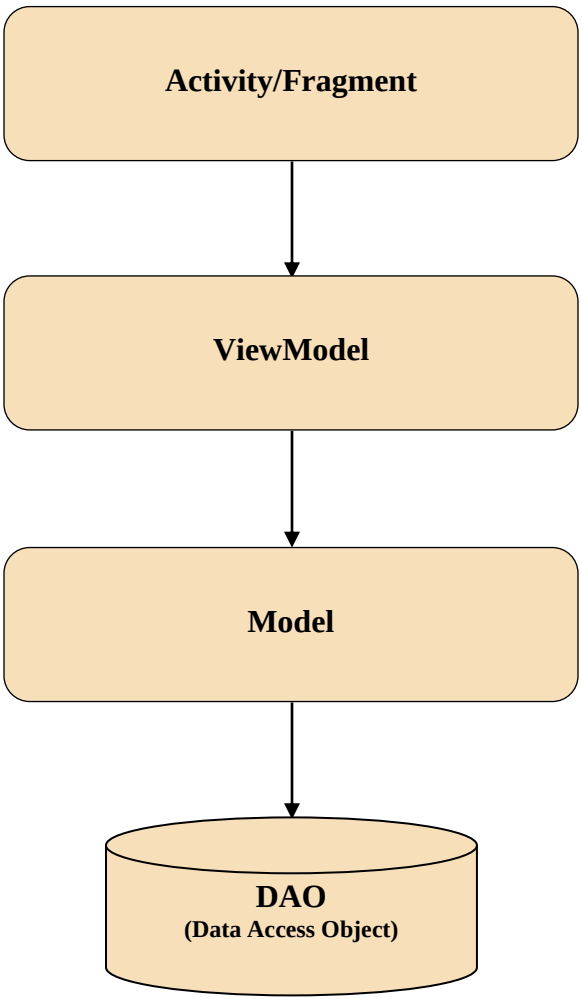

Figure 1. Software architecture diagram

Each component depends only on the component located one level below it. For example, activities and fragments depend only on a view model. The model depends on a persistent data model or data access object (DAO). In the following subchapter, the definition of each architecture component and its specific role played in the overall software architecture will be explained in more detail.

3.2.2. Architecture components – their definition and roles

Activity/Fragment

1. An **activity** is, according to developer.android.com (2020), a single, targeted undertaking or enterprise that the user can perform. This is typically one screen of the Android app's UI (one activity implements one screen in an app as a broadly accurate guide or principle, based on practice rather than theory). For instance, one of an app's activities may implement a *User Settings* screen, while another activity implements a *Select Image* screen. Android activities are commonly considered very similar to windows in a desktop application. An Android app may contain one or more activities, meaning one or more screens.

   Generally, an Android app starts by showing the main activity, which is an activity purposely declared as main, and represents the first screen to appear when the user launches the app (e.g., the Home screen, the Login screen), and from there the app may make it possible to open additional activities (even from other external apps). Most apps contain multiple screens, which means they comprise multiple activities. Each activity can then start another activity in order to perform different actions. For example, the main activity in a "To Do" task list app may provide the screen that shows a listing with all the tasks to be done by a family. From there, the main activity might launch other activities that provide screens for tasks like adding a written note or comment to a family member on a specific task or simply sharing a task by email which then launches the activity of composing the email message with the preferred default external email client app).

Almost all activities interact with the user, fact which is relentlessly federated by the Activity class that takes care of creating a window in which the UI is placed (an activity provides the window in which the app draws its UI). This window typically fills the device screen, but there are also use cases when it may be smaller than the screen and float on top of other windows. While activities are often presented to the user as full screen windows, they can also be used in other ways, such as floating windows (via a specific theme), multi-window mode or embedded into other windows.

Traditionally, in the past, while programming a desktop application, the application was typically launched via a `main()` method, while the Android system initiates code in an Activity instance by invoking specific callback methods that correspond to specific stages of its *activity lifecycle*, for example, `onPause()` method.

Figure 2 illustrates the activity lifecycle and its associated methods.

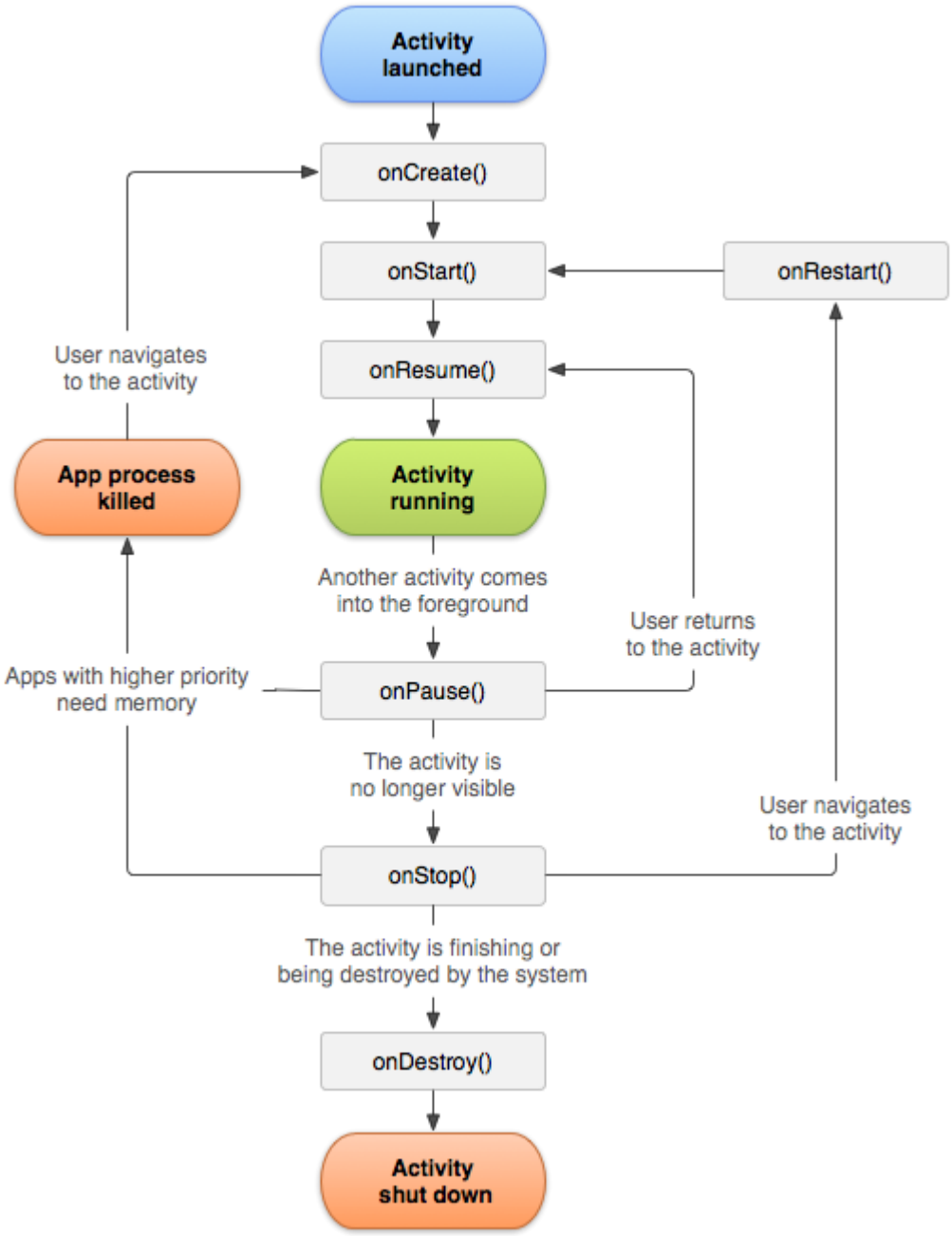

Figure 2. Android activity lifecycle (source: developer.android.com)

Using specific activity lifecycle methods, the Activity class is designed to call only specific methods when necessary. For example, when one app invokes another app, the calling app invokes an activity in the other app, rather than the app as a

monolithic entity which could seriously impact the response time or loading time. In this way, the activity serves as the entry point for an app's interaction with the user.

Although activities work together to form a cohesive user experience (UX) in an app, each activity is generally decoupled from the other activities (there are usually minimal dependencies among the activities in an app). As time passes, an app could gradually get bigger or heavier, with an increased number of activities and increased complexity. In order to keep the app easy to maintain and scalable, it is good practice to loosely bind activities between them and only when, or, if necessary.

2. A **fragment** represents, according to developer.android.com (2020), a reusable class implementing a portion of an activity or a portion of the UI (like a sub-activity that could be reused in other activities). It is basically a combination of two files: a layout file (XML) and a Java class file. Multiple fragments can be combined into a single activity to build a multi-pane UI and reuse a fragment in multiple activities. They are standalone components that can encapsulate views, events, and logic so that it is easier to reuse within activities. Fragments enable device-specific aspects, such as the reuse of shared elements, while also providing support for managing differences in layouts (for example, between smartphones or tablets, in portrait or landscape mode the layout of the same activity might look substantially different).

   A fragment could also be considered a modular section of an activity (in its core, it represents a particular operation or interface that is running within the activity), which has its own lifecycle, receives its own input events, and which could be added or removed while the activity is running. A fragment must always be embedded in an activity (is closely tied to the activity it exists in, and cannot be used apart from it) and the fragment's lifecycle is directly affected by the host activity's lifecycle (it cannot run independently of its associated activity). Despite the fact that a fragment defines its own lifecycle, the latter is dependent on its activity’s lifecycle. If the activity is paused, then all the fragments in it are also paused; if the activity is stopped, then the fragments inside cannot be started; when the activity is destroyed, all fragments will be destroyed as well. However, while an

activity is running (it is in the resumed lifecycle state), each fragment can be manipulated independently, such as adding or removing them. Figure 3 shows the lifecycle of a fragment while its activity is running.

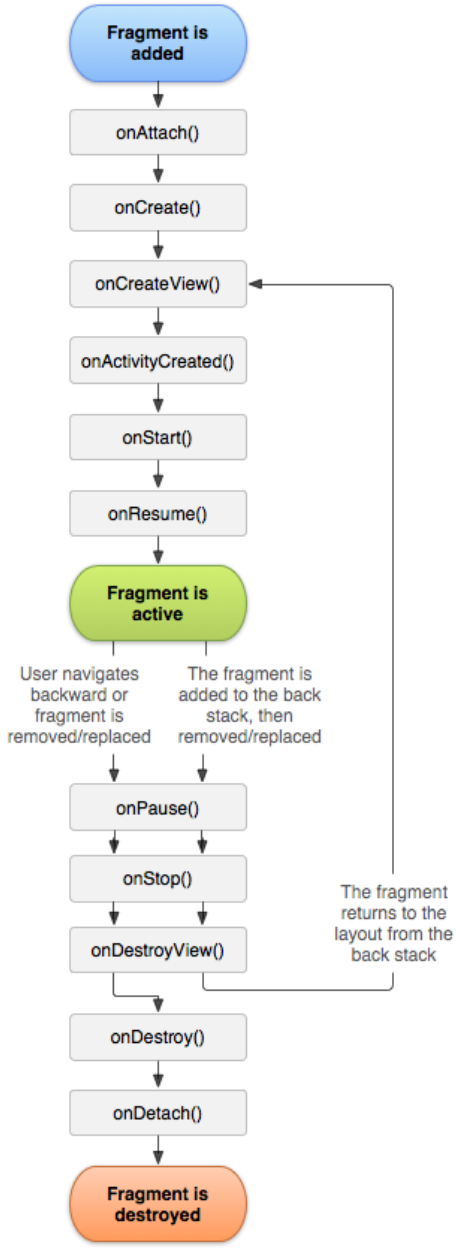

Figure 3. A fragment's lifecycle while its activity is running (source: developer.android.com)

Fragments act as content controllers and contain most views and layouts displaying app content, event logic management, error handling, and retrieval and storage of data from the persistence DAO layer through Model and ViewModel architecture components.

ViewModel

According to developer.android.com (2020), a ViewModel is responsible to acquire and manage the data for the UI components, such as a fragment or an activity, and it contains data-handling business logic to establish communication with the Model. For example, the ViewModel could call other components to load the data. In other words, it delegates these tasks, and it can also forward user requests to modify the data. The ViewModel is not aware of UI components, so it is not affected by configuration changes, such as recreating an activity when rotating the screen of the device (this fact allows data to survive configuration changes).

A ViewModel is always created in association with a defined scope (a fragment or an activity) and will be retained as long as the scope is active. The activity or the fragment should be able to observe changes in the ViewModel. The ViewModel should never access the view hierarchy or hold a reference back to the activity or the fragment. Instead, the ViewModel delegates the data-fetching process to a new architecture component called the Model.

Model

According to developer.android.com (2020), the Model architecture component is responsible for handling business data, the business logic and the business rules for the Android app. Building the app on model classes with the well-defined responsibilities of managing the data, the app becomes more testable and consistent. Model components are independent from the other view objects or app components, so they are unaffected by the app's lifecycle and the associated concerns. The UI of the app should be driven from the model which has a strong persistence characteristic supported in the background by a persistent data model and a reliable data source. Persistence is recommended and ideal for a number of reasons. For example, in case the app is destroyed in order to free up resources, but without the data being lost. Another reason is the fact the model enables the app to

continue to function even when crossing a poor or temporarily unavailable wireless network connection. This would not be possible without the full support of the DAO, the last architecture component described in the next paragraph.

DAO (Data Access Object)

In essence, according to developer.android.com (2020), the Data Access Objects are the main classes where database interactions are defined. They can include a variety of query methods. It is recommended accessing the database using a DAO class instead of query builders or direct queries in order to accomplish a better and modular strategy to access the database.

There are multiple convenience queries that can be represented using a DAO class (such as `insert`, `update`, `delete`). Also, DAO classes or methods are supporting interactions with persistent storage mechanisms such as the smartphone's internal memory (e.g. while saving an image file from the camera). One of the benefits of using the DAO design pattern is that it enables the abstraction and encapsulation of object-oriented principles, and supports simple and rigorous separation of the architecture components, that are expected to evolve regularly and independently from each other over time, but jointly fulfilling the common mission of enabling the finished app to react safely and swiftly to future changes in the persistence mechanism, logic or UI.

3.2.3. Architecture components – inter-relationship

The app's architecture components must establish relationships or interactions and support each other in a cohesive way. The ViewModel holds and prepares all the data for the UI in order to avoid having to put any of it directly into Activities or Fragments (which could make code maintenance a major burden). Instead, the Activity/Fragment connects to the ViewModel, gets all the necessary data from there, and it only has the task of displaying it on the screen and reporting the user interactions back to the ViewModel. The ViewModel then forwards these user interactions to the underlying layers of the app, either to load new data or to make changes to the dataset. So, in a sense, the ViewModel basically works as the "gateway" for the UI controller, which is the Activity or Fragment, to control the rest of

the app. Furthermore, no database operations will be initiated from the Activity directly, so that the Activity itself is not aware of what is going on in the DAO layer. In this way, the Activity/Fragment classes are kept lean and clean, and, as mentioned above, one of the main advantages about these ViewModel classes is that they survive configuration changes. When the screen of the device is rotated or other runtime configuration change is made (such as changing the text size of the device) the Activity on the screen gets destroyed and recreated for the purpose of providing an alternative layout file or other relevant resources. This also means that the state of the activity is lost, together with its member variables, and a completely new one is basically started.

In the past, there have been different ways of retaining and recreating the data, such as saving and restoring variables in a different lifecycle callback methods of the activity. Also, it was necessary to start, stop and perform the cleanup of different asynchronous operations like network calls and the correct lifecycle methods, otherwise bugs, memory leaks and repeated app crashes would constantly occur. All of this made activities very heavy, very fast, because of the existence of these overloaded lifecycle methods. Generally, the whole process of saving and restoring got very complicated very quickly, it was prone to errors, and it was also wasting resources because sometimes, calls, which already have been made, needed to be re-issued. With ViewModel, this problem is elegantly avoided because ViewModels survive configuration changes. And the new activity just receives the same ViewModel instance that still contains all the data.

Finally, the ViewModel connects through the Model to the DAO as the connection point to the database where all the persistent data is safely stored. This architecture design creates a consistent and enjoyable user experience (UX) which is seamless for the user while complexity is gracefully managed and maintained by the developer who benefits from a clearly defined, structured, focused on roles and boundaries, and, more importantly, a decoupled app architecture.

3.2.4. Architecting the image processing module using color spaces

In the initial phase of the design of the image process module, the idea of capturing the sample tube by opening the video intent of the camera was explored. This idea would have eliminated the user task of taking a photo of the sample tube and probably the process could have been faster than the photo-based approach. However, soon, difficulties and questions arose around the image stabilization and color extraction of a moving element (the sample tube). Moreover, the captured image from the video frame needed to be saved to a file for data storage and retrieval which the video preview would not support without additional steps. Therefore, the paradigm between dynamic (video) versus static (captured image) would be inclined in the favor of the latter.

With the design choice regarding the object input method settled, the next step was to figure out how the "HomeDetect" app could capture the image without the need for any special setup such as mounted supports like in other approaches that were reviewed. Furthermore, it was also important to figure out how the app could analyze the captured image using an image processing technique that was specifically designed for this purpose, and, finally, how the results could be displayed on the screen. In the following paragraphs, these challenges and their solutions will be explored.

Image capture is designed to be performed by delegating the action to the camera app by invoking *Intent*. The intent describes what is desired to be done. This process involves three elements: the Intent itself, a call to start the external Activity (this time to the Activity of the camera app), and specific code to handle the image data when focus returns to the activity of the "HemoDetect" app.

In this context, the **Image Processing Module** is then responsible to analyze the captured image. For this purpose, research was performed in order to establish the appropriate color spaces which would yield the extracted color values from the image in order to be able to calculate a color index (CI) based on the measured and referenced values, which will be used in determining the hemolysis level. The approach based on the use of color spaces, provides a helpful tool to check and, if required, improve the reference values. The steps below illustrate the journey that is executed inside the image process module.

The image is captured → RGB values are extracted from the image → these values are then converted into CIELAB values in two sub-steps as listed below.

1. The plan is to convert them into CIE XYZ values as a first sub-step;
2. Then the CIE XYZ values are converted into CIELAB values (which are based on the compressed XYZ color space coordinates – this is precisely the rationale as to why the first sub-step is planned)

→ Finally, the calculation of the color index (CI) based on the measured and reference values.

Color Spaces

The International Commission on Illumination (CIE) is a worldwide non-profit organization created with the purpose to gather and share information related to the science and art of light, color, vision, photobiology, and image technology. The CIE created the first color models based primarily on the physical aspect of the light. Among the most notable color spaces, the CIE XYZ and CIELAB can be enumerated. Both of them describe how all the colors can be formed on a plane with a given illumination level.

The CIE XYZ is calculated by using the light wavelength from the physical representation of the color, while CIELAB is indirectly obtained from the CIE XYZ. CIELAB (or also known as L*a*b* or simply Lab) is one of the most used models from the CIE family. The CIELAB is formed by a lightness component and two chromatic or color components (a and b). According to Chavolla, Zaldivar, Cuevas, & Cisneros (2018) the CIELAB color space can express a wider color range, or gamut, than the RGB model, and is usually used to enhance color images.

While reviewing the literature presented in Chapter 2, it was evident that color spaces are convenient for image acquisition and displaying. In fact, they are efficient in measuring small color differences and they allow for the pixel's chromaticity to be decoupled from the pixel's intensity.

RGB Color Model

The RGB additive color model is most widely used in computer graphics. Many works use this model as a base for color filtering, segmentation, and analysis in image processing. The ease of use and intuitive model for color creation and manipulation makes it an ideal choice for drawing and coloring. Figure 4 depicts the RGB color model, together with the D65 illuminant (white point).

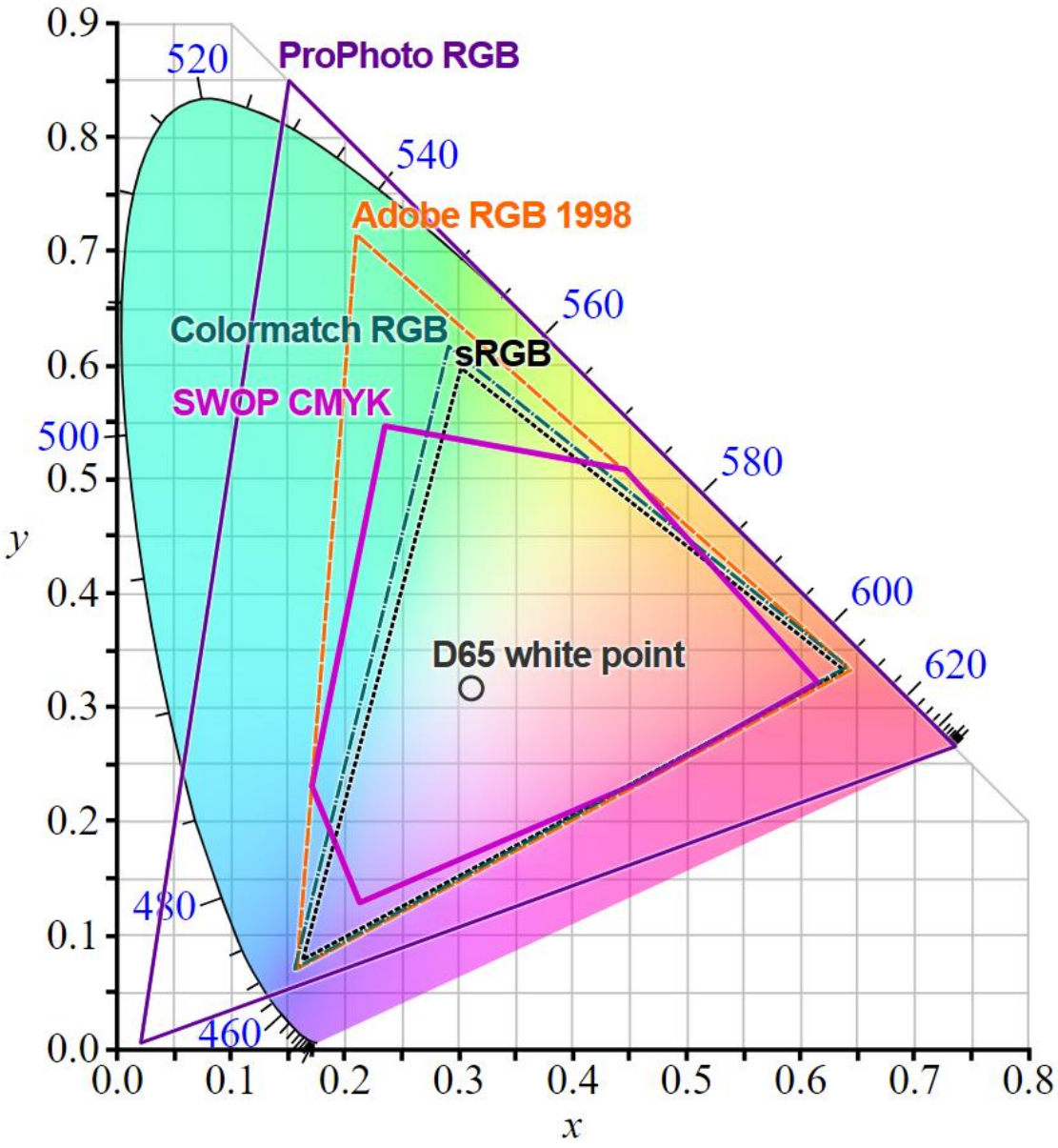

Figure 4. Comparison of some RGB and CMYK color gamuts on a CIE 1931 xy chromaticity diagram [source: based on Chapter 5: "Color Settings" figure, page 179 from (Blatner & Fraser, 2004)]

The CMYK color model is a subtractive color model, used in color printing, and is also used to describe the printing process itself. CMYK refers to the four ink plates used in some color printing: cyan, magenta, yellow, and key (black). In additive color models, such as RGB, white is the "additive" combination of all primary colored lights, black being the absence of light. In the CMYK model, it is the opposite: white is the natural color of the paper or other background, black results from a full combination of colored inks. Comparisons between RGB displays and CMYK prints can be difficult, since the color reproduction technologies and properties are very different. A computer monitor mixes shades of red, green, and blue light to create color pictures. A CMYK printer instead uses light-absorbing cyan, magenta, and yellow inks, whose colors are mixed.

Therefore, the RGB color model with the D65 illuminant is the most suitable for digital image analysis, specifically in this thesis, the analysis of the digital image of the photographed sample tube.

