The Malaria Blood Sampling System

Enhancing the quality of field prepared malaria blood smears

Master thesis by

Jorrit Kleerebezem

July 2021 | Integrated Product Design | Delft University Technology

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MSc. Integrated Product Design
Faculty of Industrial Design Engineering
Delft University of Technology

Author
Jorrit Kleerebezem

Supervisory Team
Chair: Prof. ir. Jos Oberdorf
Mentor: Dr. ir. Jan-Carel Diehl

Delft University of Technology
Faculty of Industrial Design Engineering
Landbergstraat 5
2628 CE Delft
The Netherlands

PREFACE

This thesis is the end to an era full of friends, joy, laughter, highs, lows and stress. While writing this, it feels weird to realise the time has come to leave this student life behind, while at the same time I'm very excited to see and experience what the new era will bring me. I am grateful this interesting project topic crossed my path, as the combination of healthcare and product development has gathered my increased interest over the years. I believe this thesis is a great representation of my capabilities and the knowledge obtained during my years of study at the Industrial Design Engineering faculty. I'm very thankful for my time here.

I want to use this section to express my gratitude to the people who helped me during this ‘levenswerk’ (a project that has cost some years of my life, while it took less than a year).

First and foremost I want to thank my supervisory team. Jos, thank you for the weekly meetings with cooperate brainstorms, insightful moments, sharing your expertise and providing the opportunity to prototype my final design. You sometimes were critical, hammered on making decisions and making statements on why this is the best decision, while giving me the feeling you believed in me as a designer. This brought new perspectives and eventually confidence that I had made the right decisions and this product was getting somewhere. It was a pleasure working with you!

JC, thank you for sharing your enthusiasm and expertise on the, for me unknown and difficult to imagine, African context and mind-set. Your share of information made it easier for me to get out of my ‘western’ Dutch bubble and imagine the context I developed a product for. You have connected me to a great number of people that have experience within this topic and context, which was very valuable for the project. Also, you gave me the ability to buy prototyping materials without a hassle, which made the workflow of the prototyping and testing process a lot smoother. It was a pleasure working with you!

A big thank you to my mom, dad, sisters and girlfriend for supporting me and believing in me during this project and during my entire study. You were always a safe haven to go to, providing me with the necessary love, support, confidence and food. It feels good to know your pride and feel that you will always be there for me, also during the upcoming new era. I love you.

Also a big thank you to my lovely friends. The fact that some of us graduated in similar timeframes during this weird COVID time, made it a lot more pleasant and less lonely. Great timing guys! Next to helping me out, you have always provided me with a good laugh, beer or board game that gave me that relaxed feeling I needed. I will never forget memories we made during this era and I’m sure we are going to make a lot more unforgettable ones. Cheers to that!

Thank you to Tope and Mirte for setting up this graduation project and your assistance throughout the project. Even though we didn’t meet much, the meetings we had were insightful and pleasant.

A special thank you to Brice, Henk, Chinonye, Ayemi, Nina, Bernice, Fiona, Lisette and all the others that took the time to do an interview or participated in one of the various tests. I’m truly grateful for the insights you gave me and your cooperation during this project. Without you this project wouldn’t be where it is now!

ABSTRACT

In this thesis, a new method towards enhancing the quality of field prepared malaria blood smears is developed through the use of a newly designed, innovative tool. Malaria is a life threatening febrile illness caused by parasites that are transmitted to people through bites of infected mosquitoes. If the symptoms are not treated within 24 hours, malaria can progress to severe illness, often leading to death. The golden standard of malaria diagnosis, microscopy, requires a high quality thick and thin blood smear. The smears enable distinction of the parasites and assessment of the infection rate. However, currently there is no standardized way to prepare these smears, leading to inconsistencies in quality.

The field context shows to be the context with the lowest smear quality output, due to difficulties and errors in the process. The high proportion of low quality smears lead to misdiagnosis, wrong treatment and indirectly to preventable fatalities. Estimates show that 25-50% of the smears prepared in this context is of poor quality. To get an understanding where this problem comes from, the most impactful errors on the smear quality have been mapped; uncontrolled variables in the preparation process, a lack of experienced medical staff, degradation of the smears during air drying and unavailability of proper and clean (local) resources and equipment.

The Malaria Blood Sampling System (MBSS) has been developed with the aim to increase the quality of field prepared blood smears, by reducing the impact on the quality that is caused by the human, environment and available resources. The MBSS is not just a newly developed device, it is a new system and a new approach to blood smear preparation. It provides a more precise, effective and efficient way of preparing blood smears in the field.

The MBSS makes blood smear preparation more accessible for inexperienced people, this tackles the current problem of the lack of experienced staff and reduces the human impact on the quality. The increased accessibility is achieved by eliminating two of four uncontrolled variables; angle and pressure. In addition, the MBSS minimizes the impact of the blood volume variable, leaving the movement speed the only human controlled variable in the process.

The environmental impact on the smear quality is reduced by eliminating the possibility of dust, ants, flies and other debris to settle on the smears. The ability to dry the slide inside the device enables protective air drying. This makes diagnosis easier and more efficient, by avoiding that unwanted particles colour the stain, which would lead to much noise, possible interpretation errors and misdiagnosis.

One of the key aspects towards preparation of high quality smears, is making use of high quality equipment. Since the MBSS includes a high quality glass slide and a capillary tube, the impact of the equipment on the smear quality is controlled and the quality of the smear can be guaranteed. These items are included in the MBBS package; they eliminate the risk of using non-sterile, unclean or contaminated equipment.

The MBSS has proven to deliver high quality thick and thin smears. Data from tests with experts and amateurs shows that the MBSS

scores higher on all aspects compared to the convention smear preparation method. There is an increase in ease of preparation, ability of preparation by inexperienced people, presumed smear quality, quality consistency and time efficiency. The MBSS has potential to be a game changer that will guarantee consistent high quality smears in low-resource and low-expertise settings, such as the field. Deploying the MBSS in the malaria diagnosis 'infrastructure and system' will establish and ensure higher efficiency and accuracy in malaria diagnosis, leading to an increase in the amount of correct treatments and fatality reduction.

READING GUIDE

This thesis consists of multiple chapters, each divided in sub chapters. Each chapter is briefly discussed below, so that the reader can decide to skip to chapters of interest.

Analysis – A thorough and in depth analysis of the theory behind blood smear preparation, the field context and the blood smear preparation within this context.

Synthesis – Extracting and transforming the gathered data into problem definitions and a design direction.

Ideation – The phase where creative sessions were held, in order to come up with solutions for the defined problems. This chapter also includes rapid prototyping of some of the ideas to assess some of the working principles.

Conceptualisation – Further development of the chosen idea into a concept and proof of the working principles of this concept through prototyping and testing. Also, finding the optimal variable parameters for angle, pressure and speed in thin smear preparation, through intensive testing.

Parameter determination– Finding the best parameters for the angle, pressure and speed variable, through extensive testing.

Implementation – Implementation of the found criteria from the testing phase, developing the product to a state-of-the-art concept, including materialisation, production optimisation and a final test.

Final design – presentation of the final design and its specifications, as well as a presentation of the final prototype.

Validation – Validation of the performance of the developed product with experts and amateurs.

Limitations – Project limitations per design phase.

Conclusion – The project conclusion, concluding the performance and usability of the product.

Recommendations – Recommendations on what is required to further develop this product.

This thesis has multiple conclusions:

Conclusion - conclusion of subchapter.

Chapter conlusion - conclusion of main chapter (indicated with coloured background.

From the obtained data during research and testing, criteria and challenges have been formulated.

Criteria (cr) are 'norms' that should be fulfilled.

Challenges (ch) are similar to a criteria, however is often unquantifiable and serves more as a design challenge and something that should be kept in the back of your head.