Figure 5 shows the spectral power of the D65 illuminant.

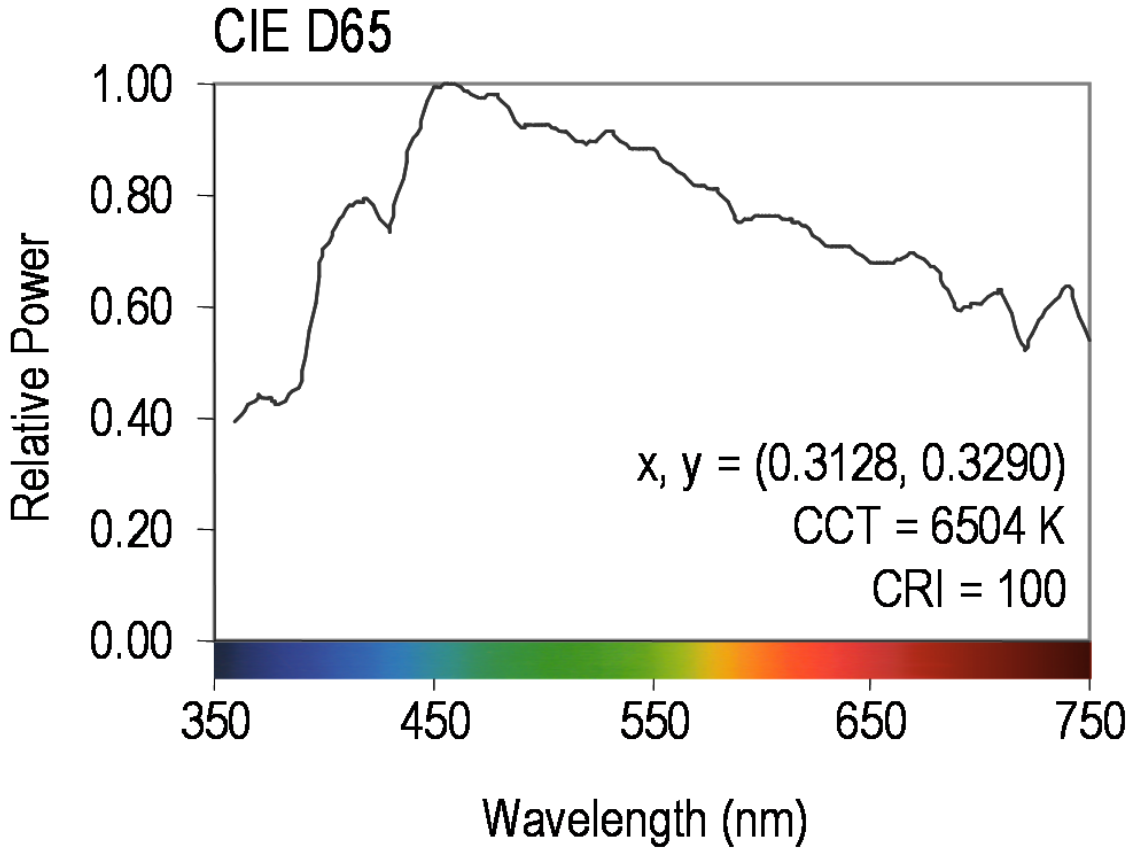

Figure 5. Spectral power distribution of illuminant D65 [source: raw spectral data from (Fairchild, 2019)]

CIE XYZ Color Space

According to the International Commission on Illumination (CIE), X, Y and Z are extrapolations of RGB values created mathematically in order to avoid negative numbers and are called tristimulus values. Y means luminance, Z is quasi-equal to blue, and X is a mix of cone response curves chosen to be orthogonal to luminance and non-negative.

CIELAB Color Space

Also annotated as CIE L*a*b*, this is a color space in which *L* represents brightness or lightness, and *a* and *b* are chrominance components, with the difference that the color values are far more wider than the human gamut (which makes it useful in image analysis where even small color differences are important to be clearly distinguished). Thus, this color space has also imaginary colors that cannot be reproduced in the physical world.

Since L*a*b* color space includes all perceivable colors, its gamut exceeds that of the RGB color model. Therefore, CIELAB is a totally different color space.

In fact, the CIELAB color space has a gamut that is greater than human vision as mentioned above. With respect to a given white point, the CIELAB model is device-independent and it defines colors independently of how they are created or displayed. The colors should not be dependent on the device they are displayed on. It is a purely theoretical color space that is used sometimes as an absolute standard to compare all other color spaces. CIELAB colors are defined relative to the white point (the D65 illuminant) of the CIE XYZ color space from which they will be converted. Figure 6 shows the CIELAB color space from a top-view.

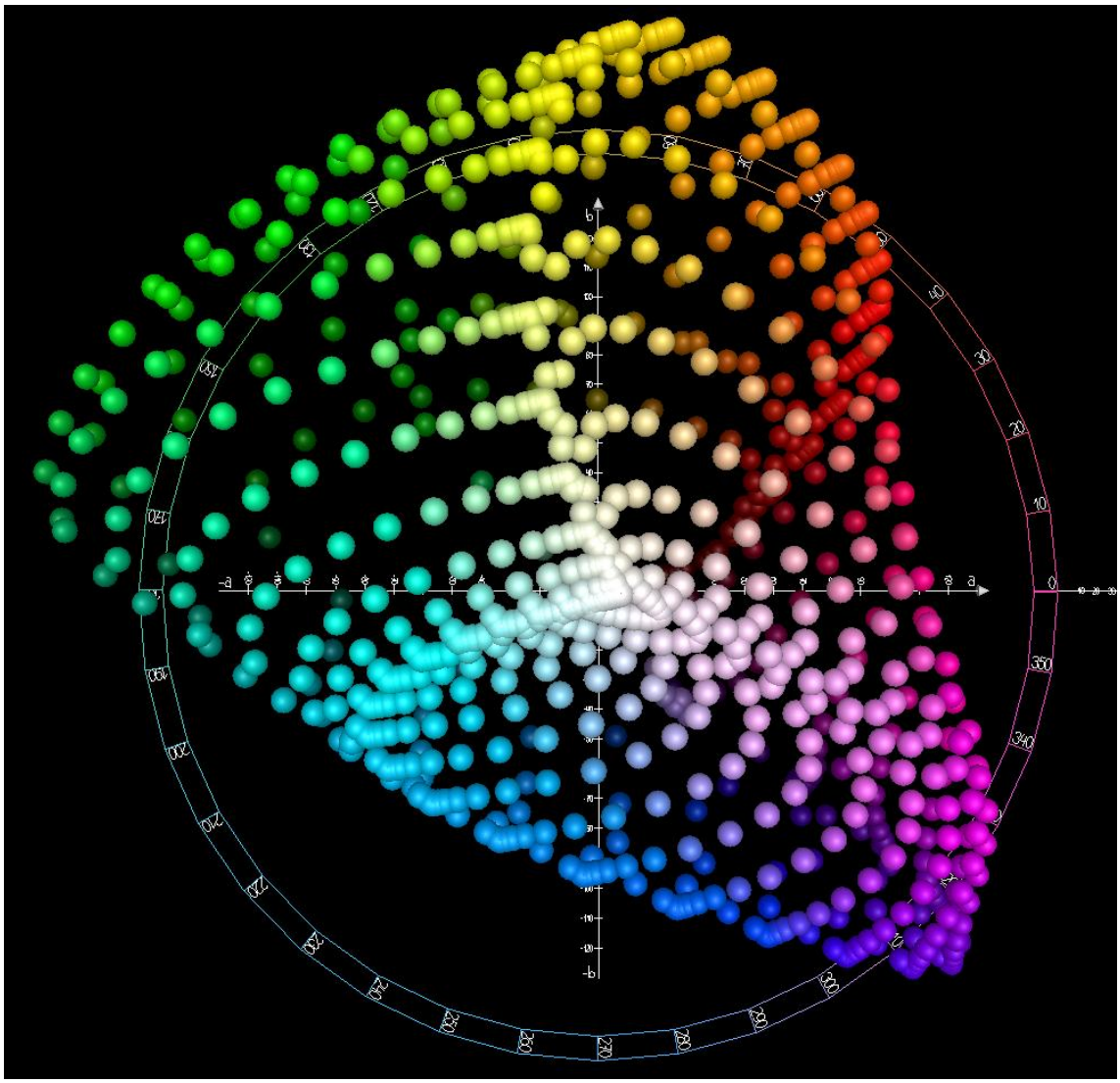

Figure 6. CIELAB color space – top-view (source: from image repository at http://cielab-farben.de/)

In the above subchapters, the theoretical advantages and characteristics of the RGB color model and CIE XYZ, and respectively, CIELAB color spaces were presented in the context of the software design concept and ideation.

Furthermore, the app's software image processing classes should be able to calculate the serum image classification on a pixel-by-pixel basis using the CIELAB color space values for this purpose. Then the results can be represented in the 3D space with the specific class color indices. The prospect of using the color models or color spaces in the analysis of the image seems promising. Then images that are captured by the camera, representing the serum samples, will be shown in a 3D graphic representation as dots in colored clouds

according to their CIELAB coordinates. Figure 7 illustrates an example of how a 3D graphical representation of serum images could look like. The colors represent the different levels of classification that were defined, more specifically in Table 4 at page 133.

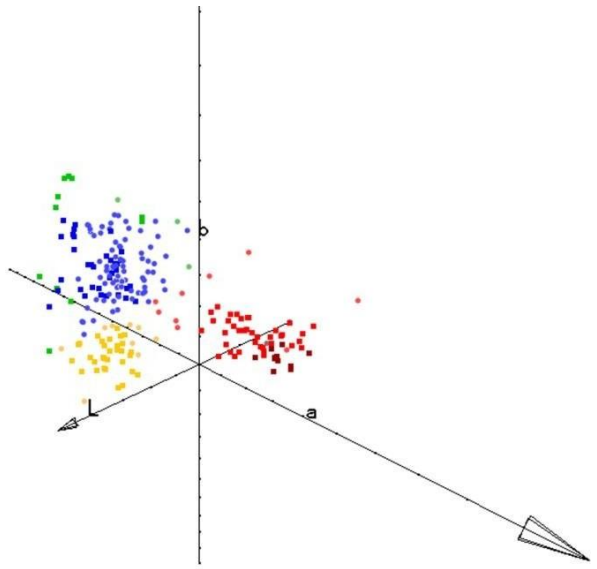

Figure 7. 3D graphic representation of serum images (source: own work)

The design of the image processing module started with a thought around which path to choose for the input method, whether dynamic (video) or static (captured image). Then, the video option was discarded due to stabilization issues and extra steps needed to be considered along with additional storage aspects. The static captured image of a sample tube seemed like a more promising alternative and it was chosen as an input object to the app's software. Then, the RGB color model values will be extracted for each pixel, from the captured image of the upper part of the sample tube (blood plasma area) and then the RGB values will be converted to CIELAB values in a two-step approach (first to CIE XYZ values and secondly to CIELAB values). Then the color index (CI) will be calculated based on the CIELAB values. The color index (CI) will then be mapped to the predefined calibration curve, which finally determines the correct measured hemolysis level result. In other words, and with a more practical flavor, in order to determine the color index of the

sample tube, a number of steps will need to be performed. In the image processing module, the starting point is to get the RGB values for each pixel, as mentioned above, and hold them in memory. Then these values will be converted in two sub-steps (in order to avoid time consuming conversions) into CIELAB values, as described above. Once the CIELAB measured values are returned, the image processing module will execute the final calculation of the color index (CI), which will determine the hemolysis level which will be displayed on the app screen. Subchapter 4.4.1 describes in detail the technical implementation of the image processing module as well as the rationale for the chosen color models or color spaces.

3.3 Materials

3.3.1. Test sample tubes

During the experiment, approximatively 37 unique sample tubes were used (a mix of sample tubes from different manufacturers, diverse tube types, various dimensions and filling volumes) in order to be able to test a diverse and comprehensive population of samples that are represented real life cases. The complete list of sample tubes is described in subchapter 5.2.1.

3.3.2. Plasma separation

In order for the plasma to separate, the real blood sample tubes will be posed in vertical stands or racks for a short period of time in order for the RBCs to gravitationally sedimentate (at the bottom of the tube). The plasma will be then present at the top of the tube (that is the layer which will be photographed with the “HemoDetect” app).

For the sample tubes which were artificially prepared, there is no required step for plasma separation since they already reflect the similar corresponding concentrations of free hemoglobin in the blood plasma as shown in Table 6.

3.4 Procedure

3.4.1. Using the prototype app

The operator is holding the sample tube and uses the "HemoDetect" app to photograph the upper part of the tube. The software performs image processing steps and determines the color of the highest density pixels and the highest luminance pixels. Then, the image processing algorithm uses deterministic rules which are predefined in the software to convert the color index (CI) to a concentration of free hemoglobin. The result is reported on the app screen to the operator as hemolysis free (≤5 mg/dl), low hemolysis (5 - 30 mg/dl), medium hemolysis (30 - 60 mg/dl), high hemolysis (60 - 300 mg/dl), or very high hemolysis (≥300 mg/dl).

3.4.2. Deterministic rules

The reference values were established according to relevant known ranges of free plasma hemoglobin based on the recommended levels of hemoglobin concentration from the Clinical and Laboratory Standards Institute supported also by research work on laboratory reference ranges measures with analytical instruments by Lippi et al. (2014).

The reference values for non-hemolytic samples is between 1-5 mg/dL. Measured values which are above 5 mg/dL represent samples affected by hemolysis as explained in the previous subchapter.

Real blood was acquired and stored in the test laboratory (for the planned experiment with real blood sample tubes). The artificially prepared sample tubes were filled with special solutions according to pre-defined rules as explained in subchapter 6.2.1.

3.5 Summary

This chapter introduced key design concepts that support the development of a smartphone-based approach to detect hemolysis. The chapter reviewed software design aspects, introduced the concept of color spaces and described in particular the CIELAB color space that will serve as the basis for the hemolysis detection mechanisms that will be applied to

digital photographs of the sample tubes. The chapter also described the plan of the experimental environment in terms of materials and methods.

Based on the concepts described in this chapter, the design of the app is made simple, focusing on easiness of usage and fast return of results. Furthermore, the materials planned to be used during the experiments are real blood samples or artificial sample tubes. The sample tubes were chosen from different manufacturers and they have different dimensions and filling volumes. The methods suggested for the experiment are easy and simple to reproduce and the results of the experiment will be carefully recorded in the database.

CHAPTER 4 - PROTOTYPE APP DEVELOPMENT

4.1 Overview

The objective of this chapter, is to present the technical implementations of the smartphone-based solution proposed, and to demonstrate that the smartphone camera, the region of interest of the image taken, the color spaces and algorithms which will be designed and implemented are working in a complementary manner to produce an end-to-end prototyped solution that can be easily used for early hemolysis detection in a point-of-care setting. While imagining the app in a potential production use, for the generic user, the complexity of the image processing should be hidden, stowed away, to make room for usability, easiness and pleasant interaction between the app and the user or operator.

For this purpose, the prototype app, called "HemoDetect" was built using the Android Studio. Most of the code implementations are written in Java. The architecture components designed and described in Chapter 3 will be exemplified later in this chapter. The technical concept design of the image processing module which was described in the subchapter 3.2.4, will be also supported by the detailed technical implementation presented later in this chapter.

4.2 Android app integrated development environment

The development of the prototype app was executed using Android Studio IDE and the programming language Java. The development guidelines were respected as much as possible as per the developer space available online[7]. Figure 8 shows a screenshot of the Android Studio integrated development environment.

[7] Consulted online (last checked on 10/10/2020): https://developer.android.com/

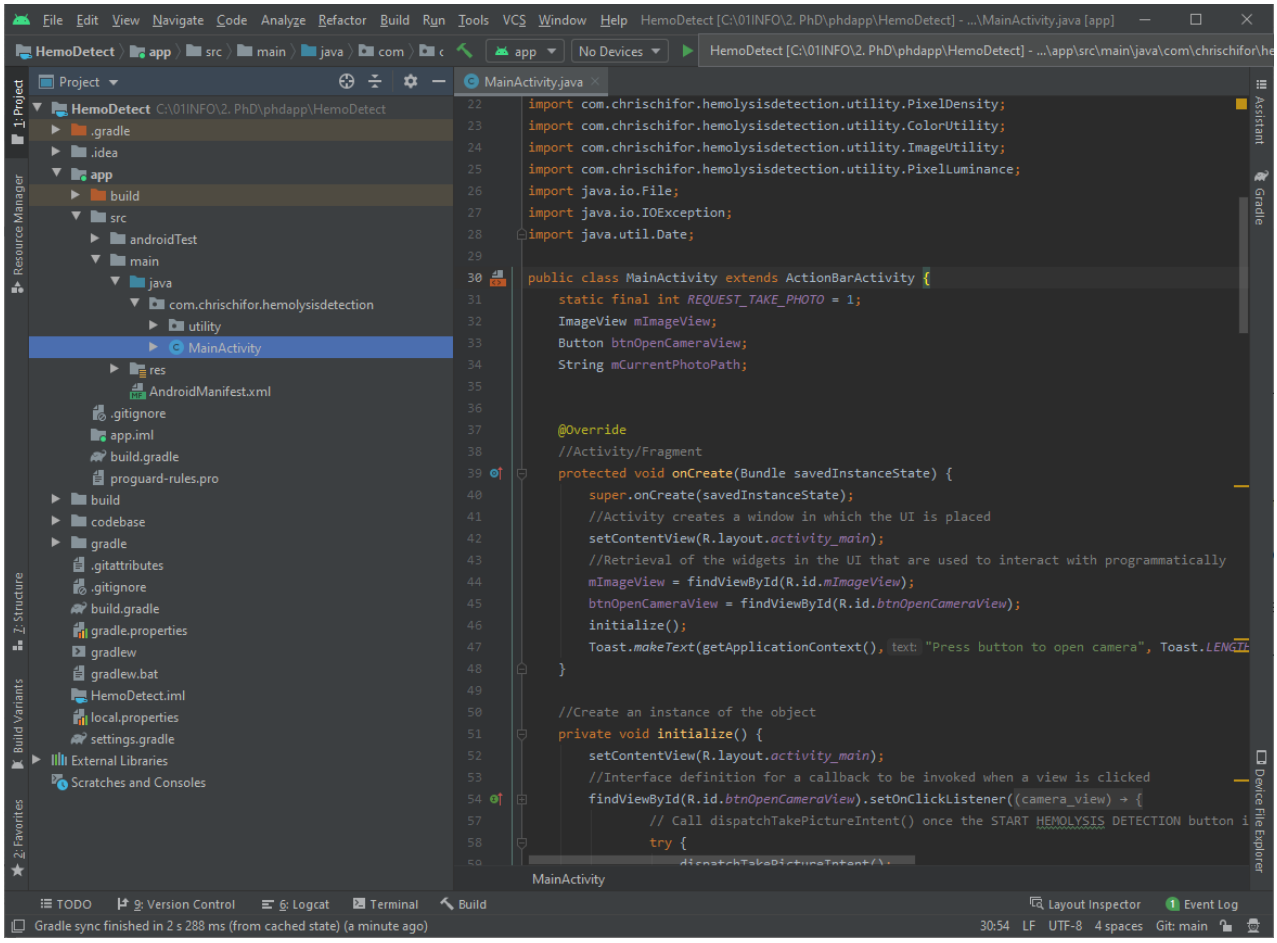

Figure 8. Android Studio Integrated Development Environment

Figure 9 shows the structure of the project which follows the Android standard development guidelines.

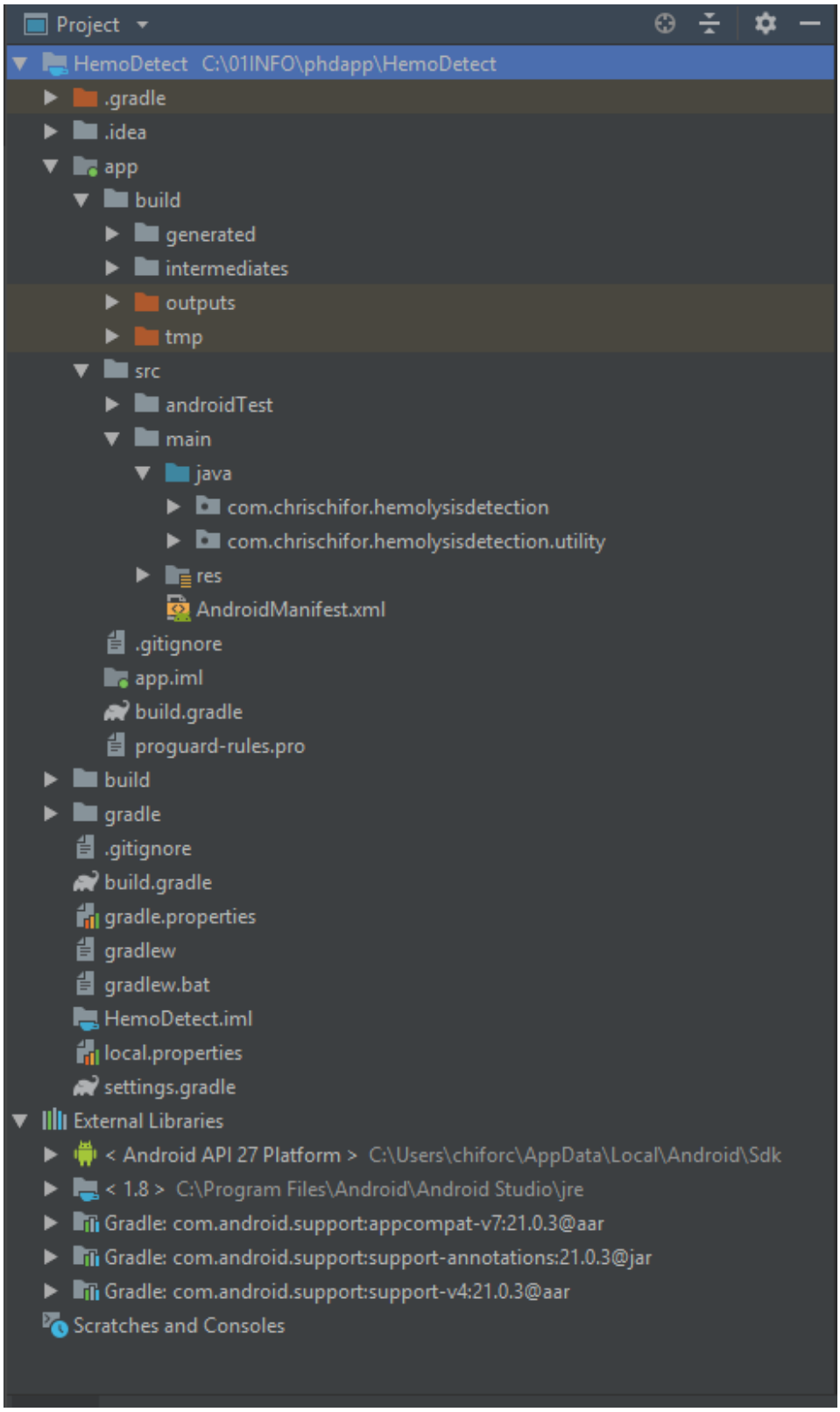

Figure 9. Prototype app folder structure

A short description of the various folders or elements of the project structure is shown in the list below.

- **app/ =** Contains the app files and folders
    - **build/** = Contains build outputs
    - **src/** = Contains all code and resource files for the app module in the following subdirectories listed below
        - **androidTest/** = Contains the code for instrumentation tests that run on an Android device
        - **main/** = Contains the "main" source set files: the Android code and resources shared by all build variants (files for other build variants reside in sibling directories, such as src/debug/ for the debug build type)
            - **java/** = Contains Java code sources
            - **res/** = Contains application resources, such as drawable files, layout files, and UI strings
            - **AndroidManifest.xml** = Describes the nature of the application and each of its components
    - **build.gradle (module)** = Defines the module-specific build configurations
- **build.gradle (project)** = Defines the build configuration that applies to all modules

4.3 Software architecture components implemented in the “HemoDetect” app (examples)

The main software architecture components were defined and described in the subchapter 3.2. Throughout the development of the app, the defined architecture components were developed or fulfilled with the recommended architectural principles in mind. In the following paragraphs they will be reiterated and supported by real code examples on how they were put into practice while building the app.