In the criteria or challenge a reference is made to the chapter the criteria or challenge is based. An example of a criteria is given below:

1.1 (cr) A downward force of 0,6N must be applied by the spreader (5.2.2).

GLOSSARY

Abbreviations

FOV – Field of view

LUMC – Leids Universitair Medisch Centrum

MBSS – Malaria blood sampling system

PET - Polyethylene terephthalate (material)

RBC – Red blood cell

RDT – Rapid diagnostic test

SLA - Stereolithography

FDM - Fused filament fabrication

WHO – World Health Organisation

Terms

Enhancing – To increase or further improve.

Challenge – An insight or problem to take into account when designing.

Criteria – A standard to which the design may be judged or assessed.

FDM 3D printing – The process of manufacturing three-dimensional objects by depositing successive layers of extruded plastic.

Monolayer – A single layer of blood where the red blood cells lay next to each other.

Plasmodium – The genus of the class of Sporazoa that includes the parasite that causes malaria.

Prototype – An early model of a product built to test a concept or process.

Slide – A piece of glass on which blood smears are prepared.

Spreader – Often referring to a slide that is used to spread a drop of blood in a thin smear.

SLA printing – The process of manufacturing three-dimensional objects by selectively curing a polymer resin layer-by-layer using an ultraviolet (UV) laser beam.

Thick smear – A large drop of blood that is spread and forms a 'thick' layer of blood.

Thin smear – A small drop of blood that is spread an forms a 'thin' layer of blood.

The field – Contextual term that refers to a remote location.

TABLE OF CONTENT

Una vez confirmado
presenta signos de deterioro importante
indicar de *inmediato* el tratamiento de Cinta Roja
siguiente:

- CUADRO
- Esquema de Tratamiento
- Plasmodium

Grupos de Edad	Cloroquina	Ter. Día
0–6 Meses	¼ Past. (37.5 mg)	Prim
7–12 Meses	½ Past. (75 mg)	½Past
1–2 Años	1 Past. (150 mg)	½Pas
	1 ½ Past. (225 mg)	1 Pa
	2 Past. (300 mg)	2 P
	Past. (450 mg)	3
	(600 mg)	3

CHAPTER 1

INTRODUCTION

An introduction to the topic and project appraoch.

1.1 INTRODUCTION

Malaria is a life-threatening febrile illness caused by parasites transmitted to people through bites of infected mosquitoes. Symptoms usually appear 10-15 days after the infective mosquito bite. If not treated within 24 hours, malaria can progress to severe illness, often leading to death. In 2018 there were an estimated 228 million malaria cases worldwide, and the estimated number of malaria deaths stood at 405.000. Africa carries a disproportionately high share of the global malaria burden. In 2018, the continent was home to 93% of malaria cases and 94% of malaria deaths (*Figure 1*). Nigeria was responsible for 25% of all malaria cases (WHO, 2021). The good news is that malaria is preventable and curable.

The 'gold standard' in malaria diagnosis, microscopy, is performed by examining a thick and a thin blood smear (*Figure 2*). The thick smear gives the ability to quantify the number of parasites per microliter of blood, the thin smear helps to identify what species of parasites are present in the blood sample. However, because of the lack of proper equipment and poor conditions in the field, where a significant amount of blood smears are prepared, smear preparation and field microscopy are often performed inaccurately (Visser et al., 2015). A research on smear quality states that '33.51% of the slides received were considered poor in smear preparations, due to poor staining, cleanliness, sizes, evenness and thickness'. It concludes that there are inaccuracies in the diagnosis of all species of malaria parasites which is contributed significantly by the poorly prepared slides and slides prepared from blood in EDTA tubes. Thus, improving the competency of laboratory professionals involved in preparing blood smears is greatly needed (Zulhainan et al, 2019). Experts from the field confirm misdiagnosis due to poor quality smears; field technician Meulah Brice states that around 25% of the smears in his previous batch had discordances in diagnoses, which is partly caused by poor quality smears (appendix 11.1). Field experienced tropical doctors Henk Schalig and Nina Korse mention that poor quality smears due to medical staff's challenging conditions and inexperience are frequently observed (appendix 11.1).

Blood smear preparation is one of the initial steps in malaria diagnosis, the smears are the core and foundation to a good diagnosis. Therefore, it's important that the quality and quality consistency of malaria blood smears can be ensured. Errors made during this stage of the diagnosis process will cause difficulties with diagnosis, forming a threat for the diagnosis accuracy. The inconsistency in quality of blood smears leads to incorrect diagnoses and therefore wrong treatment of patients, indirectly leading to preventable fatalities. This leaves opportunities in increasing the quality and quality consistency of malaria blood smears. A new procedure combined with a new product is designed to tackle this problem during this graduation project.

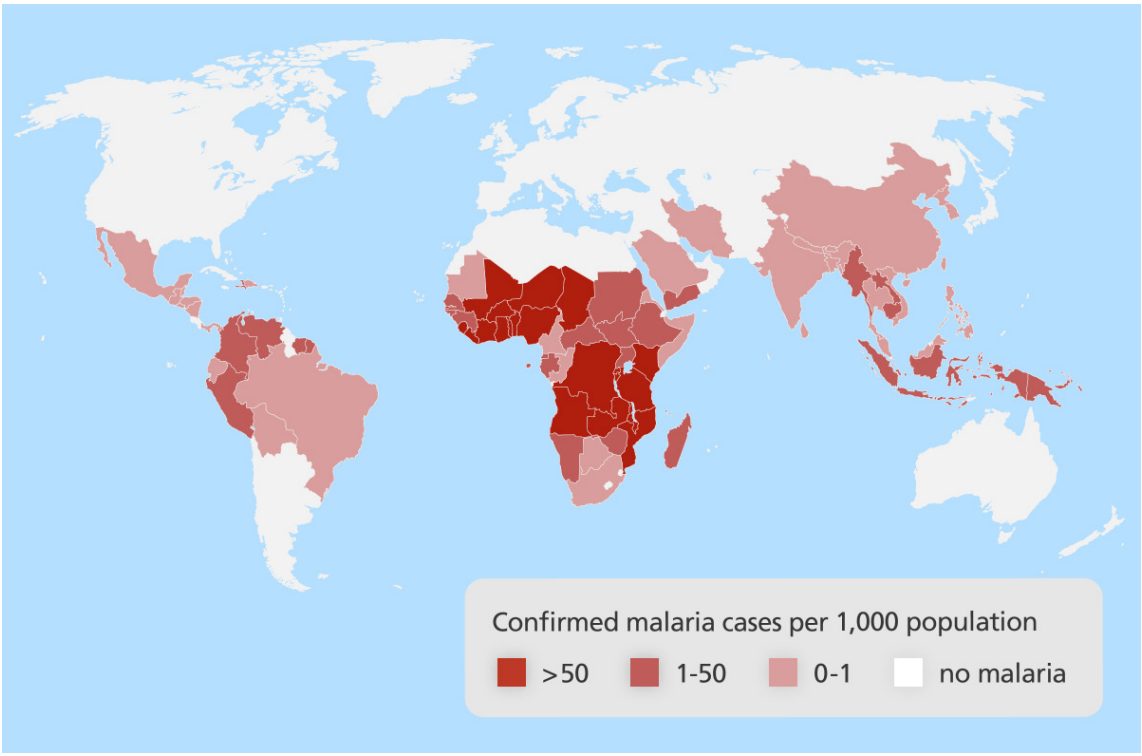


Figure 1: Malaria cases around the world.

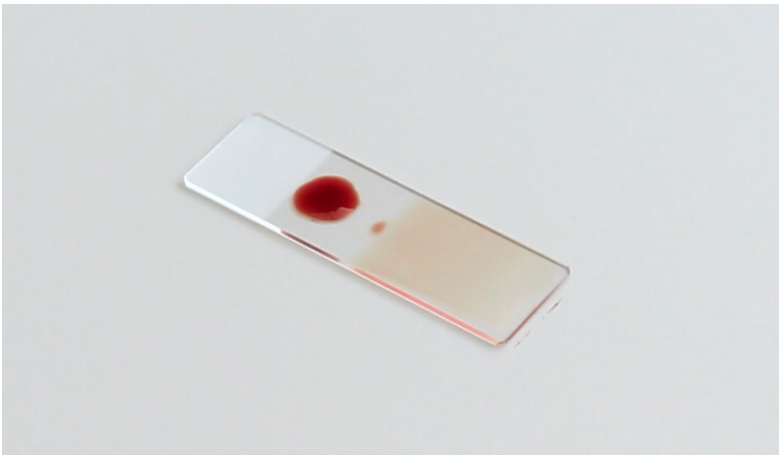


Figure 2: A thick and thin smear on a glass slide.

1.2 APPROACH

This graduation project will focus on enhancing the quality and quality consistency of malaria blood smears, through a design intervention. The report structure is inspired by the Technology Readiness Levels (TRL). TRL levels are a method for understanding and estimating maturity of technologies, developed at NASA (AcqNotes, 2021). TRL allows engineers to have a consistent reference for understanding the technology evolution (Twi-global, z.d.). In this project a method is applied that is derived from TRL, called Product Level (PL). A product will be designed, going through similar steps as TRL, the chapters in this report will be based on these levels (*Figure 3*).

To understand that the context the to-be-designed product will be deployed in, which challenges currently are being faced and which errors are being made, thorough analysis of the blood smear preparation process and the context is required. Therefore, this project starts with an analysis phase, deep diving into the working prin-ciples of smear preparation, quality requirements and sketching the current situation. The results from the analysis phase lead to defining the problem roots and a solution direction.

The next phase is about developing ideas, that serve as solution to the defined problems resulting from the analysis phase. The most promising as will be tested and validated through prototyping. Eventually one prom-ising idea will be chosen for further development into a concept. This concepts is first theoretically shaped, af-ter which it is prototyped and tested.

Eventually, based on the iterative process of prototyping and upgrading the concept, a final concept and proto-type is presented. This concept is tested and validated with experts, after which recommendations are given to further develop this concept into a market ready product.

Figure 3: Product Levels (PL)

1.3 PROBLEM DEFINITION - AIDX MEDICAL

The initiator of this graduation project is AiDx Medical. AiDx is a start-up company developing automated microscopes for malaria detection in Africa. They do this in collaboration with the parasitology department the Leiden University Medical Centre (LUMC) and the Diagnostics for all programs of the TU Delft, which aims to develop easy-to-operate devices for low resource settings. AiDx state that 'microscopy is the Gold Standard for malaria detection, but the quality of blood smears preparation is not always sufficient to reach reliable diagnostic results'. Both manual microscopy and their automated microscope require high quality blood smears, so that the parasites in the RBCs can be observed and distinguished. Their assignment is to reduce human errors in the blood smear preparation process and to develop a tool that ensures consistent preparation of high quality smears for low-resource settings, like rural parts of Africa.

Figure 4: The automated malria diagnosis device by AiDx Medical

CHAPTER 2

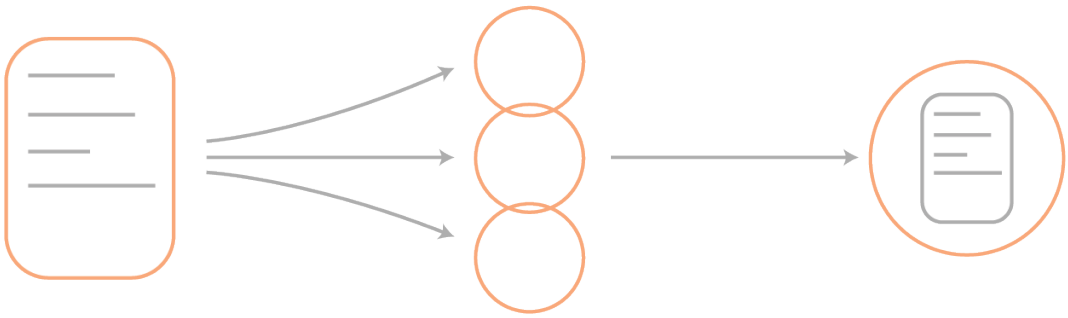
ANALYSIS

During the analysis phase the foundation for the design process is laid and the initial criteria that the to-be-developed product must meet are defined (Product Level 1). The overarching problem has been defined by AiDx Medical, however, validation and deeper investigation to the root of this problem is required. The analysis chapter dives deep into the theory behind blood smear preparation, the context where most impact on the smear quality can be made, and the current way of working within this context. By thoroughly and critically analysing the current way of working, the root problems that lead to poor quality smears and their impact are determined.

During this phase interviews were conducted with:

Meulah Brice	*Field technician Gabon*	*appendix 11.1*
Chinonye	*Field technician Nigeria*	*appendix 11.1*
Ayemi	*Medical expert and educator Nigeria*	*appendix 11.1*
Nina Korse	*Tropical doctor with field experience NL*	*appendix 11.1*
Henk Schallig	*Tropical doctor with field experience NL*	*appendix 11.1*
Fiona Geurtse	*Malaria vaccine scientist LUMC*	*appendix 11.1*

2.1 METHOD



Process in theory

Context

Process in context

First the theory behind blood smear preparation is discussed, to get familiar with the basic process and techniques. Next, we zoom out and discuss and compare multiple contexts in which blood smears are prepared, after which a decision is made on which context to focus on in this project. Then we zoom in again on this specific context and how blood smear preparation within this context is done and what challenges and errors are encountered. Based on this information the impact of the errors is defined, after which we zoom in on the most impactful section on smear quality. Within this section, clusters are defined to see what are the overarching causes to these specific errors. Eventually, based on this information a design direction, solution space and future vision are formulated.

2.2 BLOOD SMEARS

It is important to understand the differences between the two types of smears that are prepared in the malaria diagnosis process (*Figure 5*).

The thick and thin smear are prepared on a glass slide, in the field setting typically with both smears on the same one slide. As the name indicates, a thick smear is a 'thick' layer of blood on a slide. Since 16 to 30 times as much blood is examined compared to thin smears, they are significantly more sensitive and therefore used to assess parasite density (Spencer, 1986). Thus, for a fixed number of microscopic fields, thick smears allow the microscopist to examine a larger number of RBCs for the presence of parasites (*Figure 6*) and low parasitaemia can be more easily identified (Bejon et al., 2006). The RBCs in a thick smear hemolyze: this is a process where the RBCs burst open and 'drop' the parasites to the bottom of the slide, concentrating the parasite density (Geurtsen). Thin blood smears are only one layer thick (monolayer), meaning that in a perfect thin smear the RBCs are located next to each other and don't overlap (*Figure 7*) (Klassen-Fischer et al., z.d.). Thin smears are helpful in species identification because they reveal morphologic details of parasites (*Figure 8*) and erythrocytes not visible on thick smears. Thick smears are made by spreading a large drop of blood into a circular shape. Thin smears are made by pushing a small drop of blood forward with a 'spreader' slide, spreading it over the surface of the slide. Chapter 2.4 goes deeper on the preparation process of the smears.

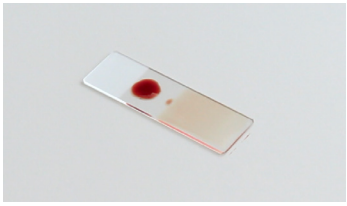


Figure 5: Thick and thin smear.