Activity/Fragment

One of the technical implementations is related to the principal activity which is usually called MainActivity in the Android development.

```
public class MainActivity extends ActionBarActivity {
```

Code snippet 1. MainActivity activity

In the case shown in the Code snippet 1, MainActivity extends another activity, called ActionBarActivity, which means that MainActivity class (subclass or child) inherits the attributes and methods from the ActionBarActivity (superclass or parent). A first example of a method which is implemented in a subclass of MainActivity is `onCreate(Bundle…)` where the activity is initialized as previously explained in Figure 2.

Inside the method shown in the

Code snippet 2

```
protected void onCreate(Bundle savedInstanceState) {
    super.onCreate(savedInstanceState);
    setContentView(R.layout.activity_main);
    mImageView = findViewById(R.id.mImageView);
    btnOpenCameraView = findViewById(R.id.btnOpenCameraView);
    initialize();
    Toast.makeText(getApplicationContext(),"Press button to open camera",
Toast.LENGTH_LONG).show();
 }
```

, it is important to call `setContentView()` with the layout resource defining the UI of the app as parameter `(R.layout.activity_main)`. The abbreviation “R” stands for the “res” folder, the “layout” for the “layout” sub-folder under the “res” folder and “activity_main” points to the “activity_main.xml” layout file which is located inside the “layout”. Also, in the same method, the `findViewById()` is called with the parameter `(R.id.ImageView)` in order to retrieve the widget (ImageView) in the UI, that is needed to be interacted with programmatically.

```
protected void onCreate(Bundle savedInstanceState) {
    super.onCreate(savedInstanceState);
    setContentView(R.layout.activity_main);
    mImageView = findViewById(R.id.mImageView);
    btnOpenCameraView = findViewById(R.id.btnOpenCameraView);
    initialize();
    Toast.makeText(getApplicationContext(),"Press button to open camera",
Toast.LENGTH_LONG).show();
 }
```

Code snippet 2. onCreate() method

Figure 10 shows the "activity_main.xml" layout file and its location in the app's folder structure.

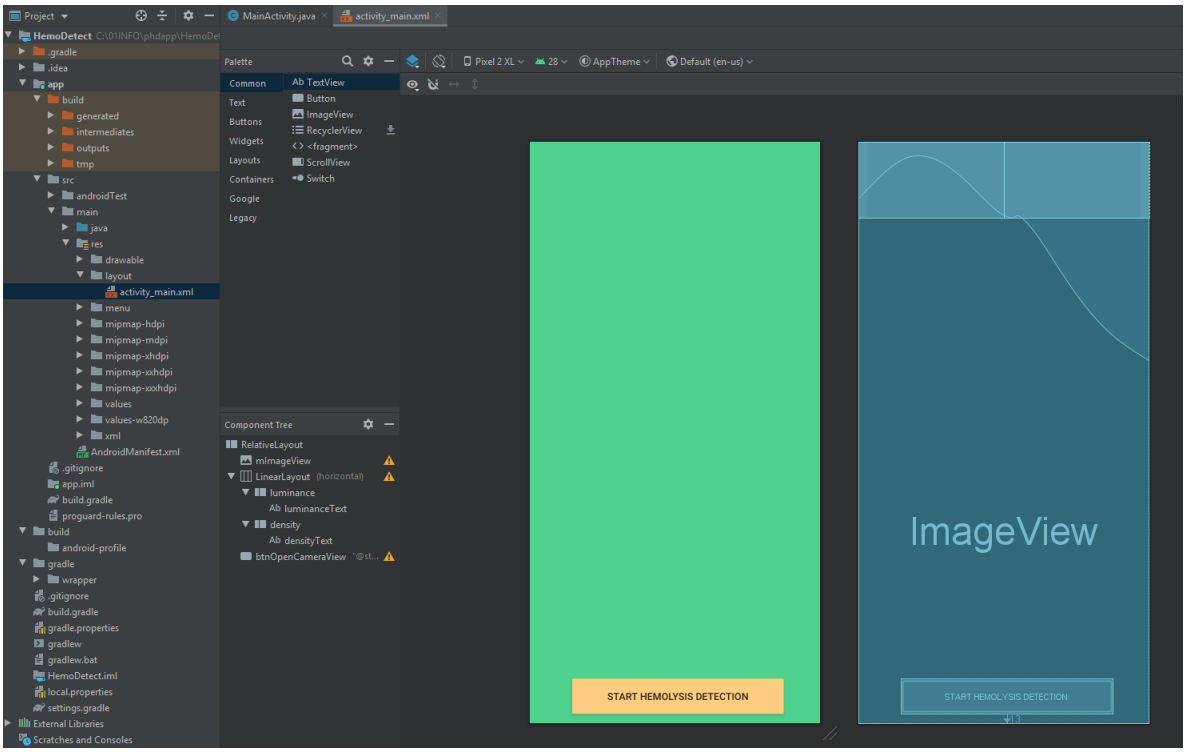

Figure 10. The activity_main.xml layout file

ViewModel

Java classes in the ViewModel layer are responsible for preparing or managing the data for the UI/View (Activity/Fragment). Also, they are responsible to forward or delegate specific tasks to classes in the Model layer. The example shown in the

Code snippet 3 below shows the `processImage()` method which is bridging the request to load the bitmap file from the model layer while, at the same time, calling the corresponding model methods to get the pixel density and luminance, and send back the results to the UI/View layer trough ImageView.

```
private void processImage() {
    Bitmap bitmap = ImageUtility.loadStandardBitmapFromPath(mCurrentPhotoPath);
    if (bitmap != null) {
        getPixelDensity(bitmap);
        getPixelLuminance(bitmap);
    }
    //Display the image resource, in this case the bitmap
    ((ImageView) findViewById(R.id.mImageView)).setImageBitmap(bitmap);
}
```

Code snippet 3. processImage() method

Model

Model classes are what are known as the domain object. The model represents the actual data and/or information. An example of a model class is the pixel object and its characteristics as illustrated in the Code snippet 4.

```
private class PixelObject {
    public int pixel;
    public int pixelCount;

    public PixelObject(int pixel, int pixelCount) {
        this.pixel = pixel;
        this.pixelCount = pixelCount;
    }
}
```

Code snippet 4. Pixel object class

The model holds the information. The model layer is usually composed of classes that contain the business data, the business logic and the business rules, as described in subchapter 3.2. One of the recommended implementation strategies of this layer is to expose its data through observables. Getters and setters are generally characteristic for these model classes as illustrated in the Code snippet 5.

```
public class ColorUtility {
[…]
public static int getRedFromColor(int color) {
    return (color >> 16) & 0xFF;
}

public static int getGreenFromColor(int color) {
    return (color >> 8) & 0xFF;
}

public static int getBlueFromColor(int color) {
    return color & 0xFF;
}
```

Code snippet 5. ColorUtility class

DAO

The DAO (Data Access Object) provides abstract interfaces or classes to underlying databases or other core data storage persistence mechanisms (internal or external). For example, the internal storage of the smartphone is considered to be the internal memory (ROM) while the external storage is supported and enabled by external memory cards with which the smartphone could be equipped (for extending the storage capacity for example).

In the "HemoDetect" app, the persistence layer is the internal storage of the smartphone where the captured sample tube images are stored. For the external storage, in order to read or write any file outside of the app-specific directories, the app needs to implement the Requesting Permissions Model declared in the AndroidManifest.xml file as shown in the Code snippet 6.

```
<uses-permission android:name="android.permission.WRITE_EXTERNAL_STORAGE" />
<uses-permission android:name="android.permission.READ_EXTERNAL_STORAGE" />
```

Code snippet 6. Extract from AndroidManifest.xml file

Upon taking the picture of the sample tube, the respective image file is stored in the internal storage of the smartphone. The code shown in Code snippet 7 implementation has been developed to save the image file to the persistent internal storage.

```
[…]
File storageDir = getExternalFilesDir(Environment.DIRECTORY_PICTURES);
File image = File.createTempFile(
        imageFileName,
        ".jpg",
        storageDir
);
//Save the file
mCurrentPhotoPath = image.getAbsolutePath();
[…]
```

Code snippet 7. Save image file on persistent storage implementation

The `getExternalFilesDir(Environment.DIRECTORY_PICTURES)` method shown in Code snippet 7 returns the absolute path to the directory on the primary storage where the app can place the persistent image files.

The parameter `Environment.DIRECTORY_PICTURES` provides access to environment variables and in this specific implementation it provides access to the standard directory in which to place the images.

Moreover, the `File.createTempFile()` method creates a new empty file in the specified directory (`DIRECTORY_PICTURES`) while the `getAbsolutePath` () method returns the absolute path of this file. An absolute path is a path that starts at a root of the file system. On Android, there is only one root ("/"). These Java DAO classes that support handling objects like images are essential for the creation and persistent storage of the exclusive input element, which is the captured image of the sample tube in the "HemoDetect" app.

4.4 Color spaces and algorithmical image processing implementation

4.4.1. High level process of determining the hemolysis level

In the subchapter 3.2.4, the theoretical process steps on how to determine the hemolysis level were described, starting with the image capture step until the step where color index (CI) is calculated. In this subchapter, more details about each component, its role and

calculation will be provided in the context of the technical implementation of the prototype app.

The image is captured with the smartphone's integrated camera. The RGB values are then extracted for each pixel of the image. The reason why the RGB color values are extracted from the image is because the RGB values will offer the needed information about the pixel characteristics (each pixel has a color numerical combination between red, green and blue). This is important, as this information will stay the base of identifying the pixels which are the closes to a specific shade of red, for example. Also, the RGB values are known for their availability and accessibility (as mentioned above most of the screen graphics use the RGB model). Moreover, according to Chavolla et al. (2018) the RGB model is used in most of the hardware and software development and it is the preferred model widely used in computer graphics.

Once the RGB values are extracted for each pixel, these values would need to be transformed in color space values which support or take in consideration the lightness component (or brightness). According to Chavolla et al. (2018) the CIELAB color space that was introduced by CIE, uses this factor, together with other two coordinates: a* or A (position between red and green) and b* or B (position between yellow and blue). The CIELAB color space is capable of wider color range or gamut than the RGB, especially when the task requires color analysis performed while considering with the luminance factor. The reason why CIELAB color space is chosen for hemolysis detection is the fact that with the a* and b* components it is possible to characterize the blood plasma (yellow color component) and the red blood cells (red color component) and the variations in between from yellowish to dark red; also, another reason why CIELAB color space is preferred when performing image analysis where pixel density and luminance is considered in the decision making process where color assessment and validation is necessary.

The CIELAB values are derived from the CIE XYZ values, where CIE XYZ is another color space introduced by CIE. One of the differences between CIE XYZ and the RGB model is the luminance factor (Y represents the luminance). CIE XYZ values are mathematically derived from RGB values. Based on the coordinates of CIE XYZ values,

the CIELAB values can be derived. The difference between CIE XYZ and CIELAB is that the latter can represent colors that are not handled by other color models or color spaces and it covers the entire range of human color perception (Chavolla et al., 2018).

The question arises as to why using a two-step approach (as step 1. RGB → CIE XYZ, and, respectively, step 2. CIE XYZ → CIELAB) and not shortcut the whole process just to one step (from RGB directly to CIELAB). The answer is simply because the process would be very time consuming (Connolly & Fleiss, 1997) while processing one single image from RGB to CIELAB. There are several research efforts that contemplate possible solutions for a faster RGB to CIELAB conversion, but, at the moment, no method is satisfactory enough in terms of processing speed.

4.4.2. Image processing algorithm

Upon capturing the photograph of the sample, the red (R), green (G) and blue (B) colors are extracted from the resulting bitmap for each pixel. The shade comparison between RGB colors of each pixel is calculated using the three-dimensional Euclidean distance in the following way as is explained by Li & Wang(2013).

$$d(\boldsymbol{p}, \boldsymbol{q}) = \sqrt{(p1 - q1)^2 + (p2 - q2)^2 + (p3 - q3)^2}$$

Where, d = distance and p, and respectively q are the points or coordinates. The Euclidean distance is the shortest distance between two points in an 3D-dimensional space.

The reason why the Euclidean distance must be calculated is because it is necessary to determine the pixel density in the region of interest or in the entire image. The result is that each pixel is assigned a value equal to the Euclidean distance to the closest pixel in the input image (a distance map of pixels that contains the closest distance of a pixel on an object of interest to any pixel in the background).

Figure 11 displays a visual representation of the above formula with the understanding of the of the p (1, 2, 3) and q (1, 2, 3) as shown in the list below.

1. p1 and q1 → R (red) where p1 and q1 are points on axis y (red)

2. p2 and q2 → G (green) where p2 and q2 are points on axis x (green)
3. p3 and q3 → B (blue), where p3 and q3 are points on axis z (blue)

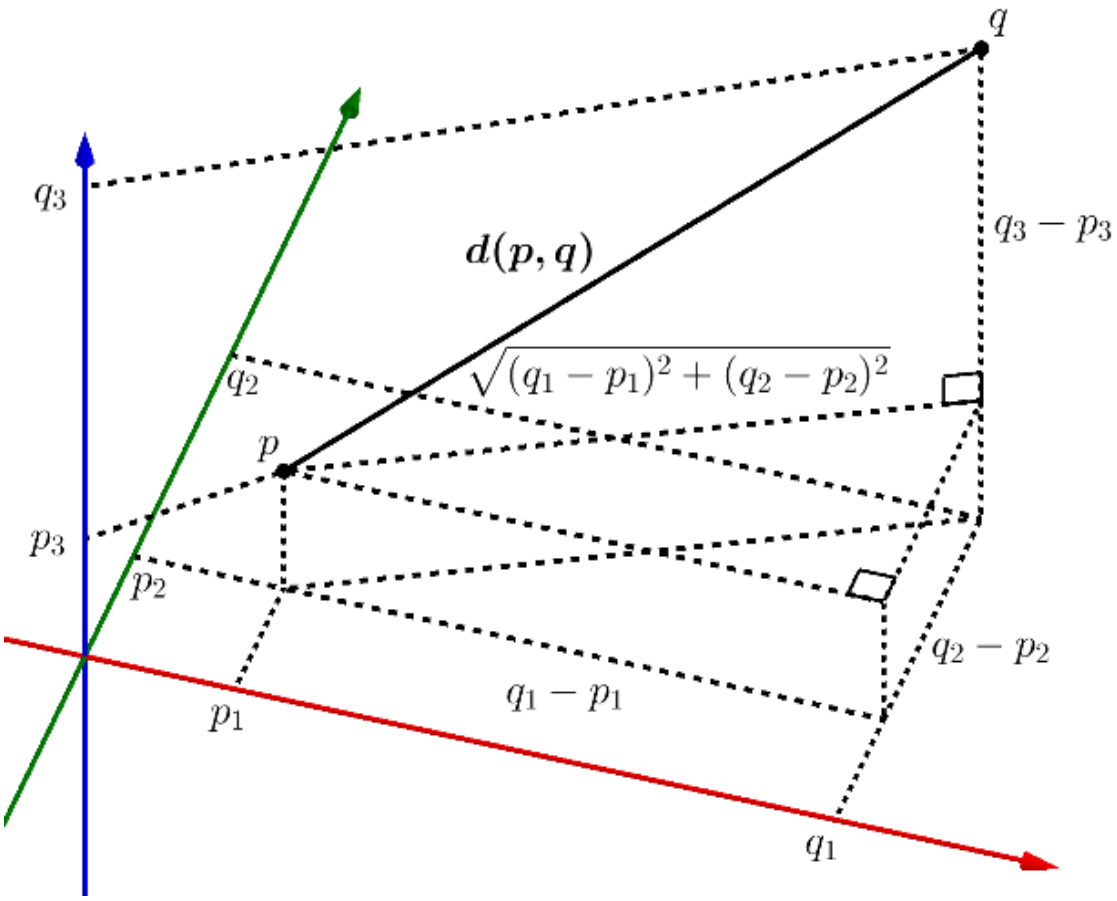

Figure 11. Visual representation of the Euclidean distance in 3D [source: (Brainard, 2003)]

The implementation in the prototype app code is made with the method `getShadeComparison` as illustrated in the Code snippet 8.**Erro! A origem da referência não foi encontrada.**

```
/**
 * Implementing the Euclidean RGB distance in 3D from the two points, each designated
by
 * (r1, g1, b1) and (r2, g2, b2) according to the below formula:
 * ((r2 - r1)2 + (g2 - g1)2 + (b2 - b1)2)1/2
 */
private double getShadeComparison(int inputColor, int comparisonColor) {
    int inputColorRed = ColorUtility.getRedFromColor(inputColor);
    int inputColorGreen = ColorUtility.getGreenFromColor(inputColor);
    int inputColorBlue = ColorUtility.getBlueFromColor(inputColor);

    int comparisonColorRed = ColorUtility.getRedFromColor(comparisonColor);
    int comparisonColorGreen = ColorUtility.getGreenFromColor(comparisonColor);
    int comparisonColorBlue = ColorUtility.getBlueFromColor(comparisonColor);

    return Math.pow((Math.pow((comparisonColorRed - inputColorRed), 2f) +
            Math.pow((comparisonColorGreen - inputColorGreen), 2f) +
            Math.pow(comparisonColorBlue - inputColorBlue, 2f)), 0.5f);
}
```

Code snippet 8. getShadeComparison() method

The RGB values are then converted to CIELAB values, (in a two-step approach) with the Lab values explained in the list below.

L = lightness (brightness)

a = red color component

b = yellow color component

The first step is the conversion from RGB to CIE XYZ, with a normalized relative luminance. The XYZ tristimulus values (set by illuminant D65 with normalization Y = 100) are listed in the Code snippet 9.

```
double Xr = 95.047;
double Yr = 100.0;
double Zr = 108.883;
```

Code snippet 9. XYZ tristimulus values

The reason why D65 illuminant is chosen is due to the fact that it supports best the daylight illumination; also it is recommended by CIE as the most commonly used standard illuminant. Moreover, during preliminary app testing, the D65 illuminant was proven to be yielding most accurate results.

Subsequently, Table 2 shows the computed values for the first conversion step, from RGB to CIE XYZ with the illuminant D65.

Illuminant	**RGB to CIE XYZ**		
D65	0.4124564	0.3575761	0.1804375
	0.2126729	0.7151522	0.0721750
	0.0193339	0.1191920	0.9503041

Table 2. The matrix values for RGB to CIE XYZ conversion

As a second step in the conversion, the CIE XYZ values are then converted to CIELAB values. The measurements, Lm, am and bm values, are then calculated using a set of conversion methods which handle the successful conversion to CIELAB values as listed below. The values of Lm (Lightness measured), am (red color component measured) and bm (yellow color component measured) are calculated based on the below conversion formulas.

$$Lm = 116\, f\left(\frac{Y}{Yr}\right) - 16$$

$$am = 500\, \left(f\left(\frac{X}{Xr}\right) - f\left(\frac{Y}{Yn}\right)\right)$$

$$bm = 200\, \left(f\left(\frac{Y}{Yn}\right) - f\left(\frac{Z}{Zr}\right)\right)$$

The practical implementation of these conversions in the app code is demonstrated in the code snippets below. Firstly, the lightness measured (Lm) is calculated using the method `calculateLightness`, as shown in the Code snippet 10. The remaining measured am, and, respectively, bm, values, are calculated in similar methods, `calculateAm` (as shown in the Code snippet 11) and, respectively, `calculateBm` (as shown in Code snippet 12).

```java
/**
 * L* for the lightness from black (0) to white (100)
 * @return Lm
 */
public static double calculateLightness(int R, int G, int B) {
    double r, g, b, Y, yr;
    // using illuminant D65 with normalization Y = 100
    double Yr = 100.0;
    // conversion from RGB to CIEXYZ values
    r = R / 255.0;
    g = G / 255.0;
    b = B / 255.0;
    if (r > 0.04045)
        r = Math.pow((r + 0.055) / 1.055, 2.4);
    else
        r = r / 12.92;

    if (g > 0.04045)
        g = Math.pow((g + 0.055) / 1.055, 2.4);
    else
        g = g / 12.92;

    if (b > 0.04045)
        b = Math.pow((b + 0.055) / 1.055, 2.4);
    else
        b = b / 12.92;
    r *= 100;
    g *= 100;
    b *= 100;
    Y = 0.2126 * r + 0.7152 * g + 0.0722 * b;
    // forward transformation from CIEXYZ to CIELAB values
    yr = Y / Yr;
    if (yr > 0.008856)
        yr = (float) Math.pow(yr, 1 / 3.);
    else
        yr = (float) ((7.787 * yr) + 16 / 116.0);
    //calculate cieLAB Lm (L measured) value
    double Lm = (116 * yr) - 16;
    return Lm;
}
```

Code snippet 10. calculateLightness() method

```java
/**
 * a* from green (-) to red (+)
 * @return am
 */
public static double calculateAm(int R, int G, int B) {
    double r, g, b, X, Y, xr, yr;
    // using illuminant D65 with normalization Y = 100
    double Xr = 95.047;
    double Yr = 100.0;
    // conversion from RGB to CIEXYZ values
    r = R / 255.0;
    g = G / 255.0;
    b = B / 255.0;
    if (r > 0.04045)
        r = Math.pow((r + 0.055) / 1.055, 2.4);
    else
        r = r / 12.92;
    if (g > 0.04045)
        g = Math.pow((g + 0.055) / 1.055, 2.4);
    else
        g = g / 12.92;
    if (b > 0.04045)
        b = Math.pow((b + 0.055) / 1.055, 2.4);
    else
        b = b / 12.92;
    r *= 100;
    g *= 100;
    b *= 100;
    X = 0.4124 * r + 0.3576 * g + 0.1805 * b;
    Y = 0.2126 * r + 0.7152 * g + 0.0722 * b;
    // forward transformation from CIEXYZ to CIELAB values
    xr = X / Xr;
    yr = Y / Yr;
    if (xr > 0.008856)
        xr = (float) Math.pow(xr, 1 / 3.);
    else
        xr = (float) ((7.787 * xr) + 16 / 116.0);
    if (yr > 0.008856)
        yr = (float) Math.pow(yr, 1 / 3.);
    else
        yr = (float) ((7.787 * yr) + 16 / 116.0);
    //calculate cieLAB am (a measured) value
    double am = 500 * (xr - yr);
    return am;
}
```

Code snippet 11. calculateAm() method

```java
/**
 * b* from blue (-) to yellow (+)
 * @return bm
 */
public static double calculateBm(int R, int G, int B) {
    double r, g, b, Y, Z, yr, zr;
    // using illuminant D65 with normalization Y = 100
    double Yr = 100.0;
    double Zr = 108.883;
    // conversion from RGB to CIEXYZ values
    r = R / 255.0;
    g = G / 255.0;
    b = B / 255.0;
    if (r > 0.04045)
        r = Math.pow((r + 0.055) / 1.055, 2.4);
    else
        r = r / 12.92;

    if (g > 0.04045)
        g = Math.pow((g + 0.055) / 1.055, 2.4);
    else
        g = g / 12.92;

    if (b > 0.04045)
        b = Math.pow((b + 0.055) / 1.055, 2.4);
    else
        b = b / 12.92;
    r *= 100;
    g *= 100;
    b *= 100;
    Y = 0.2126 * r + 0.7152 * g + 0.0722 * b;
    Z = 0.0193 * r + 0.1192 * g + 0.9505 * b;
    // forward transformation from CIEXYZ to CIELAB values
    yr = Y / Yr;
    zr = Z / Zr;
    if (yr > 0.008856)
        yr = (float) Math.pow(yr, 1 / 3.);
    else
        yr = (float) ((7.787 * yr) + 16 / 116.0);
    if (zr > 0.008856)
        zr = (float) Math.pow(zr, 1 / 3.);
    else
        zr = (float) ((7.787 * zr) + 16 / 116.0);
    //calculate cieLAB bm (b measured) value
    double bm = 200 * (yr - zr);
    return bm;
}
```

Code snippet 12. calculateBm() method

The measured values (Lm, am, bm) will then be used while calculating the color index (CI) using the three-dimensional Euclidean distance expression as shown in the formula below.

$$\mathbf{CI} = \left(\sqrt{(Lm - Lr)^2 + (am - ar)^2 + (bm - br)^2}\right)$$

Where, Lr, ar and br represent the reference CIELAB values of blood plasma. The technical implementation in the prototype app's code is depicted in the Code snippet 13.

```
//apply formula to calculate the color index
int i = 2;
double CI = Math.sqrt(Math.pow((Lm - Lr), i) + Math.pow((am - ar), i) + Math.pow((bm - br), i));
```

Code snippet 13. Color index (CI) calculation

The color index (CI) is then mapped to the calibration curve to obtain the concentration of free hemoglobin. The calibration curve corresponds to range values found in other similar endeavors reported in the literature which are based on the recommended levels of hemoglobin concentration from the Clinical and Laboratory Standards Institute supported also by research work on laboratory reference ranges measured with analytical instruments by Lippi et al. (2014), the result being displayed on the screen as hemolysis free (≤5 mg/dl), low hemolysis (5 - 30 mg/dl), medium hemolysis (30 - 60 mg/dl), high hemolysis (60 - 300 mg/dl), or very high hemolysis (≥300 mg/dl).