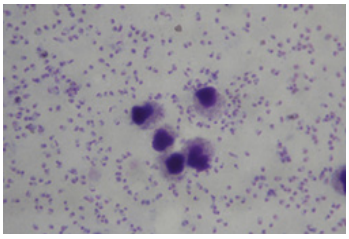


Figure 6: Microscopic view of a stained thick smear.

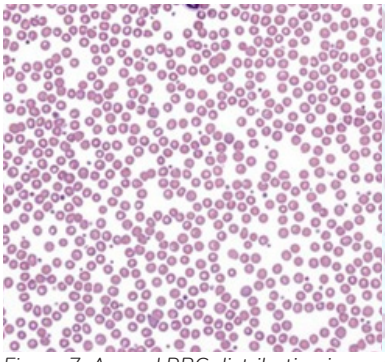


Figure 7: A good RBC distribution in a thin smear.

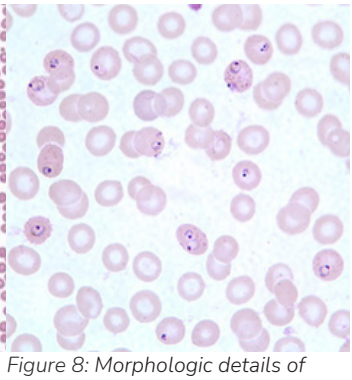


Figure 8: Morphologic details of parasites.

2.3 CONTEXT

Adaption of products to the context in which they are deployed in is a necessity in order to enhance its acceptance and to widen its application (Lindemann et al., 2013). The project's context plays a decisive role in the eventual working, mechanics, handling and appearance of the to-be-developed product. Therefore it's of importance to get thorough understanding of the product's operating context. This project aims to have maximum impact on enhancing the smear quality and increasing the accuracy of diagnosis. Therefore,

multiple contexts are defined and compared, in order to choose the context where it is presumed most impact can be made.

2.3.1 CONTEXT FRAMING

Blood smear preparation has two main purposes: research and diagnostics (*Figure 9*). For example; at LUMC they prepare smears on a daily basis for their research on a malaria vaccine, but rarely for clinical purposes. While in Nigeria the amount of prepared blood smears for research is close to negligible compared to the amount that is prepared for clinical purposes.

Figure 9: Blood smear preparation contexts.

When looking at the research side, there are two main distinguishable contexts:

- Research labs in developed countries
- Research labs in third world countries

The majority of the malaria research is done in developed countries. This is due to their, their wider budget and the fact that the labs in these countries are in general more advanced and suitable for research.

When looking at the diagnosis side, there are three distinguishable contexts:

- Diagnosis labs in developed countries
- Diagnosis labs in third world countries
- Field diagnosis

Research labs can be used as diagnosis labs, however, due to low malaria endemicity in developed countries, these labs are rarely used for that purpose. Malaria diagnosis most frequently takes place in the research & diagnostic labs in third world countries and in the field. *Figure 10* shows the full picture of the distinguisha-ble contexts.

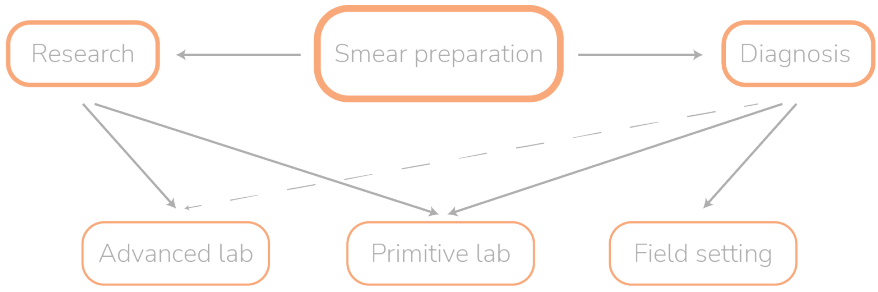
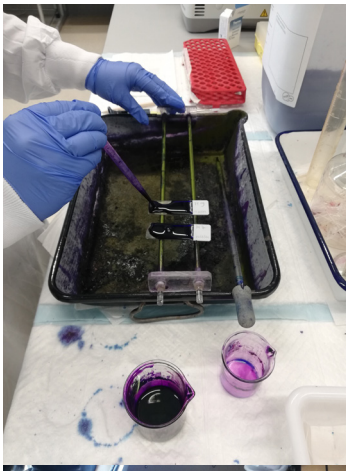


Figure 10: Blood smear preparation contexts.

2.3.2 STATE-OF-THE-ART RESEARCH LAB

Research and clinical labs (for malaria as well as other diseases) in developed countries are usually fully equipped and have a controlled and comfortable environment (*Figure 11*). According to Fiona Geurtsen working at LUMC in Leiden, they almost never experience preparing or reading poor quality blood smears that are made in their lab. She states that labs like the one in LUMC are a good facility and equipped with high-end equipment, which make the conditions 'favourable' for smear preparation. She does state that she can imagine that smear preparation can be more challenging in a more unfavourable context, with low-end equipment and non-sterile surroundings. In addition to that, the personnel working in the research labs in developed countries are in general well educated and skilled. Also, e.g. in The Netherlands, there are strict protocols for basically everything. This ensures that everything is double checked and there is quality control. When these protocols are not followed, there are consequences. These protocols enhance the general quality of labs and the smears (Korse).



Due to the low malaria endemicity and research as main purpose, the approach and mind-set in these labs are different compared to clinical labs, which indirectly can influence the smear preparation process and result. Geurtsen states that in a research lab there's not that much 'at stake', since there are no patients relying on receiving the correct diagnosis and there is an 'unlimited' supply of blood. This leaves more room for errors and if errors are made during the smear preparation process, the impact is low.

Additionally, some of the advanced labs have access to automated smear and staining machines. Depending on the type of machine, they can take over the whole process of smear preparation and staining, delivering consistent high quality smears. The reason these machines are only located in advanced labs, is because of their price and size. Besides, high quality equipment requires high quality resources to operate and maintain the equipment, something that is scarce in third world countries (Korse, Schallig).

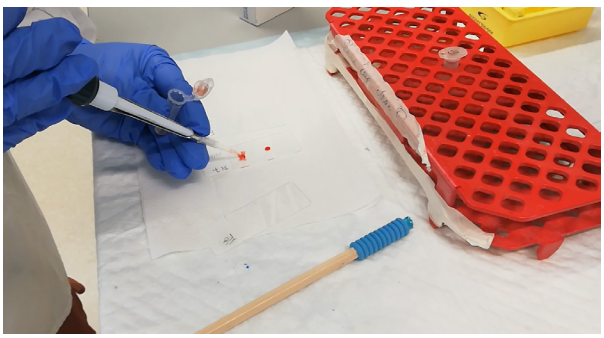


Figure 11: State-of-the-art context, with high-end equipment at LUMC

2.3.3 PRIMITIVE RESEARCH & CLINICAL LABS IN THIRD WORLD COUNTRIES

The smear preparation process in basic research and clinical labs in third world countries doesn't differ from the process in the labs in developed countries. However, the facilities are in general a bit more chaotic and less organised (*Figure 12*). Also, the protocols in countries like Nigeria are less strict or non-existent, compared to e.g. The Netherlands. According to Nina Korse, poorly followed protocols, or even not having proper protocols, leads to a poor quality environment and output. She states that there are almost no consequences for not following the protocols and that there is no quality control. This leads to chaotic workplaces, where mistakes are easily made. Protocols are essential in maintaining a sterile environment, which is often the reason why these labs are not as sterile as labs in developed countries. Besides, the lack of quality assessment and supervision leads to people assessing their own skills and then deciding whether they are capable enough to perform a certain operation like smear preparation.

It's a fact that in general the medical staff in third world countries is lower educated compared to developed countries (Korse, Schallig). Smear preparation requires experience and skills, the preparation process should be taught by experienced staff. However, due to a lack of experienced staff, inexperienced and insufficiently trained staff are preparing smears (Brice). Proper training and education is a problem. Chinonye, who is a certified microscopist and teaches at a college in Nigeria, states that she sees many poorly prepared samples by people who are educated and have followed trainings. According to her, education and training have a limited impact on the smear quality. 'Theoretical knowledge is easy to obtain, everyone knows the procedure, however executing it is not a given' (Schallig).

Additionally, the access to proper resources is limited. It frequently occurs that there's not enough equipment, equipment is missing or not cleaned (Korse). According to Korse the medical staff have to work with what they have, and that's almost never what they are used to compared to back in The Netherlands. The limited amount of resources and their poor quality and cleanliness causes problems in the smear preparation process.

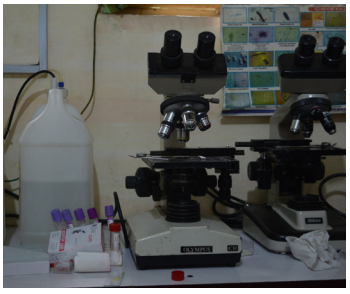


Figure 12: Primitive research & clinical lab.

2.3.4 FIELD DIAGNOSTICS IN THIRD WORLD COUNTRIES

People living in remote places in Africa don't have the possibility to go to a hospital, because they are often too far away. That is why field trips, where medical staff goes to the remote locations to collect blood, diagnose and treat patients, are necessary in the fight against malaria and other (parasitical) diseases. Field trips offer people who are living in remote places the, sometimes lifesaving, opportunity to get diagnosed and treated.

Figure 13: Improvised labs in the field.

Data from qualitative research shows that blood smears prepared in the field are in general of a lower quality compared to smears prepared in the lab. The interviewees acknowledge the problem of low quality smears coming from the field, which complicate diagnosis. Ayemi, estimates that about 50% of the samples prepared in the Nigerian field is of insufficient quality. Field technician Meulah Brice stated that in his last batch of smears, 25% had discordances in diagnosis, mainly due to poor quality smears. Also tropical doctor Henk Schallig acknowledges this problem and states that the field circumstances make it challenging to produce high quality smears. The challenging, changing and unregulated context makes that the field staff constantly has to adapt to the situation, which makes experience and imagination key in this context. Having inexperienced staff operating in a field context is doomed to fail. However, as stated in the previous chapter, there is in fact a lack of experienced staff (Brice, Chinonye, Korse, Schallig). A reason for this could be because the well-educated medical personnel often prefers to stay in the bigger cities. The field and more remote places are unattractive to go to, because of the relative poor working conditions (Korse). This leaves a relatively large number of relatively inexperienced and unskilled staff in the remote places compared to the more civilized places, while especially in the field well trained and skilled people are necessary.

In the field context resources are limited (Korse, Schallig). There is often is a lack of equipment, which causes re-usage of single-use products, which is dangerous in terms of contamination. Due to the remote locations, a limited amount of resources and equipment can be brought (Bernice, Chinonye). Frequently the staff has to work with what's locally available, making the labs slightly improvised, dependent on what each household/community has to offer (*Figure 13*). Besides the resources, the environmental conditions play a role in the poor quality of the smears. The often hot temperatures provide an uncomfortable working condition; 'with high temperatures you just want to finish as soon as possible so you can go home' (Brice). This mind-set can lead to poorly prepared slides. Besides, the hot environment is not beneficial for the quality of the smears. Korse has seen smears dry in the sun for over two hours (because of negligence of the staff), affecting the smear quality. Also, preserving blood at this temperature is difficult without affecting its quality. Next to that, the field setting is psychologically different. The staff feels less in their comfort zone, because of the changing environment, but also because of a feeling pressure coming from the community (Brice). Regarding protocols, there are WHO procedural guidelines and the staff should follow these and other set protocols. However, this context is unregulated, making it uncontrolled and error sensitive.

2.3.5 CONCLUSION

Analysis of the multiple contexts shows that in the research lab context poor quality smears are not frequently seen. This is mainly because fully equipped labs that have a controlled environment and well educated staff. Next to that, the mind-set of research labs is totally different. There are no human lives at stake, leaving more room for errors without big consequences. On contrary, smears prepared in the field setting in general of lower quality compared to smears that are prepared in a lab setting. The field is a challenging, changing, non-sterile and unregulated environment with a minimum of available resources. This makes that the field staff constantly has to adapt to the situation, which requires experience and imagination. The first presents a problem; it turns out that in this context there is a lack of experienced and skilled staff that is capable of preparing high quality smears. Besides, the field setting is psychologically different. The staff feels less in their comfort zone, because of the challenging environment, but also because of a feeling pressure coming from the community. The environmental factors also influence the mind-set, e.g. the hot conditions make the staff want to finish as possible, which could also contribute to poorly prepared slides. Due to these circumstances, errors are more likely to be made in the field compared to any other setting. The data indicates that the field context, where medical staff moves to remote villages for smear preparation and diagnosis, is the context where the biggest positive impact on blood smear quality can be made. Therefore, the field will be the context of focus in this thesis.

Figure 14: Field diagnosis in a very primitive setting.

2.4 THE BLOOD SMEAR PREPARATION PROCESS

With the field as project context, it is of importance to have a thorough understanding of the way of working within this context. This chapter is a deep dive on the theory behind blood smear preparation, which criteria high quality blood smears must meet and how the procedure is executed in the field context. It sketches the current situation, the problems that are being faced and the current mistakes that are being made that lead to poor quality smears. Once the root of these mistakes is understood, the knowledge can be used to improve the quality and quality consistency of blood smears, through eliminating the errors or reducing their impact by means of a design intervention.

Figure 15 shows a diagram that includes the steps of the overall field preparation process. Each subchapter will provide more information about the specific steps in the process.

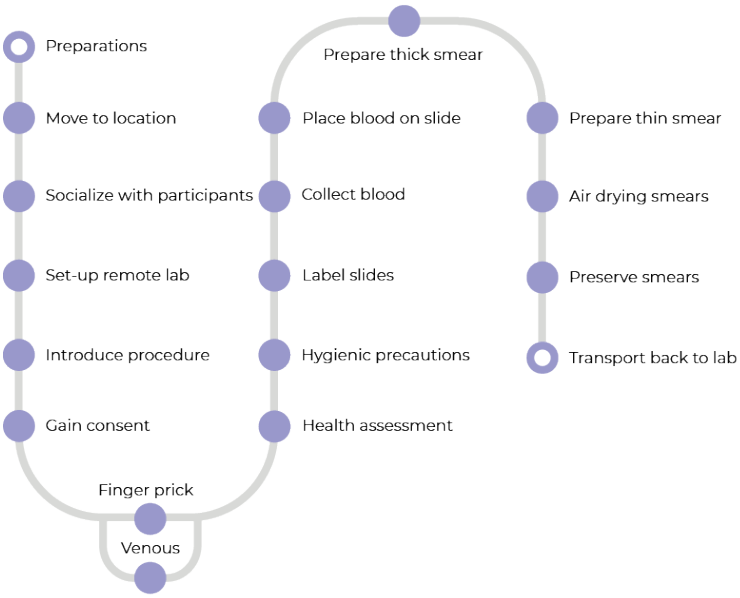


Figure 15: The overall steps of the field blood smear preparation process.

2.4.1 PREPARATIONS

Depending on the complexity of the project, field trips can take months of preparation (Chynonye). Preparations are necessary in terms of getting the permits, gaining consent from the community leaders, logistics, making a plan of action, collecting and cleaning equipment for the trip, arranging transportation and assembling staff. Being well prepared is important for the success of the field trip (Bernice, Brice).