4.5 Summary

Color spaces and image processing algorithms based on Euclidean distances provide a successful and reliable technological option to calculate the correct color index in order to detect hemolysis.

This chapter had the objective to demonstrate that a smartphone based solution to detect hemolysis is a viable option based on the preliminary test results. Practical technical topics were presented and rationale was provided as to why specific color spaces and methods or techniques were implemented.

The process steps from the moment the camera captures an image until the hemolysis level results are displayed on the screen were also explained. With the main technical developments completed, the next step is to plan the experimental environment and all the related necessary steps which will be explained in the next chapter.

CHAPTER 5 – EXPERIMENTAL ENVIRONMENT

5.1 Hardware

5.1.1. Characteristics

The prototype app is installed on a Huawei nova 3i smartphone (which is considered a medium standard smartphone on the low-high end scale) running Android [version 8.1.0 (Oreo)] mobile operating system (complete smartphone specifications available in **Appendix 1**). The smartphone is equipped with a fifteen point nine-megapixel camera (15.9 MP) comporting a resolution of 4608x3456 pixels. Figure 12 shows the front and back of the Huawei nova 3i smartphone.

Figure 12. Illustrations of the smartphone. Left: the front screen of Huawei nova 3i smartphone. Right: the back of the smartphone (with dual-lens camera visible).

Figure 13 shows the screen with the "HemoDetect" prototype app icon and the home screen of theFigure 13. Smartphone app screenshots. Left: the "HemoDetect" app icon in the center of the screen. Right: the home screen of the "HemoDetect" prototype app. "HemoDetect" app which is displayed once the app is launched.

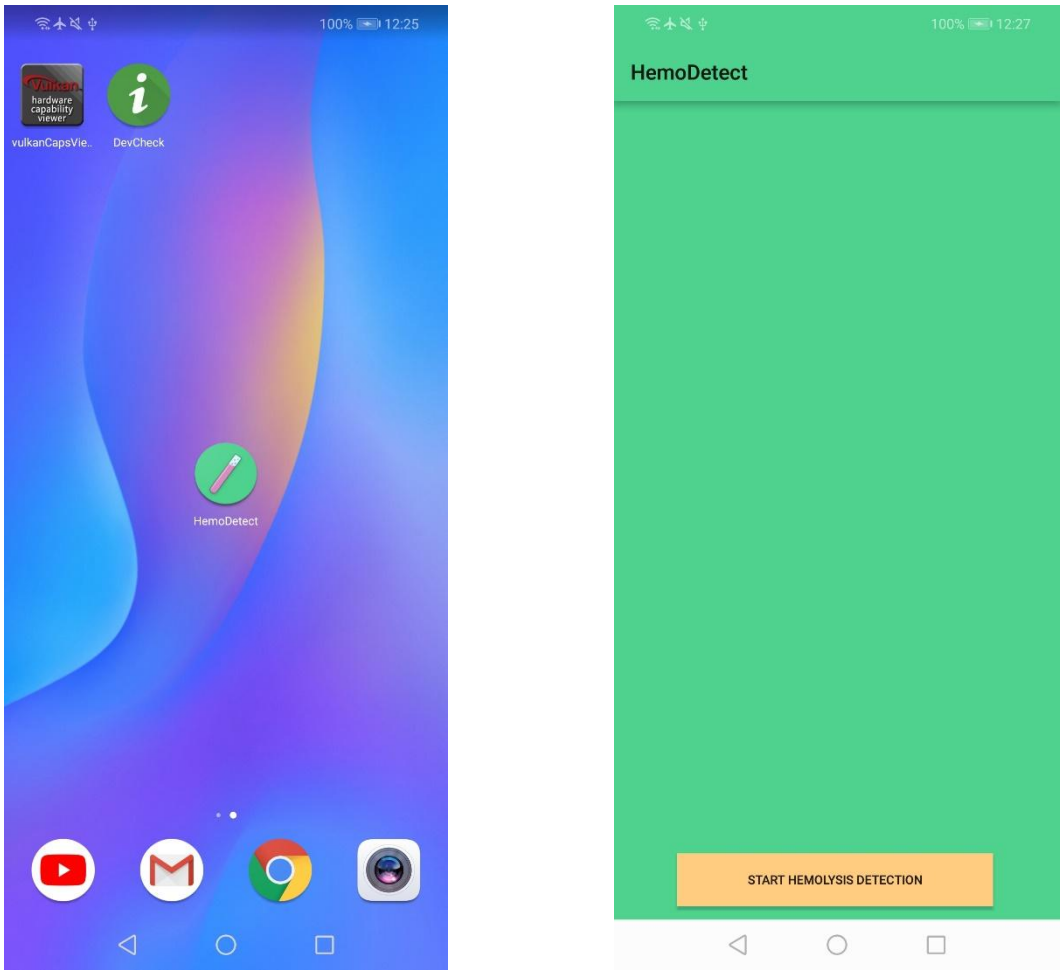

Figure 13. Smartphone app screenshots. Left: the "HemoDetect" app icon in the center of the screen. Right: the home screen of the "HemoDetect" prototype app.

5.2 Sample tubes

5.2.1. Definition of the experiment tube list

The sample tube list used in the experimented is based on 37 different or unique tubes. The selection criteria used while selecting these sample tube is in accordance with real-life usage in most of the laboratories today (the sample tubes are representative and relevant being used in laboratories, hospitals or point-of-care settings during patient sample routine testing).

Table 3 shows the list of unique sample tubes and their characteristics.

Picture	Tube manufacturer	Dimensions (mm)	Cap type	Cap color	Filling volume (ml)	REF
	Greiner Bio-One	16x100	hemogard	lavender cap - black ring	9	455036
	Greiner Bio-One	16x100	hemogard	red cap - black ring	9	455092
	Greiner Bio-One	16x100	hemogard	red cap - black ring	9	485504
	Greiner Bio-One	16x100	hemogard	gold cap - gold ring	9	455034
	Greiner Bio-One	16x100	hemogard	white cap - black ring	9	455001
	Greiner Bio-One	16x100	hemogard	green cap - yellow ring	4	455229
	Greiner Bio-One	13x100	hemogard	pink cap - black ring	6	456093
	Greiner Bio-One	13x100	hemogard	lavender cap - black ring	6	456038
	Greiner Bio-One	13x100	hemogard	green cap - yellow ring	5	456216
	Greiner Bio-One	13x100	hemogard	gold cap - gold ring	5	456010
	Greiner Bio-One	13x75	hemogard	light blue cap- white ring	2	454321
	Greiner Bio-One	13x75	hemogard	grey cap - white ring	2	454252
	Greiner Bio-One	13x75	hemogard	blue cap - black ring	3	454325
	Greiner Bio-One	13x75	hemogard	red cap - yellow ring	3.5	454071
	Greiner Bio-One	13x75	hemogard	orange cap - yellow ring	3.5	454591
	Greiner Bio-One	16x100	hemogard	red cap - yellow ring	8	455071
	Greiner Bio-One	13x75	hemogard	orange cap - black ring	3	454025
	Becton Dickinson	16x100	hemogard	yellow	8.5	367953

	Becton Dickinson	16x100	rubber	red/grey	8.5	367988
	Becton Dickinson	13x100	rubber	red	6	368660
	Becton Dickinson	13x100	hemogard	brown	5	368968
	Becton Dickinson	13x100	hemogard	yellow	5	367955
	Becton Dickinson	13x100	hemogard	ochre	5	367406
	Becton Dickinson	13x100	hemogard	translucent white	5	368970
	Becton Dickinson	13x75	hemogard	light blue	2.7	363095
	Becton Dickinson	13x75	hemogard	red	4	367812
	Becton Dickinson	13x75	hemogard	green	4	368884
	Becton Dickinson	17x120	dome-seal screw cap	blue	15	352095
	Becton Dickinson	16x100	rubber	yellow	10	364938
	Becton Dickinson	13x75	hemogard	blue	3.5	368966
	Sarstedt	15x75	screw cap	white	5.5	03.1397
	Sarstedt	11x66	screw cap	pink	2.7	05.1557.100

	Sarstedt	13x90	screw cap	brown	4.9	04.1935
	Sarstedt	13x90	screw cap	light violet	4.9	04.1931.100
	Sarstedt	11x92	screw cap	light green	5	05.1071
	Sarstedt	17x120	dome-seal screw cap	red	15	62.554.502
	Roche	13x75	hemogard	white cap - red ring	3	08128812 001

Table 3. The unique sample tube list

Off the shelf from the medical suppliers, the sample tubes come with different preparations which are part of the standard manufacturing process from the manufacturer (e.g., clot activator and/or gel for serum separation, trisodium citrate solution) or, simple test tubes, without any prior or specific preparation (just the tube and the cap, no solution or additional element physically present in the sample tube or coated on the interior walls of the sample tube).

5.2.2. Preparation of test samples with different hemolysis levels

The sample tubes listed in the experiment list were used as the testing representative population. Figure 14 and Figure 15 show several empty sample tubes which are ready to be prepared for the experiment (in the second picture, sample tubes with special preparations can be observed as well as simple tubes without any special preparation).

Based on the unique sample tube list (37), the experiment tube list was prepared (in total, 70 sample tubes with various hemolysis levels). The 70 sample tubes could also be called artificial sample tubes as they were prepared with special solutions that imitate the pre-defined hemolysis levels as shown in the list below.

1. Hemolysis free (HF): 14 tubes
2. Low hemolysis (LH): 10 tubes

3. Medium hemolysis (MH): 22 tubes
4. High hemolysis (HH): 11 tubes
5. Very high hemolysis (VHH): 13 tubes

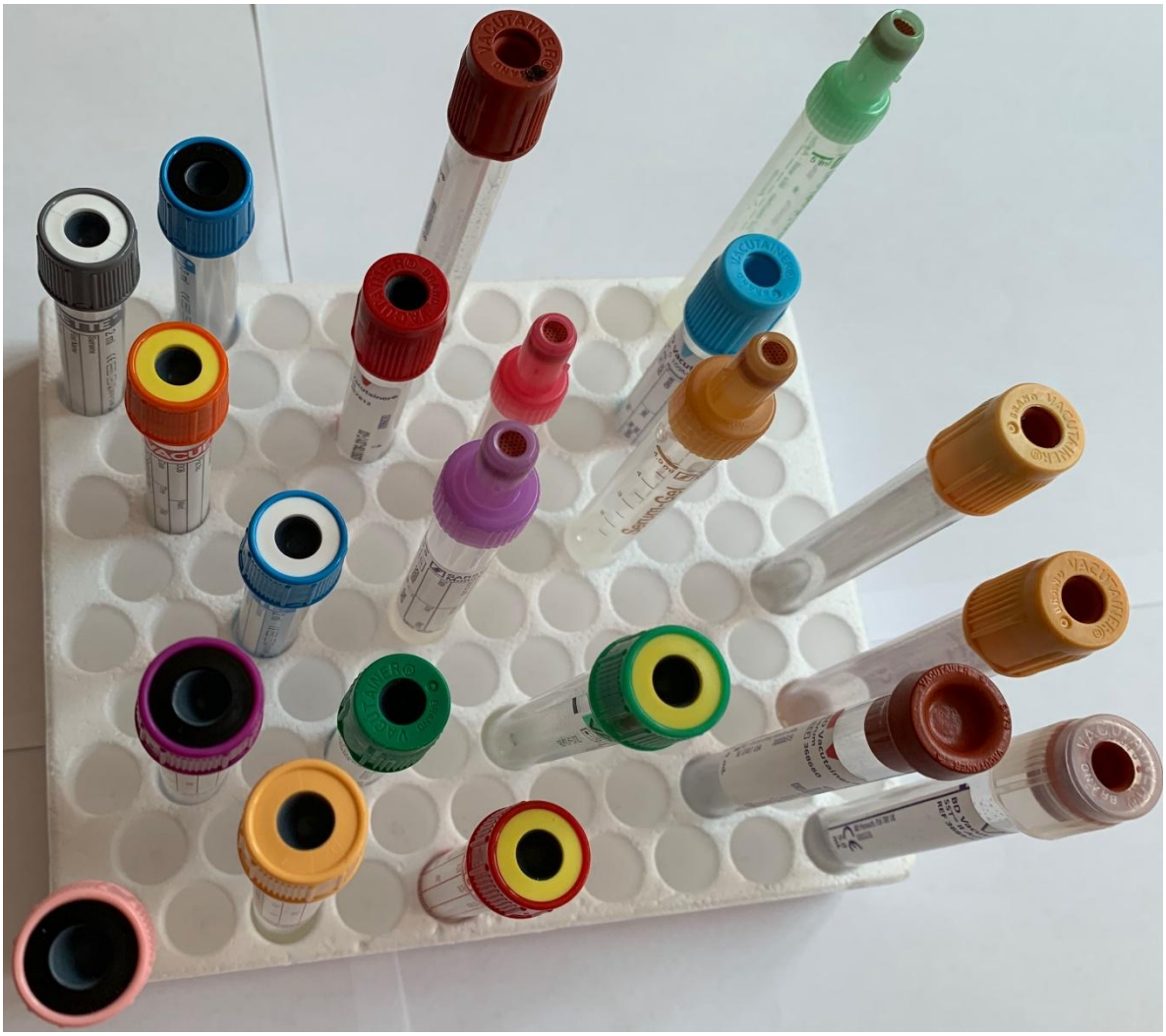

Figure 14. Samples tubes ready for preparation (< 16mm diameter)

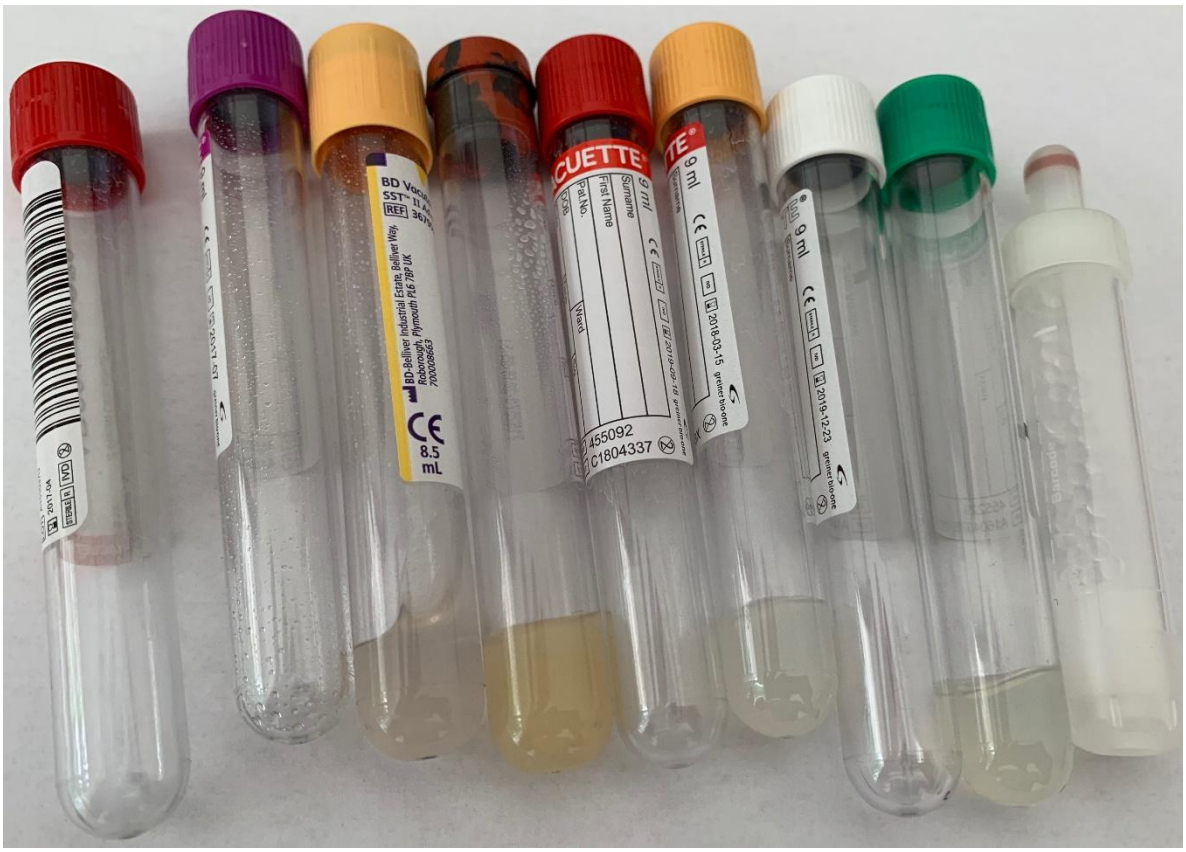

Figure 15. Sample tubes ready for preparation (16mm diameter)

In order to study and test different levels of hemolysis, the samples were prepared with solutions that simulate different concentrations of free hemoglobin. In the next laboratory study chapter, the preparation process is explained step-by-step.

5.3 "HemoDetect" app

5.3.1. Smartphone preparation

During patient blood extraction, the medical staff wear medical or surgical rubber gloves. This is part of the medical training for both the patient and the medical staff's safety. Historically, touchscreens were not responding well or were even totally irresponsive to user touch gestures if between the finger and the touchscreen there was the rubber layer of a medical glove. To make matters worse, in some particular cases the coating of the medical gloves was posing problems, too, such as leaving traces on the screen or simply interfering

with the user touch gestures. This is why, one of the selection criteria for the smartphone used in this experiment was to be equipped with a capacitive touchscreen which is naturally easy to control and manipulate while using standard medical gloves.

5.3.2. App preparation

The app does not require any special previous preparation. Once installed or deployed on the smartphone, it remains installed and ready to be used at any time.

5.4 Database preparation

5.4.1. Database software or tools

The data or measurements were recorded in a simple Microsoft Excel database. For generating reports or graphs the database file was connected to the Tableau and SPSS software.

5.5 Laboratory safety and protection rules

5.5.1. Safety and protection

In the laboratory, while preparing or testing the sample tubes, the standard laboratory rules were applied. It is important to avoid direct contact with chemicals and keep chemicals away from skin, clothing, hair, and the face. Protective equipment is strongly recommended to be worn at all times. During the experiment the three rules listed below were carefully respected.

1. Clothing; long trousers, closed toed shoes, lab coat which covered the arms
2. Gloves; medical gloves were used
3. Eye protection; safety glasses (goggles) with side shields were used

5.6 Summary

This rather short chapter introduced the experimental environment with its planned materials, such as the smartphone, the sample tubes, the “HomeDetect” prototype app which is ready, the database preparation and ultimately the safety measures that must be respected while performing tests in the laboratory.

In the next chapter, further material preparations, experiment procedures and execution of measurements will be described.

CHAPTER 6 – LABORATORY STUDY

6.1 Overview

Following planning the experimental environment explained in the previous chapter, this chapter continues the journey in the laboratory and it will present the material preparation steps in detail, the execution of the measurements and their recording into the database as well as discussions around the user interface and user interactions of the prototype app, and finally closing the chapter with a reflection on future possible design decisions to be considered in a potential production version of the app.

6.2 Experimental plan and procedure

In the preparation of the experiment, 70 sample tubes were filled up with water and food coloring to simulate the different hemolysis color levels that are encountered in a real-processing environment.

In order for the “HemoDetect” app to reliably allocate the samples to the individual quality levels during tests, a serum classifier must initially be established and should later be maintained on the basis of the sample spectrum of a laboratory.

The serum classifier is the part of the app software which is responsible for determining the quality of the processed samples. Reference images for the individual quality levels are assigned to corresponding areas for this. The reference images in their entirety are referred to as learning samples in specialist terminology. During the prototype app development and testing, the calibration of the serum classifier was performed based on the identified reference samples and their images.

The size and quality of the camera captured image depends on several parameters such as the type and manufacturer of the tube, the material properties, overlap by barcode labels or the amount of serum present in the sample tube.

When later, during testing, a serum sample is processed by the app software, its serum portion is ascertained. Afterwards, the app software compares the color values of this

portion with the reference values established and determines the serum quality (hemolysis level) based on the basis of this comparison.

The quality is expressed as reported results of the hemolysis level. The abbreviations are often cited when referring to these reported results; Table 4 shows these reported hemolysis levels based on the recommended levels of hemoglobin concentration from the Clinical and Laboratory Standards Institute supported also by research work on laboratory reference ranges measures with analytical instruments by Lippi et al. (2014).

Reported hemolysis levels	Concentration of free hemoglobin	Abbreviation
hemolysis free	≤ 5 mg/dl	HF
low hemolysis	5 – 30 mg/dl	LH
medium hemolysis	30 – 60 mg/dl	MH
high hemolysis	60 – 300 mg/dl	HH
very high hemolysis	≥ 300 mg/dl	VHH

Table 4. Reported hemolysis levels

Technically, upon image capturing, the image of the sample tube is saved in the JPEG format in the internal storage of the smartphone. Table 5 shows the folder name, path and format of an image from the experiment that is stored in the memory of the smartphone.

Internal folder name and path	Content	File format
Internal storage/Android/data/com.chrischifor.hemolysisdetection/ files/Pictures/	Images showing the captured sample tube	JPEG

Table 5. Sample image folder name, path and format

Figure 16 shows a screenshot from the smartphone, where the list of stored images can be visualized.

Figure 16. The sample images saved in the internal storage of the smartphone

In future research work or while potentially preparing the app for a production pilot in a commercial point-of-care, ward or laboratory, saving the images could be done remotely (on the laboratory internal server or in the cloud) where other software applications or the LIS could connect and consume this data so that it is fully integrated in the information system of the laboratory. This could represent a future research work idea which would implement the connectivity aspect of the app.

6.2.1. Material preparation

As previously mentioned, the main materials used in the experiment are listed below.

- Prepared artificial sample tubes and real sample tubes (containing human blood, collected from various commercial diagnostics laboratories or partners in Switzerland, and stored in the local laboratory)
- The exclusive measurement device used was the smartphone with the "HemoDetect" app installed on it

The test samples with various levels of hemolysis (HF, LH, MH, HH, VHH) to be tested, were carefully prepared according to specific determined ingredients and dosages, to be able to replicate the real-life blood samples with various levels of hemolysis. Previous work was performed to reach the correct dosage levels through continuous testing, comparisons, calibration and retesting. Each artificial sample contains a combination of specific ingredients.

Table 6. Sample preparation – ingredients and their dosagesTable 6 shows the main ingredients and their dosages for each respective hemolysis level.

Hemolysis level	Ingredient 1	Ingredient 2	Ingredient 3
hemolysis free (HF)	H_2O (distilled): 10 L	Sodium chloride (NaCl): 9.0 g	CLOU Powder Stain, color no. 151 yellow G: 2.5 g

low hemolysis (LH)	HF solution: 400 mL	VHH solution: 16 mL	-
medium hemolysis (MH)	H_2O (distilled): 400 mL	Sodium chloride (NaCl): 0.36 g	CLOU Powder Stain, color no. 155 dark red: 0.12 g
high hemolysis (HH)	H_2O (distilled): 400 mL	Sodium chloride (NaCl): 0.9 g	CLOU Powder Stain, color no. 155 dark red: 0.3 g
very high hemolysis (VHH)	H_2O (distilled): 400 mL	Sodium chloride (NaCl): 2.1 g	CLOU Powder Stain, color no. 155 dark red: 0.7 g

Table 6. Sample preparation – ingredients and their dosages

The H_2O (distilled) represents water that has been boiled into vapor and condensed back into liquid in a separate container (clean container). Impurities or residues in the original water that do not boil below or near the boiling point of water remain in the original container. Thus, distilled water is one type of purified water. It is commercially available and it was used in sample preparation in the laboratory. Figure 17 shows the H_2O (distilled) used in the experiment.