Gaining the consent of community leaders is of importance. Having the community leader support the project is necessary to gain trust and cooperation of the participants. Without the consent it's nearly impossible to get to work, so convincing the community of the importance of the project and the impact on their health is crucial. It does happen that community leader do not give their consent. 'They sometimes say that there's nothing wrong with their community, that they are all healthy, but they're not' (Chinonye).

Next to that, having a good understanding of the amount of participants, the layout of the village and its available resources, contributes to an efficient workflow during the field trip. The amount of participants can vary from 40-1000, depending on the community size and goal of the project (Brice, Bernice, Chinonye). Based on this number the amount of staff and equipment that is required can be defined. Knowing what resources are locally available helps in anticipating on what kind of resources to bring to the field. Bringing all of the required equipment and enough of course important. If for example micropipettes are forgotten, the amount of blood used for smear preparation has to be estimated. This leads to inconsistencies in smear thickness and size and therefore also in quality (Brice).

2.4.2 THE REMOTE LAB

The diagnostic labs are often primitive and reliant on locally available resources, such as tables, chairs and other basics. *Figure 16* shows a primitive lab setting, where cardboard boxes are used as 'table', the other figure shows a slightly better context with a normal table and chairs. It frequently occurs that the most basic resources are not available, meaning the staff needs to adjust to the situation and cope with what they have Not having access to proper resources negatively influences the quality of the smears (Chynonye, Brice). It is easy to imagine the difference between preparing a thin smear on a cardboard box or on a solid table. Besides limited resources, there also is limited space due to the small houses. When going from house to house, the lab and equipment is preferably set-up inside the house, especially during the rainy season. Limited space is a problem and leads to discomfort for both the staff and participants, it interferes with comfortable smear preparation (Bernice).

Figure 16: Basic labs used for preparation and diagnosis.

2.4.3 INTRODRUCING THE PROCEDURE

Patient consent and cooperation are important components of respecting patient rights. Blood cannot be collected without a consent. A patient information leaflet or poster that explains the procedure in simple terms is a helpful tool to use. It visualizes the process, which makes it easier for participants to understand (WHO, 2010). Next to that, introducing the procedure to the participants is of importance to gain their trust. There often is quite some distrust among participants about the purpose their blood is used for. Some participants think their blood is used for private purposes of the staff, or due to religion or culture they think giving blood can lead to bad things (Chinonye). Therefore, having a clear introduction on the procedure and on the importance of the procedure on their own health, helps to gain trust and get consent for blood collection and smoothen the overall process.

2.4.4 HYGIENIC PRECAUTIONS

Hygienic precautions are important to take, especially in the non-sterile field context. According to WHO guidelines, it is necessary that all personnel is trained in personal hygiene, in order to avoid contamination. Blood and blood smears can easily be contaminated when guidelines are not being followed. Blood contamination is a serious problem that has effect on the quality, readability of smears and influences the reliability of the diagnosis. To avoid contamination of products, patients and personnel, personnel should wear clean protective clothing, carry out hand hygiene before and after each procedure. During blood collection and smear preparation disposable or sterile gloves should be worn. To remove the risk of environmental contamination with pathogens, counter and work surfaces, and chair arms should be cleaned with disinfectant at the start of each shift and when visibly dirty (WHO, 2010). Besides that, smoking, eating, drinking, and keeping plants, food, drinks, smoking material and personal medicines should not be permitted in areas used for blood collection and smear preparation (WHO, 2010b). Therefore, choosing a suitable location to setup the remote lab is of importance to minimize the risk of environmental contamination. However, often these guidelines are not being followed, due to a lack of equipment or competence. Often the labs are setup, understandably, in shadow rich spots under trees, however, this can lead to an increasing amount of contamination through particles that fall from the trees (*Figure 17*) (Brice, Shallig, Tope).

The equipment that is used needs to be clean and sterile, this can either be achieved by using new disposable equipment for each participant or through thoroughly cleaning used equipment with alcohol. However, the second option is risky, leading to equipment that is often not properly cleaned and sterilized (Korse, Schallig). This leads to difficulties with smear preparation, as this requires high quality and clean equipment, to avoid conamination. Bringing disposable equipment for every participant would require a lot of it, which is challenging due to the limited space and it can bring challenges related to costs.

The field context makes it challenging to strictly follow the hygiene guidelines. Due to the 'limited' amount of equipment that can to be brought and the costs of these materials, guidelines as using new disposable gloves for each participant, properly disinfecting reused items, not reusing disposable items are not always followed (Schallig). Next to that, the environment is not sterile, meaning environmental contamination plays a role in affecting the smear quality.

Figure 17: A lab set-up under the trees increases the risk of environmental contamination.

2.4.5 LABELLING

Labelling of the slides and EDTA blood tubes is necessary in order to distinguish the patient data and link a diagnosis to the right corresponding person. The slides should be labelled clearly with the patient name, location, date and time and sample number (Irvine, z.d.). Labels should be resistant to smudging and the effects of fixation, staining, immersion oil and cleaning of blood smears with inorganic solvents. The label should firmly adhere to the slide and not be easily removed. Printed labels especially should be checked for possible fading over time and for sensitivity to immersion oil and solvents commonly used in the laboratory. Labelling of blood smears by hand can be done by pencil; crayon (not sensitive to alcohol or other solvents); permanent marker pen; or diamond pencil at the frosted end of the slide (Corrons et al., 2004).

Wrongly labelling of slides and blood tubes is a frequently seen error in the field (Korse, Schallig, Chinonye). Due to the often large scale of participants, the risk of making errors is significant. Schallig stated that he sometimes sees unlabelled slides, which belong to 'no one'. Labelling mistakes obviously have great impact on the patient's diagnosis. Patient safety is the utmost concern, and specimen labelling errors can place patients in dire life-or-death situations (Houwen, 2002). Errors in labelling lead to a higher number of inaccurate test results, which can delay the patients results and treatment options. This is not only dangerous for the health of patients, but negatively impacts the trust in the medical staff.

2.4.6 BLOOD COLLECTION & PRESERVATION

Blood samples can be obtained by venous draw or skin puncture (finger prick), these procedures have been standardised. Depending on the amount of required blood and the research objective either one or the other is selected. Sometimes alongside smear preparation, RDTs and other blood tests are necessary. In this case a relatively large volume of blood is required and a finger prick would not be the appropriate method (Corrons et al., 2004).

Figure 18: Collecting blood from the finger, using a capillary tube.

There is a difference in who is allowed to draw venous blood and blood from a skin puncture. Venous blood sampling should only be performed by health workers for whom the procedure is in the legal scope of practise for their position in their country and who have demonstrated proficiency after formal training, whereas everyone is allowed to perform a finger prick (WHO, 2010). Venous blood is collected in vacuum tubes containing anticoagulant (EDTA). The anticoagulant prevents the blood from clotting. Ideally, when smears are prepared from blood collected into an EDTA tube, they should be prepared within 1 hour after drawing. However, due to the limitations of sample transport, this will not always be possible. Yet, blood with EDTA should be stored for no longer than three hours at room temperature (WHO, 2010). Blood from a finger prick is not collected in an EDTA tube, since it has a too small volume. It is often stored in a capillary tube or micropipette straight from the finger (*Figure 18*), after which is it directly used/applied. Plastic capillary tubes are recommended for placing blood drops onto the slide. Glass tubes should be avoided because of the possibility of breaking, causing a biohazard (Corrons et al., 2004).

Figure 19: Applying blood straight from the finger to the slide. This results in use of inaccurate blood volumes.

For malaria diagnosis in the field, a finger prick is the standard method (Schallig). The main problem found during blood collection is that often the blood volume requirements for smears are not met. Because of placing blood directly from the finger onto the slide (*Figure 19*), or due to the use of inaccurate tools, the applied blood volume is not consistent, leading to inconsistencies between smears. Applying the required blood volume is one of the variables that influence the quality of the smears and should therefore be concisely applied. Another problem is that it's sometimes difficult to collect the required amount of blood from a finger prick, especially with young children this is challenging. Bernice states that this leads to discomfort with patients, since they need to firmly press the fingertip or perform a second finger prick.

2.4.7 THICK SMEAR

Procedure

A thick blood smear is made by spreading a large drop of blood (6 µL) in a small area of about 1,2 cm (TDR & WHO, 2016). There are variations possible regarding the perfect combination of used blood volume and the surface area of the smear (see appendix 11.2). Thick smears with the correct thickness can be consistently prepared using measured volumes of blood spread over a fixed surface area. In order to consistently prepare similar quality thick smears, a frequently used method is the usage a template that indicates the required size of the thick smear (*Figure 20*). The template is placed underneath the slide during preparation, the larger circle is used as size reference to spread the blood in.

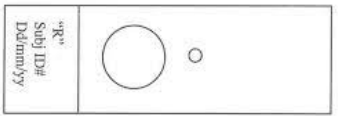


Figure 20: A template that can be used to put underneath the slide during preparation.

The thick smear preparation procedure is as follows (*Figure 21*):

1. Place a labelled slide on a template.
2. Place a 6 µL drop of blood from a pipette in the centre of the larger circle of the template
3. Use an applicator stick, spread the drop in a circular pattern until it is the area of the template is completely filled. Another technique is to use the corner of another slide (see appendix 11.3).
4. Lay the slide flat and allow the smear to dry thoroughly, protect it from dust and flies (CDC - DPDx, 2020).

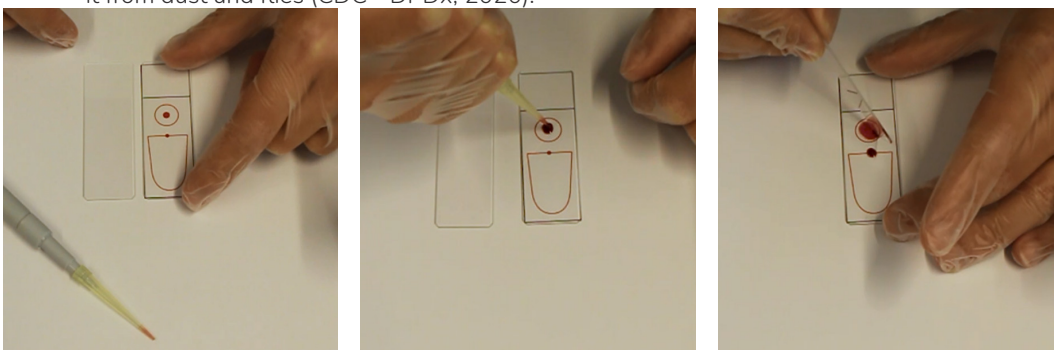


Figure 21: The thick smear preparation process, using a template.

Assessment & diagnosis

The flowchart in appendix 11.4 presents the procedural steps of diagnosing a thick smear. A minimum of 100 high-power fields must be examined before a thick smear can be declared as having “no malaria parasites seen”. If possible, the whole thick smear should be scanned. If parasites are observed, a further 100 fields must be examined before final identification of the species, ensuring that a mixed infection is not overlooked (WHO, 2016). This indicates that the thick smear must be at least contain 200 readable FOVs. *Figure 22* shows the order in which a thick smear should be examined. Results of microscopy of the thick smear may show a ‘normal’ result and an ‘abnormal’ result. Normal means no parasites are present in red blood cells. An abnormal diagnosis means there are parasites present in the blood. The density

of RBCs infected by the parasite is determined by counting the amount of cells and the amount of parasites (Healthwise, 2020). The infecting parasite species must be identified through examining the thin smear.

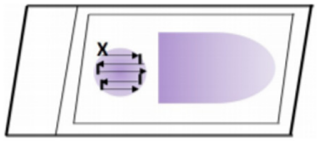


Figure 22: Thick smear examination order.

Quality requirements

In order to assess a thick smear on its quality, it must meet the following quality requirements:

- Has a minimum of 200 readable field of views with multi-layered red blood cells (CDC - DPDx, 2016).
- Has a proper density when a newsprint is barely readable through a wet slide (CDC - DPDx, 2020).
- Requires 6 µL of blood and have a diameter of 1,2 cm (CDC - DPDx, 2020).
- Are evenly spread, have 10-15 white blood cells per field of view at 1000x magnification (TDR & WHO, 2016).
- The red blood cells must be lysed (CDC - DPDx, 2020)
- Are free of dust and other debris to avoid confusion with parasites.
- The surface should be as uniform as possible (Zulhainan et. al, 2018).
- Is properly air dried for at least 10 minutes.

In practice

Preparing the thick smear is relatively easy and doesn't require much skills (Brice, Schallig, Korse). However, there are some common problems that occur during the process that affect the smear quality and readability. The first one being; not making use of the template and a micropipette. As stated, depending on the used amount of blood there is an optimal diameter for the thick smear. Not using a template and/or micropipette results in estimations in blood quantity and smear diameter. This results in smears that can be either too large and thin or too small and compressed. It frequently appears that surface areas and smear thickness differ from slide to slide (*Figure 23*). This influences the readability of the smear. With smears that are too thick, the cells will be too concentrated and the FOV will appear messy, making it difficult to determine cellular morphology. Besides, with a minimum of 200 field of views required to diagnose a thick smear, it is necessary that there is at least this amount of readable field of views available on the smear. In some cases the smear is of such poor quality that this requirement cannot be met.

A frequently observed problem that is found in thick smears is the non-uniformity of the smear's surface, this non-uniformity can also be seen in *Figure 23*. Because the blood is manually spread with the edge of a slide or with an applicator stick, there are differences in blood layer thickness. As discussed, for a good thick smear with the proper layer thickness, a newspaper should be barely readable through the blood. However, because of the inconsistency in uniformity, there are parts that are too thick and too thin, which are less suitable for diagnosis. This results in the microscopist having to search for a readable and

diagnosable spot in the thick smear. This can make reading the thick smear more time consuming than necessary.

A problem that arises with thicker smears are their relatively long drying times. Besides that fact that this is inefficient, it can also lead to problems when storing not fully dried slides. Slides stored in slide boxes are positioned on their side, allowing the blood to flow of the slide when not properly dried. This can lead to unusable slides and contamination of other slides. Another problem with thicker smears is that the stain may not be able to penetrate the entire thickness of the thick smear.