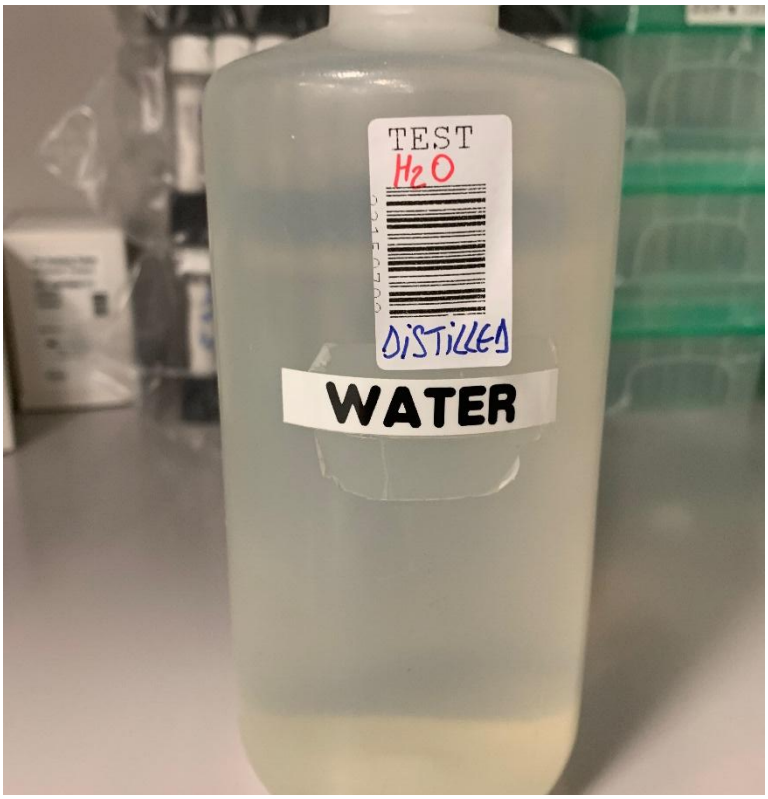

Figure 17. H_2O (distilled)

Sodium chloride (NaCl) or commonly known as salt is an ionic compound with the chemical formula NaCl, representing a 1:1 ratio of sodium and chloride ions. With molar masses of 22.99 and 35.45 g/mol respectively, 100 g of NaCl contains 39.34 g Na and 60.66 g Cl. Sodium chloride is the salt most responsible for the salinity of the extracellular fluid of many multicellular organisms. Figure 18 illustrates the salt used in the experiment.

Figure 18. Sodium chloride (NaCl) – salt

CLOU Powder Stain is a commercially available product, which is a dry powder (mix of synthetically produced water-soluble monoazo dyes), ready to be mixed with water. Prescribed concentrations vary from 50 grams of powder per litre of water for certain color tones to as little as 20 grams per litre for lighter colors. Average is around 30 grams per litre. Colors can be mixed to create custom colors. The shelf life of the dry powders is at least several years. An important characteristic of the CLOU Powder Stain is the color-fastness (the color will not fade away or be washed out) unless subject to prolonged exposure to sunlight. Figure 19 is an extract from the official product technical data sheet that shows the colors used in the experiment (151 yellow G and, respectively, 155 dark red).

Figure 19. Extract (colors) from the product technical data sheet

Figure 20 and Figure 21 illustrate the front packaging of the two colors used in the experiment.

Figure 20. CLOU Powder Stain 151 yellow G

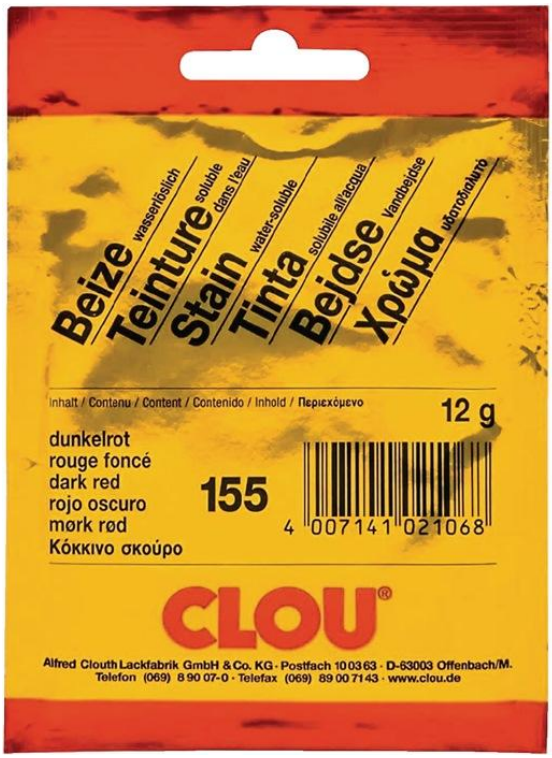

Figure 21. CLOU Powder Stain 155 dark red

6.2.2. Container preparation

The solutions which were prepared as per strict dosages shown in Table 6, were stored in small plastic containers, labeled HF, LH, MH, HH and respectively, VHH. Figure 22 shows some of the capped containers (upper part of the combined picture) and some of the uncapped containers with pipettes inside (lower part of the combined picture).

Figure 22. Containers with the solution prepared and pipettes

6.2.3. Sample pipetting

Five sample tubes were pipetted first, to exemplify and do a first check of the hemolysis levels. Figure 23 shows the hemolysis levels as defined in Table 4Table 4. Reported hemolysis levels; from left to right, the color difference is visible (from yellow, hemolysis free sample to dark red, very high hemolysis sample).

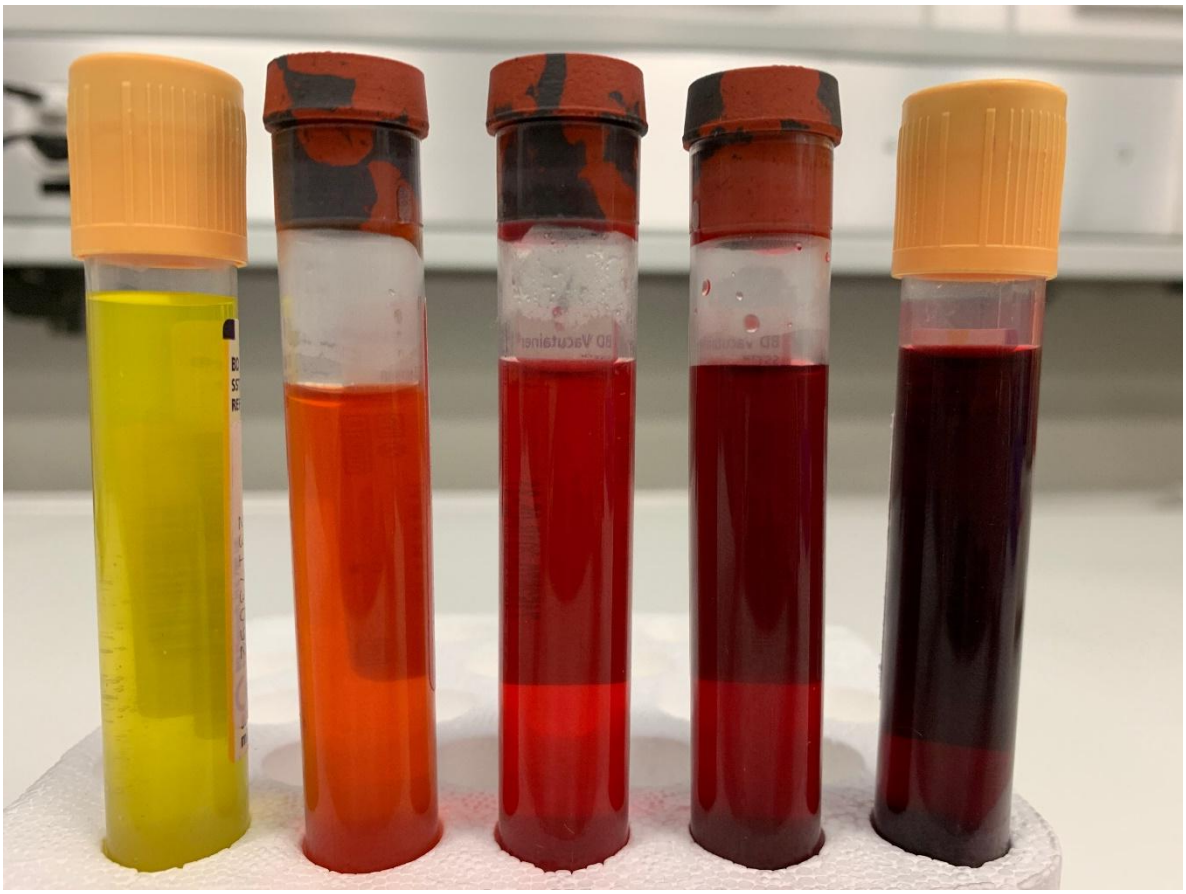

Figure 23. Hemolysis levels, from left to right: HF, LH, MH, HH and VHH

Figure 24 shows the same sample tubes, but labelled with small white labels for identification and illustration: HF, LH, MH, HH and respectively VHH.

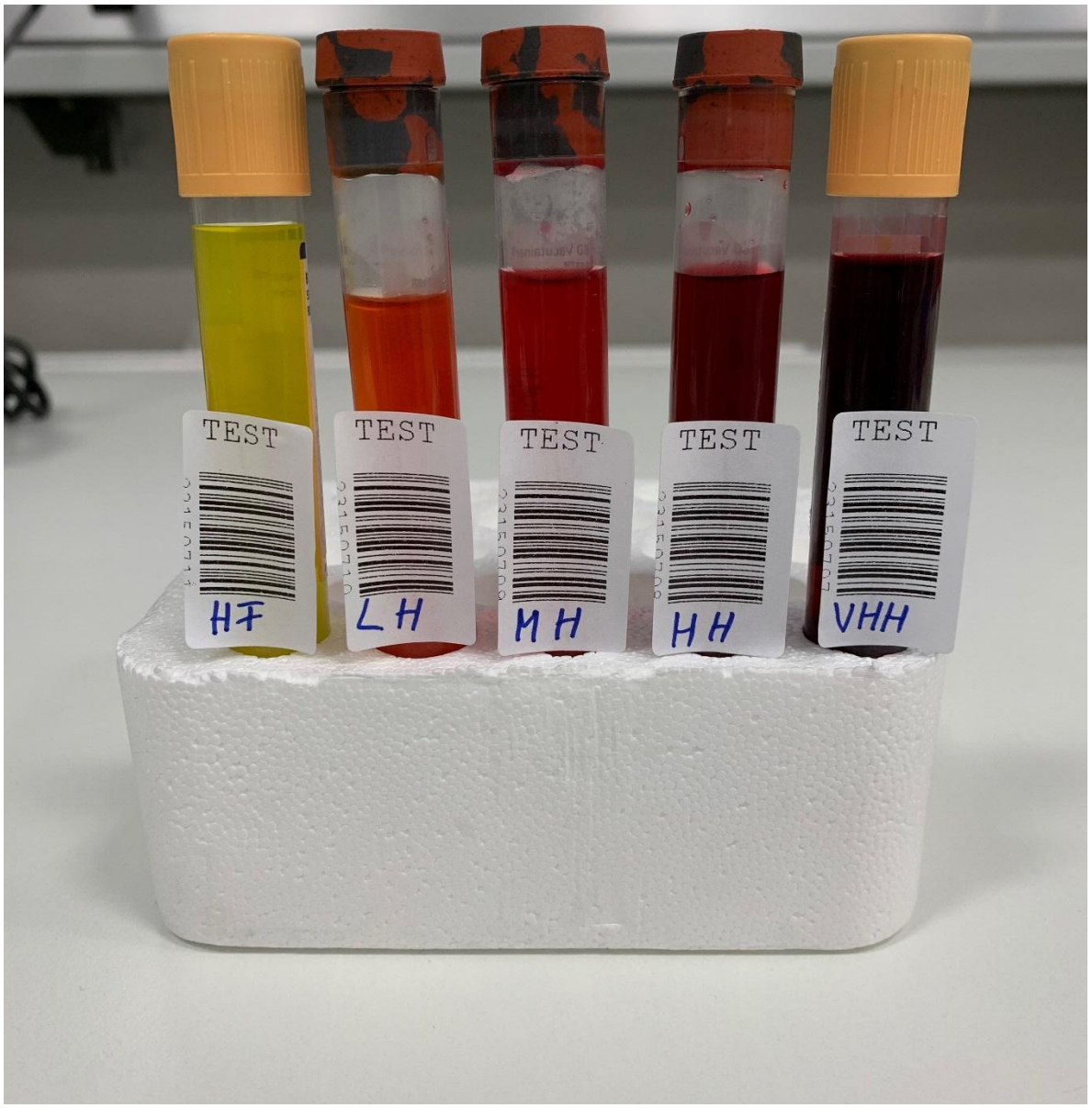

Figure 24. Labelled sample tubes with hemolysis levels, from left to right: HF, LH, MH, HH and VHH

The rest of the sample tubes were mixed and then filled with the solutions from the containers. For each container a dedicated pipette was used (to prevent any sort of influence on the color status). The samples tubes were temporarily placed on racks, unsorted. Figure 25 shows such a rack, with mixed or unsorted sample tubes after they were filled.

Figure 25. Sample tubes placed temporarily on a working rack

After all the samples tubes were filled, and placed temporarily on working racks, they were then sorted and organized in final racks; one rack for each hemolysis level. This process is depicted in the images starting with Figure 26 through Figure 34.

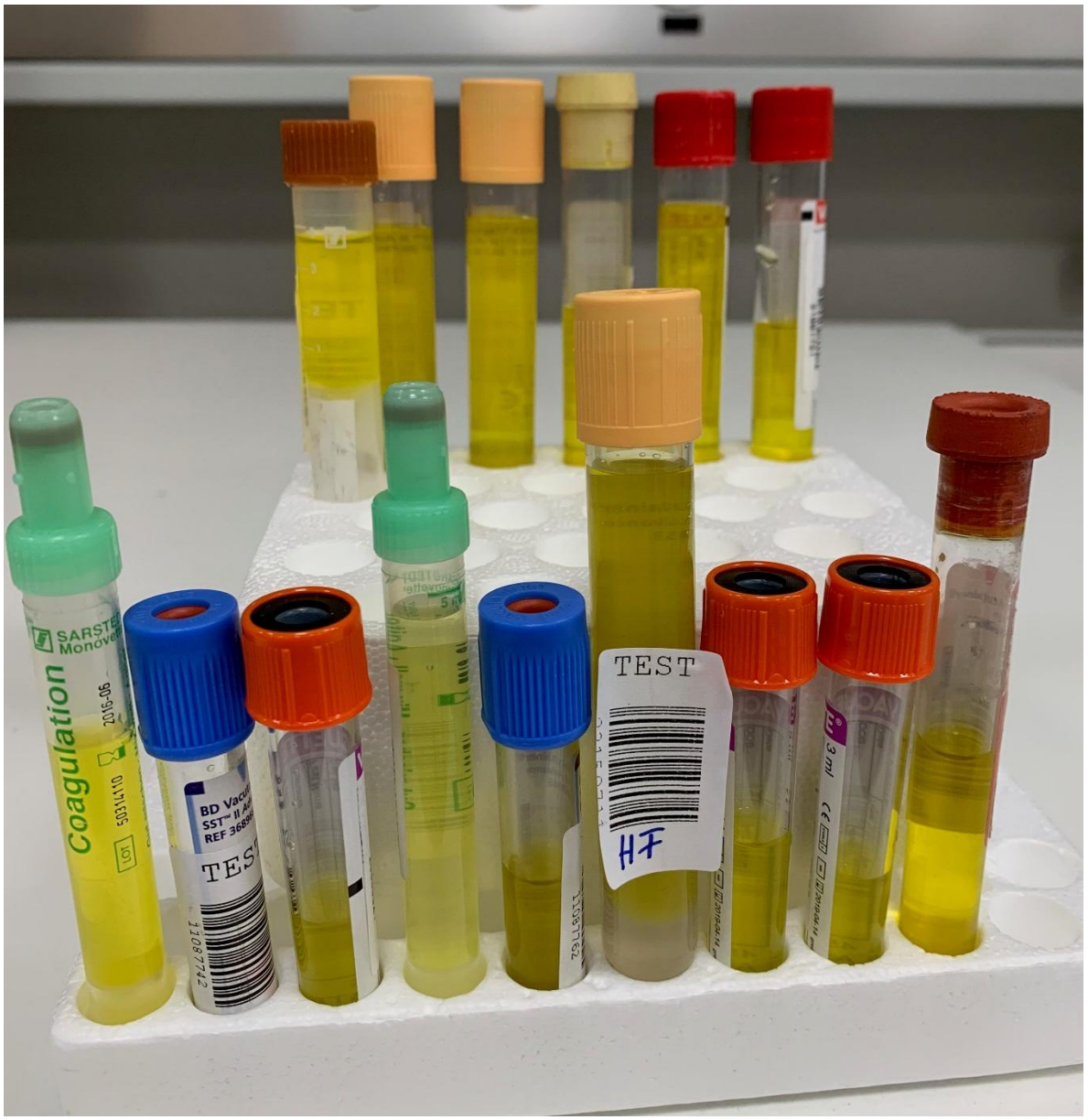

Figure 26. HF samples temporarily sorted in a working rack

Figure 27. HF samples organized and labelled in a final rack

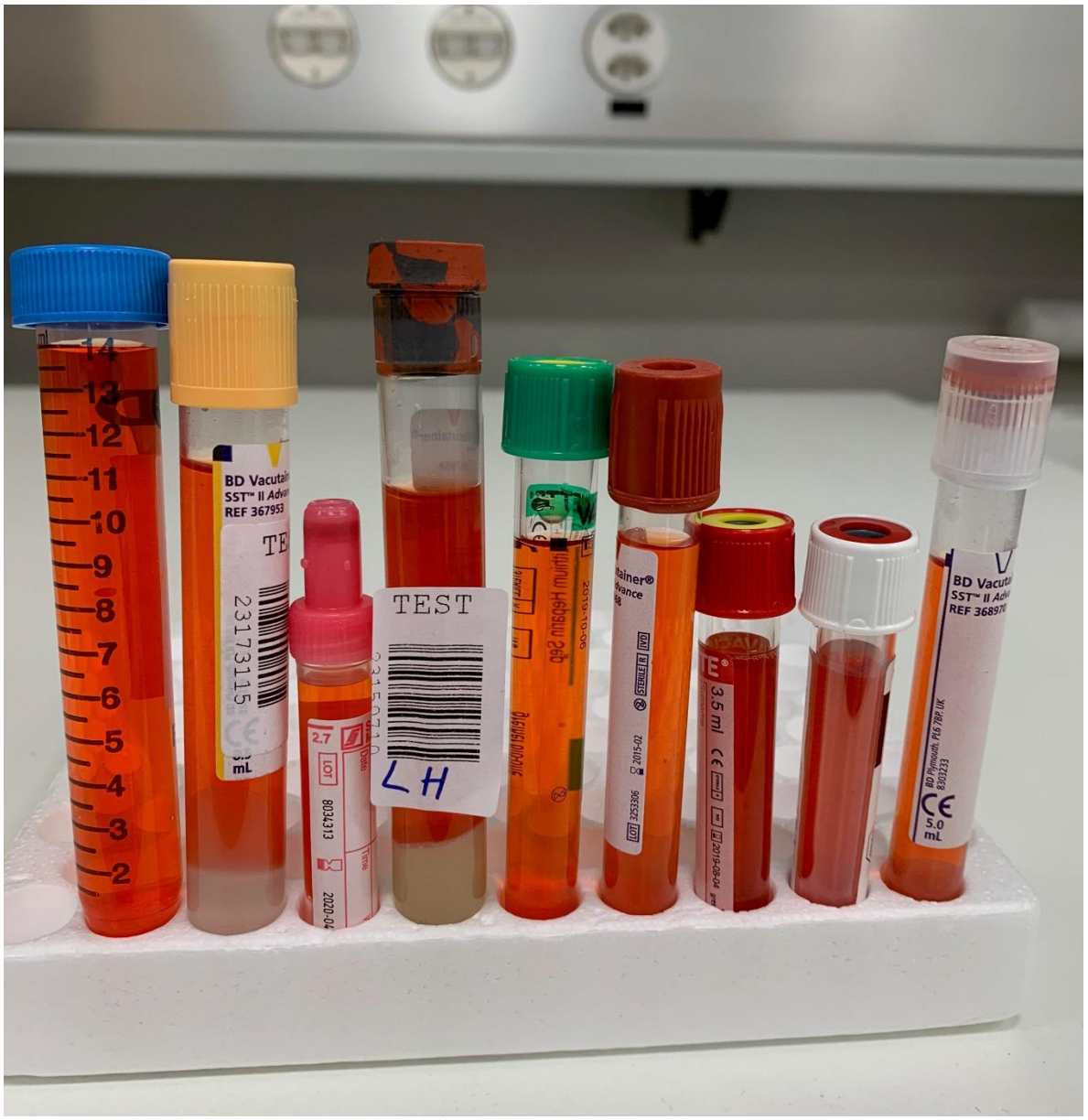

Figure 28. LH samples temporarily sorted in a working rack

Figure 29. LH samples organized and labelled in a final rack

Figure 30. MH samples organized and labelled in a final rack

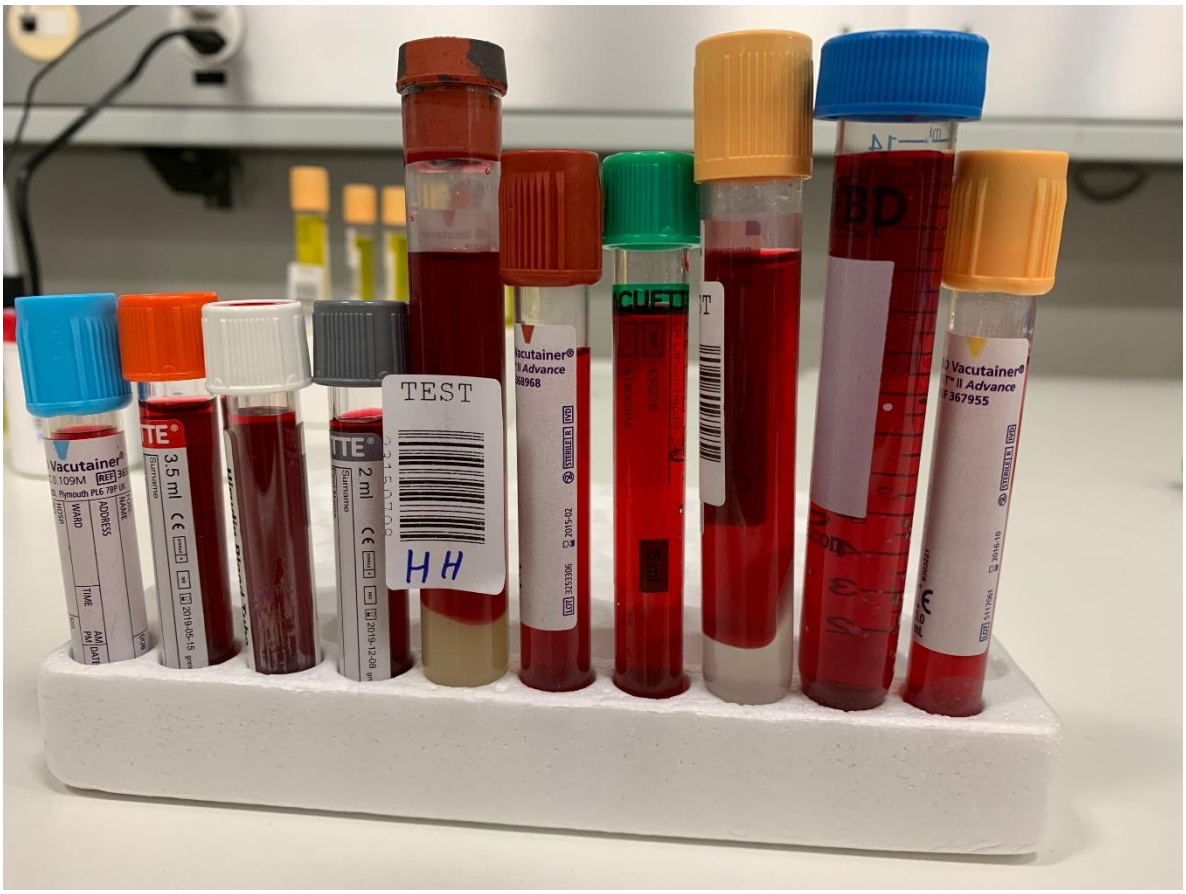

Figure 31. HH samples temporarily sorted in a working rack

Figure 32. HH samples organized and labelled in a final rack

Figure 33. VHH samples temporarily sorted in a working rack

Figure 34. VHH samples organized and labelled in a final rack

6.2.4. Measurement of variables

The overall aggregated sum of sample tubes tested is 70 (count of all the sample tubes grouped in the final racks, by their respective hemolysis level: HF, LH, MH, HH, and VHH).

The sample tubes were then photographed one-by-one using the smartphone app. The sample tube was hold with one hand while the other hand was used to operate the

smartphone and take the picture of the upper part of the sample tube as closest as possible (in real blood samples, the hemolysis caused by the rupture of red blood cells, RBCs, will be present in the upper part of the sample tube). Figure 35 shows how a sample tube is being photographed using the “HemoDetect” app. It is important that the upper part of the tube is as close as possible to the smartphone rear camera (or the camera zoom could be used as needed).

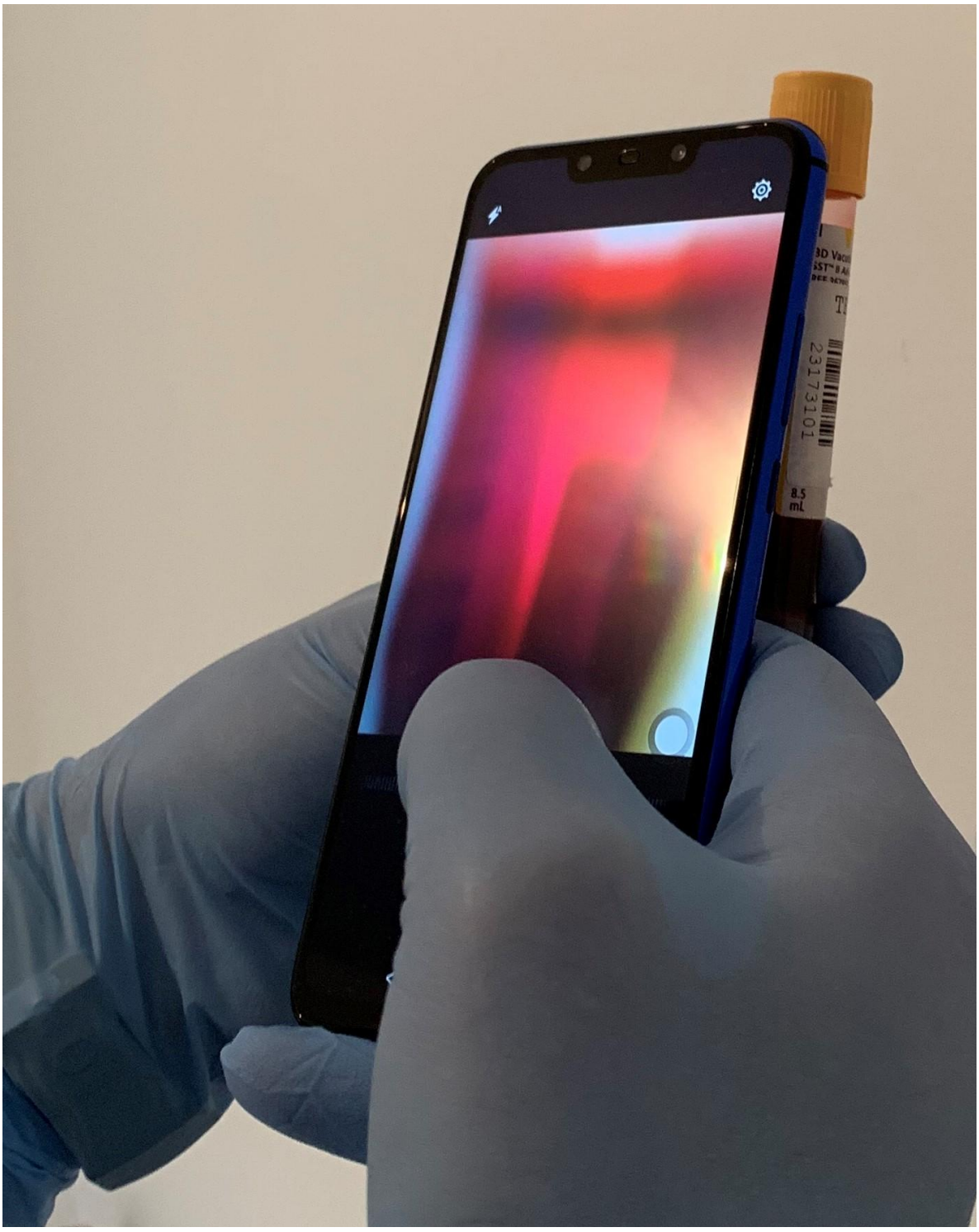

Figure 35. Sample tube being photographed with the “HemoDetect” app

Once the photograph is taken and confirmed by simply tapping a checkmark button, the “HemoDetect” app displays the hemolysis level result on the screen. Figure 36 shows the

hemolysis level detected by the "HemoDetect" app; in this particular case, a VHH (very high hemolysis) sample was tested and the result is displayed in the "HemoDetect" app's screen.

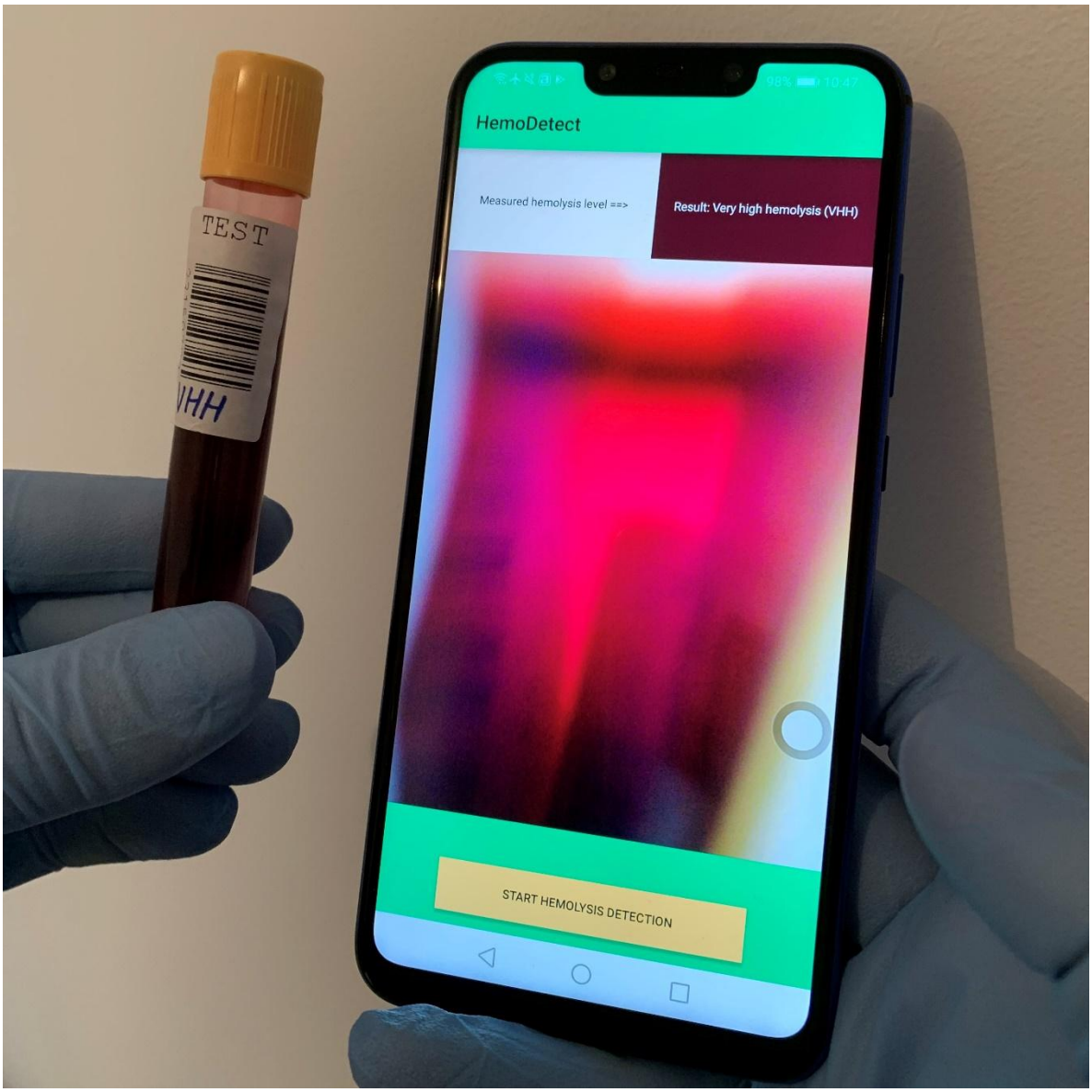

Figure 36. To the left: a VHH sample tube which was just photographed; to the right: the result displayed by the "HemoDetect" app on its screen

The corresponding hemolysis result, which was displayed on the smartphone screen, was respectively recorded in the database. After all the 70 sample tubes were photographed, and their result recorded in the database, the test cycle was closed and a new one was started. A

test cycle means that all 70 sample tubes were tested and the results were stored in the database. The number of test cycles completed during the experiment was 4; with the exception of HH sample tubes where 6 test cycles were performed (due to additional test cycles needed for calibration purposes and further checks). Figure 37 illustrates how the results were recorded for each sample tube tested during one of the early test cycles.

REF	Tube manufacturer	Dimensions (mm)	Cap type	Cap color	Filling volume (mL)	Defined HL	Measured HL	Test result	CI	Time (s)
367953	Becton Dickinson	16x100	hemogard	yellow	8.5	HF	HF		18.59	13.39
455071	Greiner Bio-One	16x100	hemogard	red cap - yellow ring	8	HF	HF		24.53	13.45
5.1071	Sarstedt	11x92	screw cap	light green	5	HF	HF		19.56	10.25
364938	Becton Dickinson	16x100	rubber	yellow	10	HF	HF		22.71	11.1
367953	Becton Dickinson	16x100	hemogard	yellow	8.5	HF	HF		14.86	10.15
367953	Becton Dickinson	16x100	hemogard	yellow	8.5	HF	HF		18.96	12
368966	Becton Dickinson	13x75	hemogard	blue	3.5	HF	HF		17.12	12.21
368966	Becton Dickinson	13x75	hemogard	blue	3.5	HF	HF		18.16	11
368660	Becton Dickinson	13x100	rubber	red	6	HF	HF		19.32	9.81
455071	Greiner Bio-One	16x100	hemogard	red cap - yellow ring	8	HF	HF		16.20	9.82
454025	Greiner Bio-One	13x75	hemogard	orange cap - black ri	3	HF	HF		10.46	11.03
454025	Greiner Bio-One	13x75	hemogard	orange cap - black ri	3	HF	HF		13.18	14.14
454025	Greiner Bio-One	13x75	hemogard	orange cap - black ri	3	HF	HF		21.51	9.93
5.1071	Sarstedt	11x92	screw cap	light green	5	HF	HF		41.34	15.69
367988	Becton Dickinson	16x100	rubber	red/grey	8.5	LH	LH		71.29	7.53
454071	Greiner Bio-One	13x75	hemogard	red cap - yellow ring	3.5	LH	MH		75.16	11.12
05.1557.100	Sarstedt	11x66	screw cap	pink	2.7	LH	LH		64.69	10.16
368968	Becton Dickinson	13x100	hemogard	brown	5	LH	LH		59.34	10.61
62.554.502	Sarstedt	17x120	dome-seal screw cap	red	15	LH	LH		63.78	10.49
08128812 001	Roche	13x75	hemogard	white cap - red ring	3	LH	LH		54.73	11.42
456216	Greiner Bio-One	13x100	hemogard	green cap - yellow ri	5	LH	HF		38.07	14.89
367953	Becton Dickinson	16x100	hemogard	yellow	8.5	LH	LH		59.62	9.48
368970	Becton Dickinson	13x100	hemogard	translucent white	5	LH	LH		59.36	9.48
352095	Becton Dickinson	17x120	dome-seal screw cap	blue	15	LH	LH		63.09	9.12
456093	Greiner Bio-One	13x100	hemogard	pink cap - black ring	6	MH	MH		83.29	12.5
455036	Greiner Bio-One	16x100	hemogard	lavender cap - black	9	MH	LH		68.72	9.51
5.1071	Sarstedt	11x92	screw cap	light green	5	MH	MH		75.46	9.78
456038	Greiner Bio-One	13x100	hemogard	lavender cap - black	6	MH	MH		78.69	11.68
04.1931.100	Sarstedt	13x90	screw cap	light violet	4.9	MH	MH		76.48	10.61
455092	Greiner Bio-One	16x100	hemogard	red cap - black ring	9	MH	MH		81.79	10.04
367953	Becton Dickinson	16x100	hemogard	yellow	8.5	MH	LH		70.35	9.95
454071	Greiner Bio-One	13x75	hemogard	red cap - yellow ring	3.5	MH	MH		79.11	11.28
352095	Becton Dickinson	17x120	dome-seal screw cap	blue	15	MH	LH		71.79	11.25
367812	Becton Dickinson	13x75	hemogard	red	4	MH	MH		77.52	13.85
367988	Becton Dickinson	16x100	rubber	red/grey	8.5	MH	LH		73.00	9.48
62.554.502	Sarstedt	17x120	dome-seal screw cap	red	15	MH	MH		75.84	10.77
367988	Becton Dickinson	16x100	rubber	red/grey	8.5	MH	MH		75.78	10.67

Figure 37. Database extract showing part of the results recorded for one test cycle

During the test cycles, all the test tubes from different manufacturers were tested. Figure 38 illustrates the test tubes grouped by manufacturer represented in color, and with their measured color index, hemolysis level and timings in seconds.

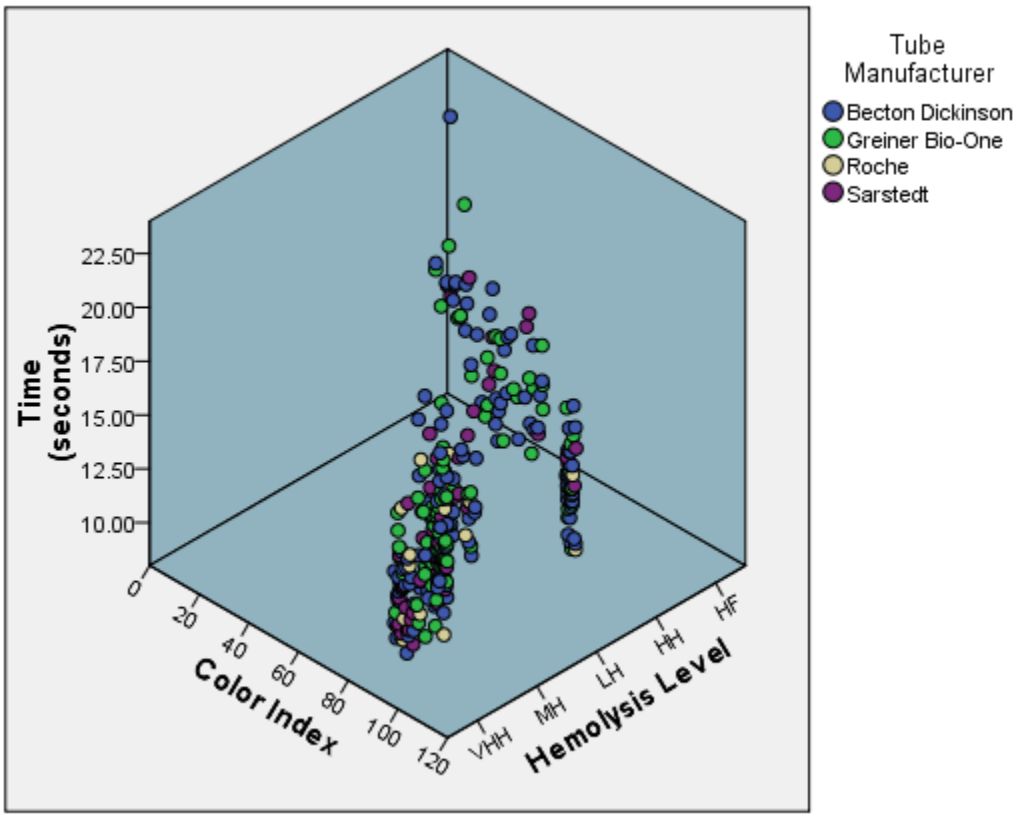

Figure 38. 3-D scatterplot showing the test tubes by manufacturer, with their measured color index, hemolysis level and timings

6.2.5. Real blood sample tubes

Real blood sample tubes were also prepared. The real blood sample tubes with various hemolysis levels were collected from various commercial diagnostics laboratories or partners from Switzerland and stored in the local laboratory. However, due to the coronavirus situation, it was not possible to test them in the laboratory with the "HemoDetect" app, due to restrictions to the office work and laboratory spaces. Additionally, the "work from home" mode was privileged since MAR-2020 (when the coronavirus pandemic started in Switzerland). The level of confidence related to the accuracy of the results obtained on the artificial samples would be equally comparable with the level of confidence derived from testing the real blood samples. However, for

demonstration purposes, it is foreseeable that in future research work, and depending on the future coronavirus situation, testing on real sample tubes will definitively be in scope.

6.3 Experimental results and discussion

6.3.1. Hemolysis free samples

The artificial hemolysis free (HF) samples, with ≤5 mg/dL concentration of free hemoglobin were tested in 4 test cycles. The findings indicate a precision percentage of 99.90 – 100% in various lighting conditions. The average time recorded between picking up the sample and obtaining the result was of approximatively 11.47 seconds. This time would add up to the gravitational sedimentation time required for a real blood sample. This is applicable to all samples with different hemolysis levels. The sedimentation time needed for real blood samples would vary between 10 – 60 minutes (depending on the volume of the sample tube). Since the samples used in the experiment are artificially created, there is no need for gravitational sedimentation since they reflect directly the blood plasma.

Figure 39 shows a HF sample just after being photographed.

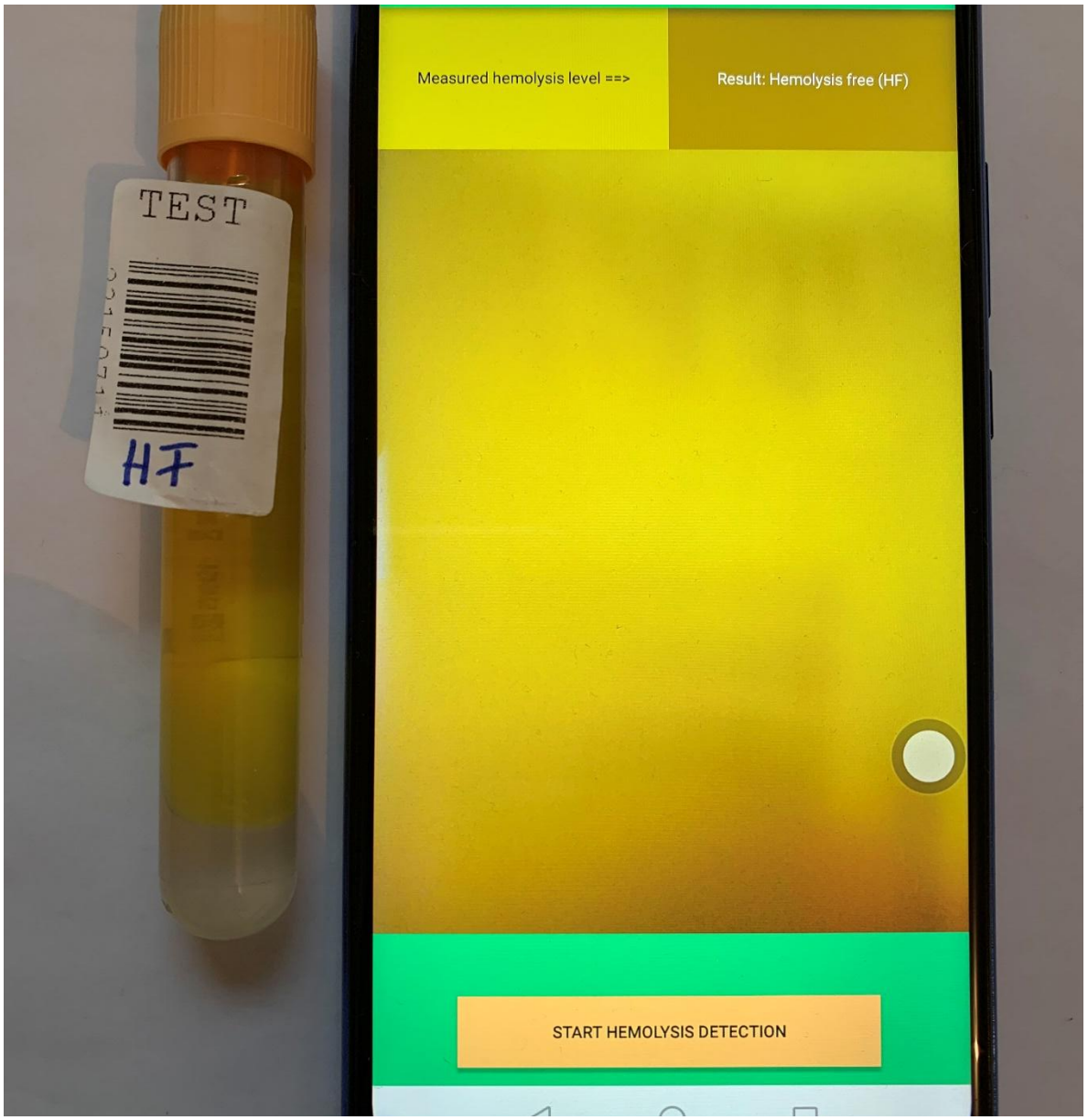

Figure 39. Hemolysis free sample – result

6.3.2. Low hemolysis samples

The artificial low hemolysis (LH) samples, with 5 – 30 mg/dL concentration of free hemoglobin were tested in 4 test cycles. The findings indicate a precision percentage of 98.90 – 100% in various lighting conditions. The average time recorded between picking up the sample and obtaining the result was of approximatively 10.43 seconds.

Figure 40 shows a LH sample just after being photographed.

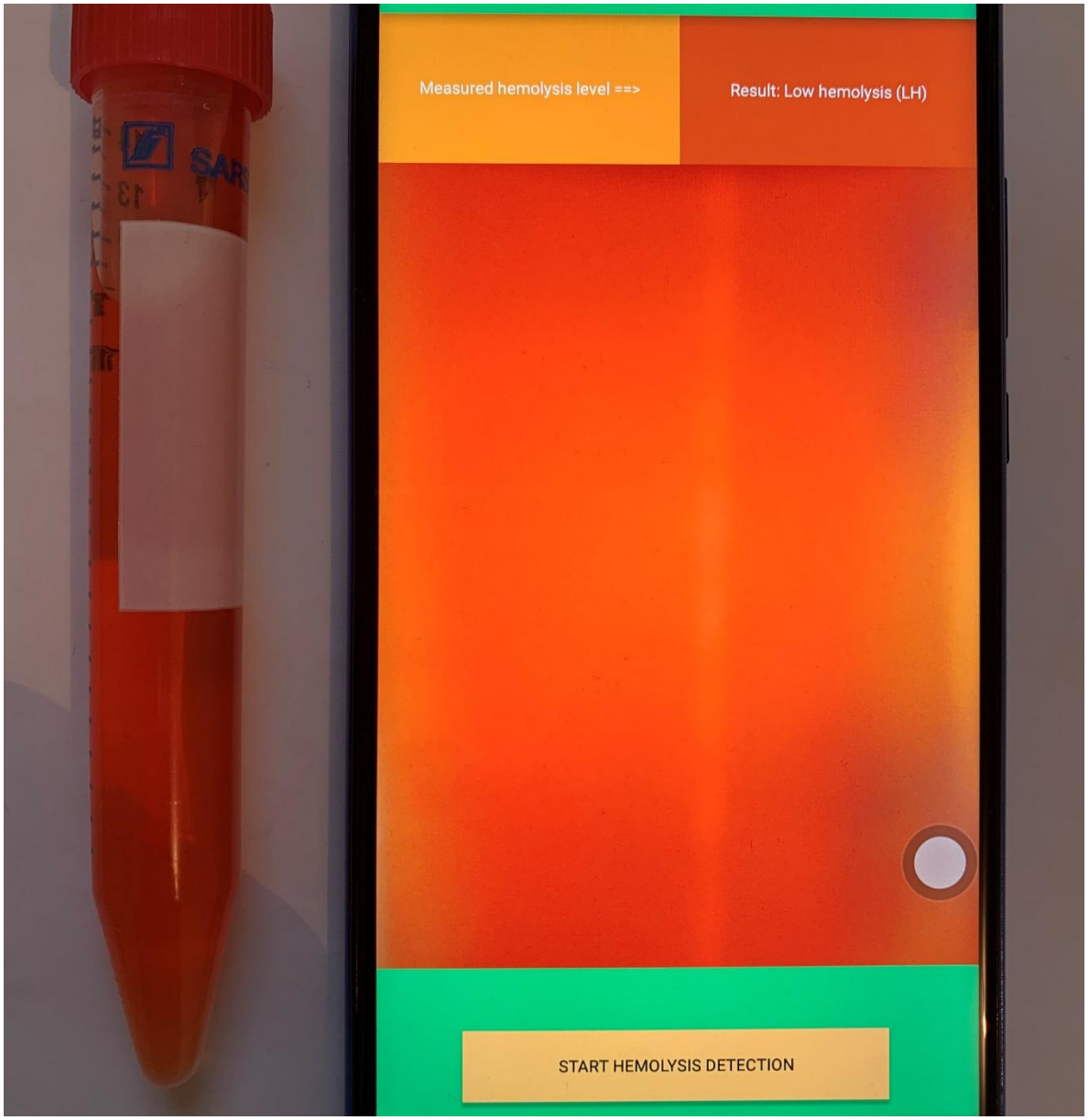

Figure 40. Low hemolysis sample – result

6.3.3. Medium hemolysis samples

The artificial medium hemolysis (MH) samples, with 30 – 60 mg/dL concentration of free hemoglobin were tested in 4 test cycles. The findings indicate a precision percentage of 97.90 – 100% in various lighting conditions. The average time recorded between picking up the sample and obtaining the result was of approximatively 10.97 seconds.

Figure 41 shows a MH sample just after being photographed.

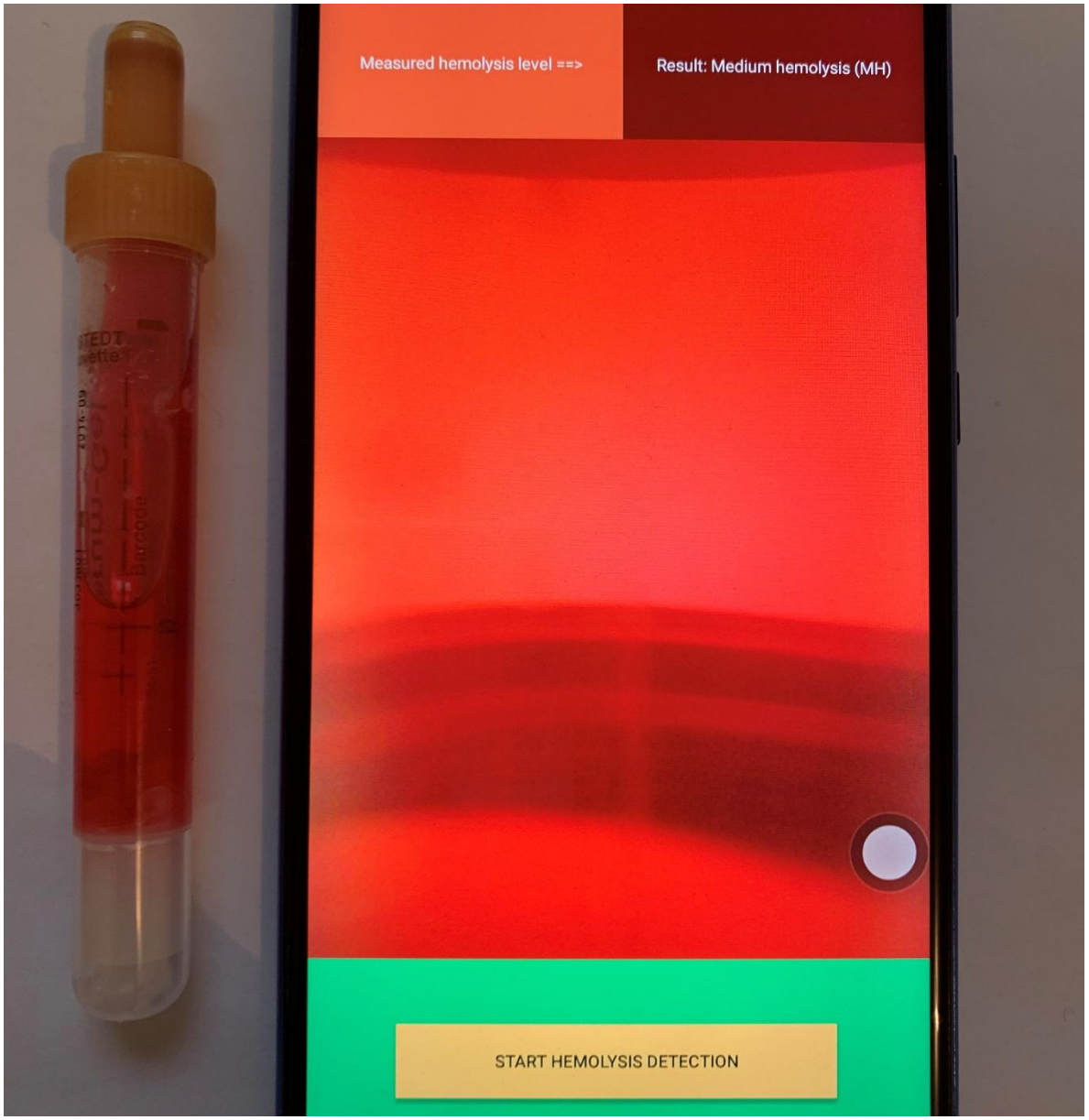

Figure 41. Medium hemolysis sample – result

6.3.4. High hemolysis samples

The artificial high hemolysis (HH) samples, with 60 – 300 mg/dL concentration of free hemoglobin were tested in 6 test cycles. The findings indicate a precision percentage of 97.90 – 100% in various lighting conditions. The average time recorded between picking up the sample and obtaining the result was of approximatively 10.97 seconds.

Figure 42 shows a HH sample just after being photographed.

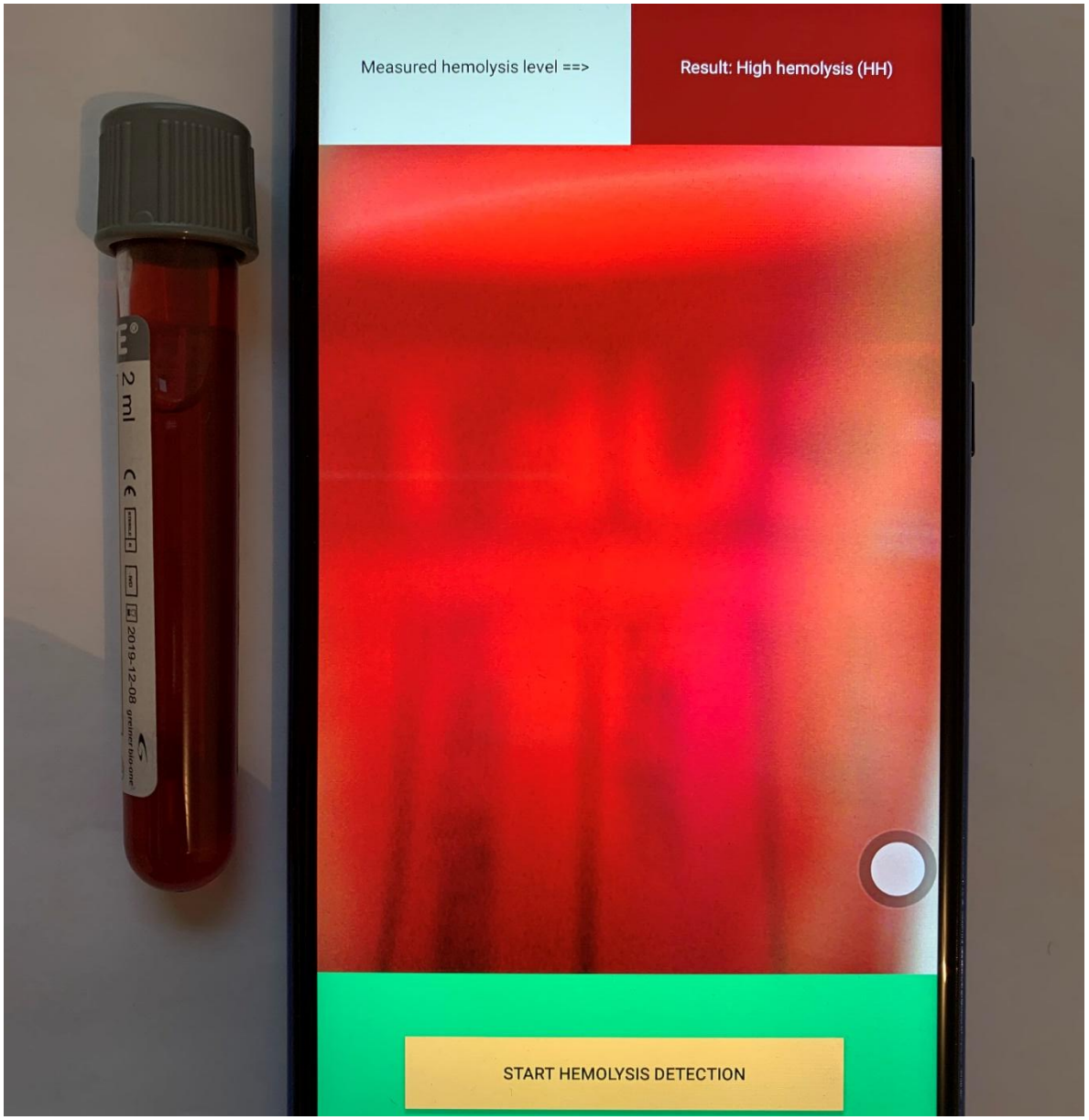

Figure 42. High hemolysis sample – result

6.3.5. Very high hemolysis samples

The artificial very high hemolysis (VHH) samples, with ≥ 300 mg/dL concentration of free hemoglobin were tested in numerous test cycles. The findings indicate a precision percentage of 97.90 – 100% in various lighting conditions. The average time recorded

between picking up the sample and obtaining the result is of approximatively 10.97 seconds.

Figure 43 shows a VHH sample just after being photographed.

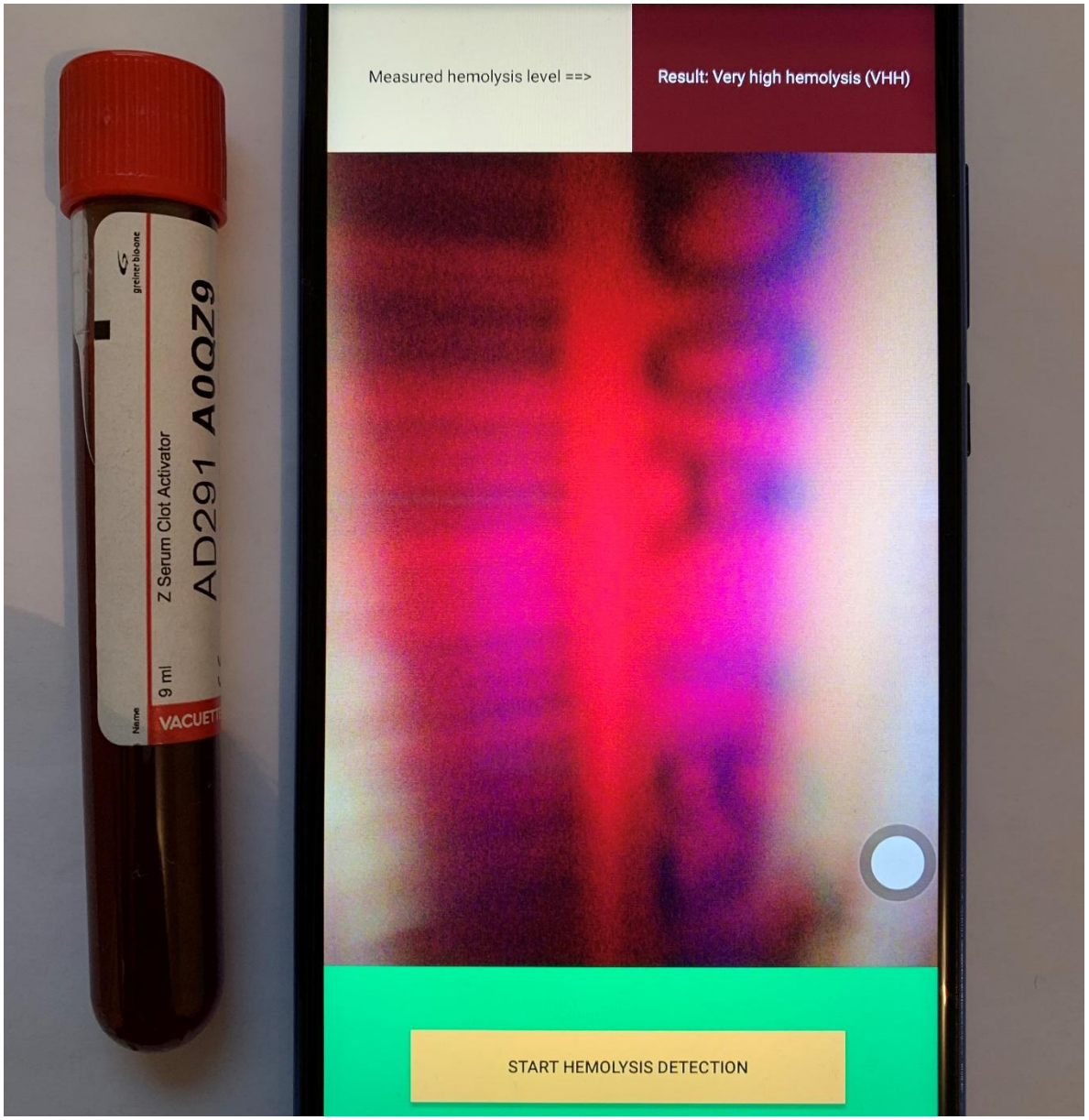

Figure 43. Very high hemolysis sample – result

6.3.6. Assessing the prototype app assistance quality

A useful implementation for the end-user is the *toast message*. A toast message provides a simple feedback about an operation in a small popup. It only fills the amount of space

required for the message and the current activity remains visible and interactive. Toasts are not clickable and they automatically disappear after a determined timeout. Another useful implementation of toast messages in the app code is in case of errors or malfunctions (e.g., "Failed to open camera" or "Failed to create image file"). Figure 44 illustrates two examples of toast messages, one briefly instructing the user to press the button to open the camera and another one is in fact the confirmation of the successful image processing.

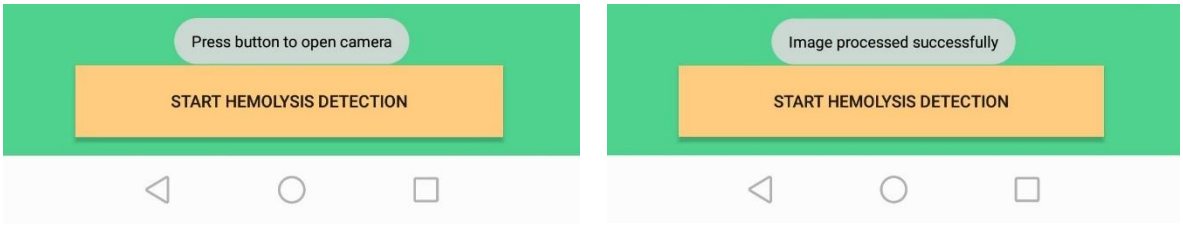

Figure 44. Examples of "HemoDetect" app toast messages

6.3.7. Assessing user interaction and usefulness

The app's interface is simple to use, and the app controls can be operated easily while wearing medical gloves. The buttons are large enough and they react quickly at the user touch event. The camera zoom can be operated by using two fingers (pinch-to-zoom in or out). Smartphone users are very familiar with these common touch gestures. The steps or touch events required to photograph a sample tube are just three or four, as explained in the list below.

1. Open "HemoDetect" app
2. Press "START HEMOLYSIS DETECTION" button
3. Zoom in or out (as needed) – optional step
4. Confirm image by clicking the checkmark button

After these simple steps, the result screen is displayed to the user. The user interaction is straightforward and the result is displayed very quickly reaching the ultimate objective in a fairly short span of time.

Another visual element which was implemented and may be helpful to the user was the color of the two upper sections where the hemolysis result is displayed as depicted in Figure 43 (left box the highest pixels brightness or intensity, as observed in the background, and the right box represents the color of the hemolysis which is measured).

6.4 Further discussion and design implications

6.4.1. Production app version

Undoubtedly, for a production version of the app, a highly regulated product development process must be followed, to ensure patient safety and reliability of results according to the standards set by the medical regulatory official bodies. The prototype app developed during this research work and while writing this thesis, does not make this claim (it is just an experimental app, therefore called a "prototype" app) and it is not to be used in any real-life scenario or in a production setting under no circumstances.

Considering security (e.g., user identification, password management) or connectivity (e.g., transmitting images to a server, or send results by email) in a production version of the app will certainly introduce new elements of interaction to the user, but even with additional elements the app will still remain easy to use.

"HemoDetect" is a native prototype app developed for Android powered devices. In terms of user interface design and release on other non-Android powered devices, the "HemoDetect" app will need to be re-worked and tested accordingly. Technologies, such as HTML5 and JavaScript, enable the developer to have just one code base and deploy the app to many different types of mobile operating systems because it would effectively be an app executed in the context of a web browser. This represents a cost-effective alternative to developing native individual apps for each mobile operating system.

6.5 Summary

In this chapter, the experiment performed in the laboratory was presented. The material preparation steps were described in detail. The measuring process was also presented step-

by-step and the user interaction with the prototype app was described. The laboratory study was immensely useful to get insights about the material preparation (solutions, containers, preparation of artificial samples, collection and storage of real blood samples) and the actual measurements of all the test samples (except the real blood samples due to coronavirus situation) in repeated test cycles. The "HemoDetect" app performed very well, returning results very fast, and the timings were recorded in the database. Finally, while executing the experiment, new elements were identified, especially related to which specific enhancements or aspects the "HemoDetect" app will need to have in order to evolve from the prototype app status to a full production one. The results obtained in the experiment will be discussed and compared to other results from other studies in the next chapter.

CHAPTER 7 – RESULTS, DISCUSSION AND FUTURE RESEARCH

7.1 Overview

7.1.1. Results and discussion

For whole blood sample tubes, shortly after collection, a time period is required for the gravitational sedimentation of the RBCs. This time period is determined by the erythrocyte sedimentation rate (ESR). ESR test measures the rate at which the RBCs, or erythrocytes, in a sample of whole blood settle at the bottom of the sample tube. This process of settling is called sedimentation. The Westergren method is widely used in measuring the sedimentation of RBCs in citrated, diluted blood after 1 hour in an open- ended glass tube of 30 cm length mounted vertically on a stand, with results reported in mm (Jou, 2012). The normal reference range for ESR results is 1 – 15 mm/hr for males and 1 – 20 mm/hr for females.

For example, the unique tube list, which was defined for the experiment, consists of tubes widely used in point-of-care practice with filling volumes ranging between 2 – 15 mL.

The real blood samples tubes will require between 10 – 60 minutes of sedimentation time in order to have at least 2 mm of plasma layer on top. This layer will then be photographed using the "HemoDetect" app. Based on the need to capture the interest area, the camera zoom is recommended to be used. However, as stated above, the real blood samples were collected and stored in the laboratory but could not be tested with the "HemoDetect" prototype app due to the coronavirus restrictions in the work offices and laboratory spaces.

For artificially prepared sample tubes, there is no need for gravitational sedimentation time, as the solutions prepared reflect the corresponding free hemoglobin levels (in mg/dL) in the blood plasma. Therefore, these samples can be photographed immediately.

7.1.2. Results compared to traditional methods

The typical TAT for hemolysis detection in a traditional automated laboratory is approximatively 4 hours or more. With the proposed solution, the TAT is reduced to maximum 1.10 hours.

The accuracy of the method for both real and artificial samples is ~1 mg/dL.

Automated pre-analytical systems (e.g. cobas p 612 from Roche) return a similar level of accuracy for low hemolysis (corresponding to LH from the experiment) and hemolytic samples (corresponding to MH, HH and VHH from the experiment). One key observation that was mentioned previously in this thesis is, the fact that traditional pre-analytical systems which detect hemolysis in the laboratory, are precise on a limited number of hemolysis levels (2, or 3) and lack more granularity, for example, when trying to distinguish between HH and VHH samples.

7.2 New research achievements

7.2.1. How and why use medical apps?

Domestic mobile device users are very familiar with health apps that measure their vital signs (e.g., heart rate or a representation of an electrocardiogram) which are running typically on their wearable devices (e.g. smartwatch). It is observed that their usefulness is not questionable, however, when seeking or needing professional medical help, the "connected" user becomes a "mere" patient. A patient that would need professional and reliable results for a correct diagnosis. Although the domestic wearable data is useful, it is not advanced enough to match the specific need or contribute in a robust manner at identifying a medical complex condition.

Healthcare professionals must always be certain that they use medical apps which are subjected to medical regulations in place. Without this certification, the patient results are at risk. Also, it is important that contracts of support and service are in place with certified vendors to ensure the continuity and optimal functioning of the medical apps, following the same procedures as applicable to the traditional pre-analytical systems.

Medical apps provide, in most cases of diagnosis, a cost-effective, highly portable, small footprint solution, especially for point-of-care settings. The ability of a medical app to provide meaningful, accurate and fast results will drive up the adoption rate among HCPs.

Finally, the usage of mobile apps in a professional medical environment will not only provide the expected benefits as abovementioned, but open up a new fresh window of learning opportunities which will certainly be useful in continuous research and development of new healthcare solutions and applications.

7.2.2. How to enhance user interfaces and user interactions?

As observed so far, medical apps used in a professional medical environment need certain specifications or considerations. For example, medical personnel, when working with samples or when performing tasks like blood collection, will always use medical gloves (which represents an extra layer of material between the human finger which operates a touchscreen and the touchscreen itself). In certain cases, medical goggles or special equipment is necessary which might have an impact on how the colors of an image are rendered for example in special lightning conditions. In other cases, the medical facility requires special illumination or a certain room temperature.

When developing apps for medical purposes, all these elements must be taken into consideration (it is not the same as while developing mobile apps for domestic usage).

Finally, testing and retesting the medical apps in real or artificial induced conditions or specimens will lead to findings which will help addressing all the aspects for a high quality product. For example, during the experiment in the laboratory, medical gloves were used, to test the responsiveness of the touchscreen while using the “HemoDetect” prototype app.

7.2.3. Planning and implementation of the experimental environment

Running experiments in laboratories might prove, in most of the cases, dangerous and prone to risks due to contamination, risk of infection when it comes to working with real blood sample tubes. That is why, during the experiment, attention to detail was effectively

employed while patiently performing each task. Although real blood samples were not tested, special attention was given to collect and store them in a cold unit. Creating artificial samples which imitate the real hemolysis level was particularly useful, cost-effective and helped eliminate risks posed by real blood sample tubes. Preliminary calibration tests, followed by repeated test cycles were utterly useful to prepare the experiment.

7.2.4. Empirical study

The study started with observing the real-life hemolysis detection in various setups, planning the experiment, carefully considering building the necessary framework and individual materials and always leaving room for uncertainty or unforeseen changes. Then, the study continued with a direct analysis of each sample from the batch of samples categorized by their hemolysis level. Each sample tube was photographed multiple times while executing the test cycles. Tested through experimentation, direct observation and behavior of the "HemoDetect" app, the hemolysis result, color index and the timing taken to photograph the sample were carefully recorded in the database. The data gathered represents the fundamental evidence and knowledge based on which research conclusions were drawn.

7.2.5. Practical results

The results are based upon image data and not upon analytical results. That means that the sample tubes were not decapped and pipetted to obtain a small biological sample of the blood plasma layer for a clinical chemistry study or to be analyzed under the microscope.

"HemoDetect" prototype app is simply yielding results only based on image analysis and not on biological analysis.

The results recorded by the "HemoDetect" prototype app have an accuracy of 97.90% or ~1 mg/dL for artificial samples only. Based on measurements made on artificial samples, the assumption is that the TAT for a real fresh whole blood sample is of maximum 1.10 hours (from the moment of blood collection until the result displayed by the app). However, this

would need to be proven in future research work and by performing testing on real blood samples.

7.3 Research contributions

7.3.1. Results and characteristics compared to other results from other research works

The research contributions and the solution proposed in this thesis must be scrutinized and compared to other relevant research works with the purpose of providing insights into what is the additional value proposed by the research contributions in this present thesis, and their differentiating factors, but to also emphasize the limitations or gaps, for scientific clarity, which may be tackled as future research work topics.

Table 7 shows the comparison of main characteristics and results between the research work described in the present thesis and two other relevant research works.

Characteristic	**This thesis and research work – HemoDetect app**	**A Mobile Phone–Based Approach to Detection of Hemolysis (Archibong et al., 2017) – Hemolix app**	**Point-of-care testing of plasma free hemoglobin and hematocrit for mechanical circulatory support (Shin et al., 2021) – *hemo*CAM system**
Hardware	Smartphone (with camera and flash)	Smartphone (with camera and flash) attached to a sample holder (custom-built)	Point-of-care device, custom-built (minimized centrifuge system, camera module, channel cartridge holder, touchscreen, power source)
Hardware weight	Smartphone weight (~160 g)	Smartphone weight (~135 g) and holder weight (not specified)	1100 g
Additional light source (excluding camera flash)	No	No	Yes

Software/image processing algorithm	Custom-built	Custom-built	Custom-built
IDE	Android	Android	Qt Creator
Programming language	Java	Java	Python
Code base accessibility	Yes (Appendix **2**)	Not published	Not published
Science	Photometry	Photometry	Colorimetry
Color spaces or models	RGB, CIE XYZ, CIELAB	RGB, CIELAB; (CIE XYZ not specified)	RGB, CIELAB; (CIE XYZ not specified)
Illuminant	D65	Not specified	Not specified
Calibration curve (built-in)	Yes	Yes	Yes
Samples/specimens	Conventional sample tubes (different types and manufacturers)	Capillary tubes/micro tubes	Cartridges (custom-built)
Sample/specimen material	Solutions prepared with different free hemoglobin concentrations	Solutions prepared based on purchased plasma and hemoglobin	Animal blood (swine)
Required additional steps for real-life blood sample measurement (e.g. blood pipetting/dropping)	No. Blood sample tubes (directly in which the blood is drawn into).	No. Blood capillary tubes/microtubes (directly in which the blood is drawn into).	Yes. Blood dropped in a cartridge.
Centrifugation of samples/specimens	No	No	Yes
Chemical reagents, compounds or reactions	No	No	No
Reported hemolysis levels	5 (including hemolysis free)	5 (including hemolysis free)	1 (mild hemolysis only)
Mobility and	Yes	Yes	No

connectivity			
Accuracy of measurements	~1 mg/dL	~1 mg/dL	~1.4 mg/dL
Intended use	Point-of-care	Point-of-care	Point-of-care
mHealth (mobile health)	Yes	Yes	No
TAT (Turnaround Time)	Up to 70 minutes depending on the sample tube filling volume (including gravitational sedimentation); for example, 2 ml - up to ~9.3 min; 15 ml - up to ~70 min	Up to 10 minutes for capillary tubes and up to 30 minutes for microtubes (including gravitational sedimentation)	5 minutes (including centrifugation)
Cost	Cost of the smartphone (excluding the software app): ~199 USD	Cost of the smartphone + custom holder (excluding the software app): ~195 USD	Cost of the device not specified

Table 7. Results compared to other results from other research works

7.3.2. Similarities and differences of image analysis algorithm proposed in this thesis compared with other similar algorithms from other research works

During research review and comparison of characteristics and results with other research works, it was observed that, typically, the image processing algorithm is custom-developed by researchers and its code implementation depends very much on the programming language and development environment which is chosen. Therefore, an exhaustive comparison between lines of code might not be precise enough or in some cases might be irrelevant. Also, this comparison might be simply not possible due to the fact that many research works did not publish their code base on a public code repository. For example, the two research works mentioned in Table 7 do not publish their custom algorithm software algorithm code.

However, it is important to mention the similarities and differences in the principles and steps applied in the reviewed research works which employ image processing algorithms. Probably the most important similarity is the fact that the color spaces and color models are being used in the algorithms. The starting point is extracting the RGB color values for each pixel. Then, another similarity is the use of CIELAB color space and a calibration curve. Then a color index is calculated which is mapped to the calibration curve or reference values to obtain the corresponding hemolysis levels based on the concentration of free hemoglobin in blood plasma.

The difference between the image processing algorithm proposed in this thesis and the rest of the proposed approaches, is the fact that it uses the illuminant D65 (subchapter 4.4.1) and treats the luminance or brightness factor without the need of having an extra light source or obscure chamber to keep light conditions constant. The objective and original research idea was to use the standalone smartphone acting as the measurement device in the experiment. Any additional hardware components or extensions were excluded from the start for absolute mobility and avoidance of any additional hardware nuisance and cost. The surrounding light is taken into consideration in the image processing and calculated in the code (Lm) together with the other two measured values (am, and respectively, bm) which are then used to calculate the color index (CI) taking into consideration the predefined Lr, ar and br (which represent the determined, carefully calibrated reference CIELAB values of blood plasma).

7.3.3. Specific research contributions of this thesis; limitations to be considered in future research work

"HemoDetect" prototype app was built to run on an Android smartphone (standalone) equipped with a camera. No additional hardware devices were designed or intended to be built in order to put the focus only the modern smartphone device as the standalone and exclusive measurement device which represents a key differentiating factor of this thesis. The footprint of the solution was also considered, to be reduced at the minimum possible.

Artificial sample tubes were prepared in the laboratory with 5 different hemolysis levels that were measured during the experiment (including the hemolysis free level).

The image processing algorithm incorporated in the prototype app code, employed the RGB model and CIE XYZ and, respectively, CIELAB color spaces to calculate the color index of each hemolysis level.

The differentiating factor is the use of illuminant D65 which helps portraying standard illumination conditions with the Y (relative luminance) set at 100. During tests it was observed that the precision of the measurements would be at the highest accuracy level with conversion from RGB color model values to CIE XYZ color space values with illuminant D65 and Y=100 (relative luminance). Then the CIE XYZ color space values were converted to the CIELAB color space values. As explained previously in this thesis, a direct conversion from RGB color model values to CIELAB color space values, although theoretically possible, in practice, it would have been too slow and time consuming, therefore it was discarded.

The measurements were executed in normal lightning conditions (e.g. laboratory lighting). More than 70 sample tubes were measured. Another differentiating factor of this thesis is the ample range of test tubes that were selected and used in the experiment (37 different tube types from various manufacturers). These sample tubes also cover, very conveniently, different age groups of patients, ranging from babies to adults (the volumes of patient blood drawn depends on age, weight and other possible medical factors). The accuracy of the measured results during the experiment is of 97.90% or ~1 mg/dL with a turnaround time (TAT) of up to 70 minutes (depending on the filling volume of the sample tubes as listed in Table 3). The sample tubes had filling volumes ranging from 2 ml up to 15 ml and were carefully selected based on what is frequently and mostly used in the laboratories today. For example, for a 2 ml sample tube the entire process would take up to ~9.3 min (including the gravitational sedimentation) while, larger sample tubes, of 15 ml filling volume, would take up to ~70 min (including the gravitational sedimentation).

Another value added contribution of this thesis is the fact that sample tubes with normal volumes were used instead of micro-volumes of blood or blood drops or very small

quantities of blood. In a real-life point-of-care facility, the patient blood is drawn in standard sample tubes and rarely in capillary or microtubes. Moreover, any other preparation step which might tamper with the integrity of the patient sample, such as blood pipetting or blood dropping, was avoided in this thesis, since it puts another stress level on the red blood cells (RBCs) which may result in potential blood lesions.

In terms of limitations or further problems to be considered in future work, they are detailed and explained in subchapter 7.4.1.

7.3.4. Enhancing early detection of hemolysis

Firstly, a solution such as this one, presented in this thesis, called "HemoDetect", could potentially be at the forefront of early detection of hemolysis for patients in point-of-care facilities who need a quick diagnosis and the TAT of a traditional laboratory is simply too long. From the moment the gravitational sedimentation of the RBCs is sufficient enough to allow capturing a good image of the blood plasma layer, the "HemoDetect" prototype app will need additional ~11.47 seconds until the hemolysis result is displayed on the screen. The mobile solution simply comes into action as soon as the sample is ready, eliminating, thus, the unnecessary waiting or dead time that is needed in case the sample tube is sent to a traditional laboratory (be it on-site, next to the medical facility, or a centralized commercial laboratory).

Secondly, the cost factor is also undisputed and very important; it is less expensive to run a hemolysis detection test right in the point-of-care facility compared to issuing a test order to a traditional laboratory, which later has to be billed and paid (even if the sample tube is hemolyzed and no blood tests were deemed to be performed).

7.3.5. Smartphone-based sample analysis

"HemoDetect" prototype app uses image analysis algorithms and a defined classifier of rules to analyze an image and return the hemolysis level as a result.

Image analysis performed by a smartphone is an undeniable fact at present. Smartphones get better and better every day. Better camera resolutions, outstanding zoom capability, object tracking function, and ultimately, camera AI capabilities, make modern smartphones excellent hardware candidates for mobile apps for point-of-care, hospitals, laboratories, and medical centers.

Image analysis of samples, performed by apps such as "HemoDetect" prototype app will spread and evolve in tackling other applications, helping to diagnose other medical conditions (an example was mentioned in the subchapter 2.3.5 about the sickle cell detection). Sample tubes represent excellent mediums or containers due to their material's physical transparency and smartphone cameras are capable to capture the required image or the portion of image needed for a test. Any liquid or solution which is carried by a sample tube could be potentially analyzed for different factors or analytes (substances of interest to be measured from a human body fluid) using a standard smartphone equipped with a camera running a medical app (with image processing algorithms and programmatically established rules).

7.3.6. An extensible experimental environment

The existing experimental environment defined in this thesis could be extended to incorporate tests performed on various fluids carried by a sample tube. For example, the "HemoDetect" prototype app could become, in future research work, the "EasyDetect" app, by expanding the existing app with additional functions for analysis of lipemic samples (lipemia is the presence of a high concentration of lipids or fats in the blood; when blood is lipemic it causes the plasma to have a "milky" appearance) or icteric samples (icterus or hyperbilirubenemia is the presence of high levels of bilirubin in the blood; icteric serum or plasma ranges in color from dark to bright yellow, rather than normal or standard plasma color).

Image analysis of samples could apply not only to blood and its serum indices but to other human body fluids as well. For example, urine samples, could also be analyzed using

mobile app image analysis to determine the hematuria level (the concentration of blood in urine) which could help discovering or preventing medical conditions.

7.4 Additional extensions and future work

7.4.1. Addressing current problems and limitations

Lm represents the lightness (or brightness) CIELAB measured color space value as defined in the subchapter 4.4.1 while explaining the developed image processing algorithm. While the Lr represents the same CIELAB reference value for blood plasma, it would be interesting to test the behavior of the "HemoDetect" app in both extreme low light and high brightness conditions. Although it is unlikely that these extreme light conditions are to be found in a point-of-care facility, it would certainly be an aspect worthwhile to be considered.

7.4.2. Extending mobile apps to other healthcare areas

Like many other industries, healthcare is embracing the Internet wave, connected apps and things. Due to increasing healthcare costs, the insurance policies increase year by year to the point that consumers cannot afford to pay their premium health insurances anymore and prefer cheaper alternatives instead. Healthcare insurance companies, under pressure from consumers and the state governments, started to offer telemedicine services, or telehealth services. Although many of these attempts are merely a phone call to a healthcare trained person who is filling a web form and sends the caller (patient) to the specialist, some of these services go beyond a simple registration formality, especially in these coronavirus times where digitalization and remote completion of tasks is almost the norm.

Technological advancements and increased Internet bandwidth have boosted communication (mainly through videoconferencing) and collaboration between patients, doctors, insurance companies and medical facilities, especially, during the pandemic.

For example, insurance companies have developed apps which track the sports activity of a customer and, based on the recorded results, the customer could benefit by having a lower

price for the health insurance policy. Also, paper work (e.g., invoices, medical bills, letters) is exchanged or processed through mobile apps (personally and family wise, it is the case since 1 year now, that all the medical bills and communication is performed through the app of the health insurance company). For consumers it is mostly related to the convenience and easiness of use, while for insurance companies is all about cost optimization (e.g., savings related to staff, and office supplies). Also, this is becoming an increasing topic especially when there are measures in place to limit the human-to-human interaction or the exchange of items (by post) due to the global sanitary crisis.

Consumers, at their end, have become more careful about their lifestyle, nutrition, sports or individual health in general. Wearable devices are helpful in this regard.

In some hospitals, doctors use mobile devices to access patient files, records, and expert databases for cancer diagnosis, for example. Usually these apps are configured to work only in internal networks and medical personal is formally trained on each app. Patients are still outside of this enclosed circle, but there are initiatives, particularly at a regional level, to have simple digital access to individual medical records.

In automated laboratories, mobile apps are used by laboratory operators to monitor the sample workflow and status of each medical device. Orders are also operated via mobile apps in some cases. Communication from the issuer of the order and the laboratory is often performed via desktop applications, less on mobile apps. In this interaction, the patient is unfortunately, in most of the cases, excluded, mainly due to lack of infrastructure to provide accessibility to digital services that is partially justified by a high investment efforts versus presumed value added for patients due to their low training level in specialized medical terms.

The usage of mobile apps in healthcare is undeniably increasing. However, it can be observed that there is still a long journey until the digital loop can be closed with all the actors playing in harmony (insurance companies, patients, doctors, laboratories, and medical clinics).

7.4.3. Extending the range of mobile app characteristics

Mobile apps in healthcare infrastructures or networks require certain accommodations and shifts in how these infrastructures should be approached and designed. Mobile apps require certain infrastructure requirements which go beyond the traditional local area network of a point-of-care or medical facility. Mobile apps, in general, evolve very quickly, and they go through a continuous transformation. In healthcare, mobile apps are subjected to the same phenomenon. So it is important, before designing the system architecture for healthcare mobile apps, to understand the characteristics of these apps.

In their vast majority all these apps make use of a key characteristic which is the wireless connectivity (in most of the cases, through Internet) in order to exchange data or send messages to other mobile apps or computers. Apps are increasingly becoming heavy consumers of resources and they have to process vast amounts of data, sometimes over bandwidth constrained wireless networks. Persistence, caching or data synchronization mechanisms could help mitigate problems with potential data loss while experiencing short "network disconnects" or temporary heavy load on the network. Another important app characteristic is the location services capability which could be very useful to identify the dynamic hot spots of where most mobile devices operate at a certain point in time and have an intelligent load balancing of network bandwidth to meet the needs of these apps and devices which execute critical data tasks.

Finally, a mobile app such as "HemoDetect" prototype app, could integrate additional functions such as user authentication while using facial recognition or using technologies such as NFC or scanning a QR code at a laboratory door terminal to gain access or using a mobile app to scan the barcode labels of sample tubes.

While designing apps for healthcare, developers or vendors need to be experts in mobile app technologies to be able to educate and demonstrate to healthcare professionals the benefits of extending the usage of mobile apps by leveraging their supported characteristics in complete harmony with security best practices and healthcare regulatory requirements.

7.4.4. Creating an automated sample analysis ecosystem

What has been science fiction 30 years ago, applied robotics, in many industries or areas, as well as in healthcare, has become a reality and a fact today. For example, researchers at the Rutgers University, U.S., have developed a robot[8] which draws blood from the arm of a patient and analyzes it.

The system contains three separate parts. The first is a "venipuncture robot". This robot locates the patient's blood vessels using near-infrared (NIR) and ultrasound imaging. Then, it reconstructs the vessels in 3D using image analysis. Finally, it inserts the needle right into the center of a vein. The next part of the platform is the "sample handling module." This extracts the blood and pumps it to the third part of the system, which is called the "centrifuge-based blood analyzer". The analyzer itself contains an acrylic chip that houses the blood sample, a centrifuge that separates the blood into its various parts, and an optical microscope system that determines the cell count. The system was set up to replicate the two most common blood tests in the U.S. (a 3-part white blood cell differential and hemoglobin measurement).

This is an outstanding example of robotics applied in phlebotomy and blood analysis. To be able to reach a fully automated, sample analysis ecosystem, robotics must have probably the main role to play in it. Hemolysis detection, such as performed by the "HemoDetect" prototype app in this thesis, could very well be integrated in a robotic solution that performs sample quality check and sample analysis at the point-of-care. This represents a possible future research work idea. Another idea would be to take "HemoDetect" prototype app at the next level and evolving it into an AR solution with the powerful camera capabilities of the AR kit Microsoft HoloLens 2. For example, the advantage of using a device like HoloLens 2 is the fact that in most use cases it is very ergonomic (touching a physical screen is no longer necessary, thus the user is untethered and free to move). The HoloLens 2 has a powerful front-facing camera, which enables the app on the smartphone to which it can be connected to, to see exactly what the user sees while visualizing holograms with

[8] Consulted online (last checked on 01/03/2020): https://news.rutgers.edu/rutgers-researchers-develop-automated-robotic-device-faster-blood-testing/20180612#.Xg8_p0dKiHs

natural touch, grasp, or move gestures that respond a lot like real objects. A sample tube could be picked up and by just pointing at the real object (sample tube) the patient hemolysis result is displayed on both the holographic display and on the smartphone app simultaneously.

7.4.5. Other application areas

Besides sample analysis, other healthcare areas have an important lead in the journey of using robotics. For example, robot surgeons are used in complex surgery activities and they help close the gap between the human-doctor and the machinery. These robots perform complex tasks with a high degree of precision, and, in some cases, outperforming the doctors. That does not mean that doctors will be replaced by machinery, instead, it will be a perfect symbiosis where, the machine, its artificial intelligence, its immense medical knowledge base and expertise will help the human-doctor taking the best diagnostic decisions or administer the best treatment to patients. The human-doctor, thus, takes on a more strategic role, leaving part of the medical routine tasks to the robot while focusing more on strategic and decisional tasks which the machine cannot do.

Other application areas where robotics is used, is in caretaking of patients, for example, training patients who suffered a motor disability following an accident, to walk again. Companion robots for elderly long-term patients help them with basic tasks such as measuring blood pressure or reminding them to take their medicine.

In a medical facility, cleaning is a major topic as well. Cleaning robots are used in some hospitals or clinics to perform this very important task which secures a high degree of hygiene for both patients and medical staff. Supply chain robots also perform basic tasks such as managing logistics, ordering additional supply when the stock is low or storing and distributing supplies as per internal orders.

Undoubtedly, robotics, machine learning, artificial intelligence and augmented reality are gaining more and more terrain in the healthcare space, putting technology advancements at the cornerstone of better patient care.

7.5 Final remarks

This thesis proposed, as research objective, the design, development and testing of a smartphone app solution to detect hemolysis in blood sample tubes. Thorough literature review and study of recent related research work have been a great foundation for preparing the pillars of building the prototype app and the corresponding experiment presented in this thesis.

Designing, coding and testing in repeated cycles, the "HemoDetect" prototype app has been a very enjoyable experience. Preparing the materials in the laboratory (ingredient dosages, solutions, containers, pipetting and sorting the sample tubes, handling the real blood tubes) has been immensely insightful to deeply understand the challenges of the laboratory and point-of-care life.

Photographing tirelessly each sample tube with the "HemoDetect" prototype app, in repeated cycles, and recording the first hemolysis level results in the database has been a culminating and defining moment. The research findings during the experiment confirmed that the "HemoDetect" prototype app results were reliable and comparable to traditional medical device systems and other comparable research works. During the experiment, 70 artificial sample tubes were tested in repeated test cycles. The accuracy of the "HemoDetect" prototype app measurement was of 97.90% or ~1 mg/dL.

CONCLUSION

"HemoDetect" Android prototype app represents a cost-effective, small footprint, and easy to use mobile solution for detecting hemolysis in point-of-care facilities. The proposed solution consists of an Android prototype app which runs on a standard modern mobile smartphone equipped with a rear camera (Huawei nova 3i). The test sample tubes consisted of two categories: real whole blood samples (from anonymous donors in Switzerland) that could not be tested during the experiment (due to coronavirus situation), and artificially prepared sample tubes that mirrored exactly the defined hemolysis levels in accordance with their concentration of free hemoglobin, defined as hemolysis free – HF (≤ 5 mg/dL); low hemolysis – LH (5 – 30 mg/dL); medium hemolysis – MH (30 – 60 mg/dL); high hemolysis – HH (60 – 300 mg/dL), and very high hemolysis – VHH (≥ 300 mg/dL). In case of real blood sample tubes the blood plasma was separated from the whole blood using gravitational sedimentation (while considering the ESR according to the Westergren method) and thus the hemolysis test could be done immediately after without the need for previous automated blood sample centrifugation (however the tests on real blood samples could not be performed due to coronavirus situation). Using CIE L*a*b* color space that can differentiate varying concentrations of free hemoglobin in the blood plasma, the "HemoDetect" Android prototype app was developed to capture the sample image with the smartphone camera in normal lightning conditions (including zoom capabilities where needed) and perform fast digital image analysis. The color index calculated (CI) was mapped in the app's code to the defined hemolysis levels and the results were immediately displayed on the app screen. More than 70 sample tubes were photographed using the prototype app in repeated test cycles and the results were recorded in the database. The accuracy of the measured results during the experiment was of 97.90% or ~1 mg/dL.

The proposed smartphone app solution can reduce pre-analytical errors related to wrong visual inspection results and can definitively improve the TAT compared to the TAT of standard medical pre-analytical devices in a laboratory. While the TAT of traditional systems to detect hemolysis is at least 4 hours, the solution proposed in this thesis found that the TAT could be of up to 1.10 hours depending on the filling volume of the sample

tubes as explained in 7.3.3 (the time measured between the fresh blood sample is drawn and left on the vertical stand or rack for gravitational sedimentation of red blood cells and until is photographed by the “HemoDetect” prototype app and the result is returned to the user on the prototype app’s screen).

This mobile device enabled solution could support improved and faster results for a number of hemolytic conditions, particularly helpful in point-of-care facilities, where results are needed faster and early detection of hemolysis is critical.

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APPENDICES

Appendix 1: Technical specifications of the smartphone "Huawei nova 3i" used in the experiment

Huawei
nova 3i

HARDWARE
HiSilicon Kirin 710
Hardware: kirin710
Cores: 8
CPU:
4 x Cortex-A73
4 x Cortex-A53
Process: 12nm
Frequencies:
480 MHz - 1709 MHz
807 MHz - 2189 MHz
Governor: schedutil

GRAPHICS
Vendor: ARM
GPU: Mali-G51
OpenGL: OpenGL ES 3.2 v1.r10p0-00cet0.968d3d22ccad8a5f729e8d0a4de7195d
Resolution: 2340 x 1080
Screen density: 409.73096 ppi
Screen size: 6.29 in / 160 mm

RAM

RAM size: 4.0 GB

OTHER HARDWARE

Bluetooth support: yes

Bluetooth LE support: yes

USB host support: yes

Infrared transmitter: no

NFC support: no

DEVICE

Model: INE-LX1

Codename: HWINE

Manufacturer: Huawei

SYSTEM

Android Version: 8.1.0 (Oreo)

Build: INE-LX1 8.2.0.130(C432)

EMUI: EmotionUI 8.2.0

Security patch: October 1, 2018

Architecture: aarch64 (64-bit)

Instruction sets: arm64-v8a armeabi-v7a armeabi

Kernel: 4.4.103+

BATTERY

Technology: Li-poly

Health: Good

Capacity (reported by system): 3340 mAh

NETWORK

MAC address: 34:79:16:1C:BC:1C

5GHz band support: no

Phone type: GSM

Dual SIM: yes

REAR-FACING CAMERA

Resolution: 15.9 MP (4608x3456)

Focal length: 3.81 mm

35mm equivalent focal length: 25.6 mm

Sensor size: 5.16x3.87 mm

Crop factor: 6.7x

Field of view: 68.2° Horizontal

Pixel size: 1.12 µm

Aperture: 2.2

Focus modes: infinity, auto, macro, continuous-video, continuous-picture

Flash modes: off, auto, on, torch

ISO sensitivity range: 50-3200

RAW capable: yes

Image format: JPEG

FRONT-FACING CAMERA

Resolution: 23.8 MP (5632x4224)

Focal length: 3.81 mm

35mm equivalent focal length: 23.4 mm

Sensor size: 5.64x4.23 mm

Crop factor: 6.1x

Field of view: 73.2° Horizontal

Pixel size: 1.00 µm

Aperture: 2.0

Focus modes: fixed

ISO sensitivity range: 50-3200

Image format: JPEG

Appendix 2: Prototype App Code Repository

GitHub link: https://github.com/hemodetect/codebase