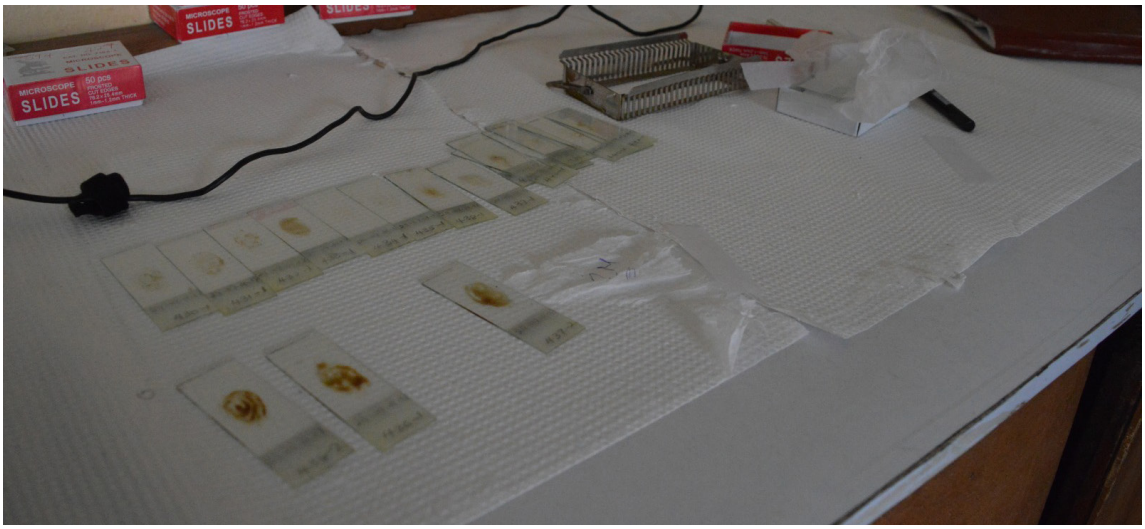


Figure 23: Thic smear laying to air dry. Their quality is inconsistent.

2.4.8 THIN SMEAR

Procedure

The template used for thick smears is also used for thin smears. It indicates the location of the drop of blood and its size. Some templates also indicate its supposed shape (*Figure 24*). The goal is to produce a blood smear with evenly distributed RBCs in the monolayer area of the smear (Allison & Meinkoth, 2007).

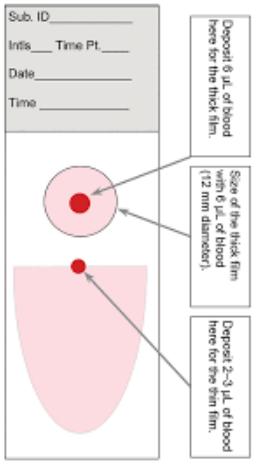


Figure 24: A preparation template indicating the size and width of a thin smear.

The thin smear preparation procedure is as follows (*Figure 25*) (CDC - DPDx, 2020):

1. Place a labelled slide on a template.
2. Place 2–3 µL of blood on the slide at the spot indicated by the template.
3. Bring another slide, the spreader, with the unfrosted end at a 30-45° angle up to the drop, allowing the drop to spread along the contact line of the two slides.
4. Quickly, but smoothly, push the spreader slide towards the (unfrosted) end of the lower slide.
5. Dispose or thoroughly clean the spreader slide before re-use.
6. Lay the slides flat and allow the smears to dry thoroughly, protect it from dust and flies.

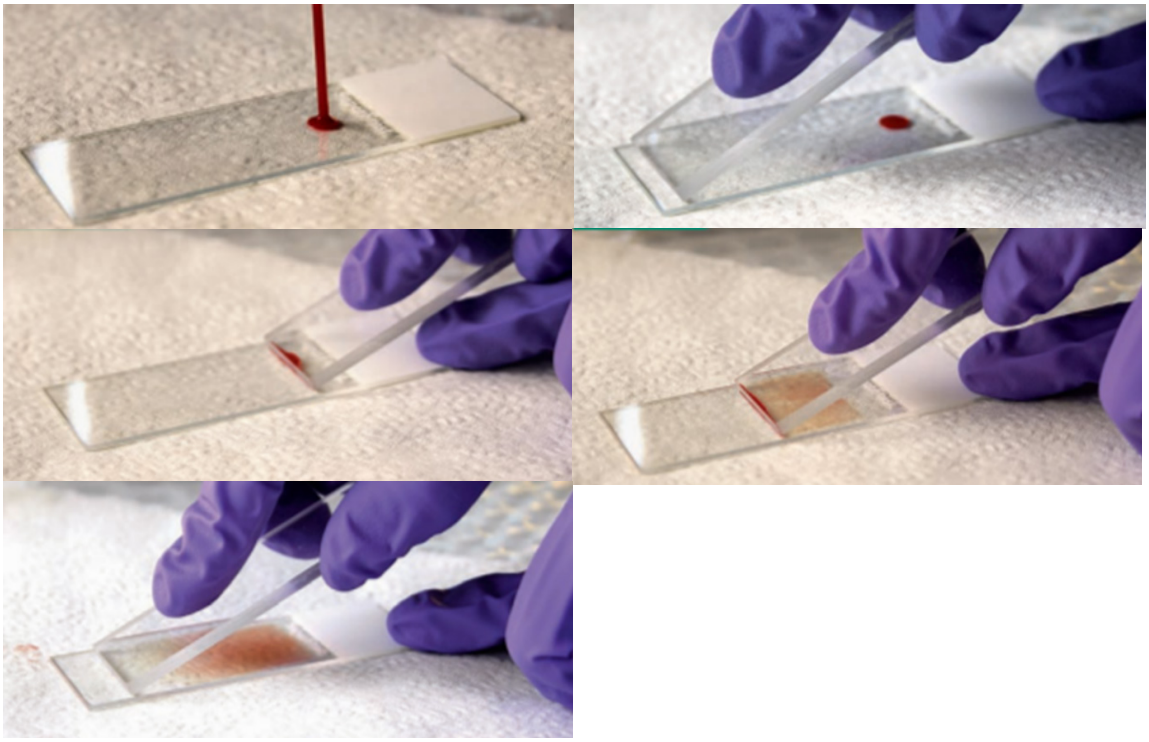


Figure 25: The thin smear preparation process.

Technique

- Unlike the thick smear, thin smears are relatively difficult to prepare and require a certain well-developed technique. To obtain blood smears of consistent quality, one must use the same technique every time (Sysmex, 2013). Therefore, producing a good quality smear requires practice (Dacvp, 2012). Thin smears are most commonly prepared using the 'spread' or 'wedge' technique, another less frequently used technique is the spin technique (see appendix 11.5). A well-prepared thin smear becomes progressively thinner with good separation of cells towards the tail of the smear. The so-called 'monolayer', the area of optimal thickness for light microscopic examination, should be at least 2 cm in length (Sysmex, 2013). The quality of the smear is influenced by four main variables: speed, angle, push force and blood volume
- The faster the spreader slide is moved, the longer and thinner the smear will be. The slower the movement, the shorter the smear (Sysmex, 2013).
- An angle greater than 30° makes the smear thicker; less than 30° makes it thinner. In general it is recommended to keep the angle between 30-45°. However, the angle varies based on the viscosity level of the blood (appendix 11.6) (Sysmex, 2013).
- Apply only enough pressure to keep the spreader slide on the glass, excessive pressure will push too much blood forward without allowing development of a good body and monolayer (Neel, 2017).
- A small drop of blood may be insufficient to prepare a slide of sufficient length, a too large drop may cause the smear to extend beyond the length of the slide or will result in a too thick smear (CDC - DPDx, 2020).

Technique

The flowchart in appendix 11.7 shows the process steps of diagnosis of the thin smear. Examination of the thin smear should be done by reading the monolayer area of the smear. During diagnosis, move along the edge of the smear, then move the slide outwards by one field, inwards by one field, returning in a lateral movement and so on (*Figure 26*). The examination should be continued until the presence and species of malaria parasites have been confirmed. All the observed species must be identified and recorded (Osorio et al., 2007). The guidelines to the amount of field of views for proper diagnosis vary, some recommend scanning a total of 2000 RBCs, others a minimum of 300 monolayer field of views (CDC-DPDX, 2016).

Figure 26: The diagnosis order of a thin smear.

Quality requirements

Well-made thin blood smears must meet the following requirements:

- Has a minimum of 300 readable field of views with mono-layered red blood cells (CDC-DPDX, 2016)
- Has a dense body and a blood thickness that decreases towards a 'feathered edge' at the end (Neel, 2017)
- Requires 2-3 µL of spread out blood and takes ½ to 2/3 of the slide's length (Al Sadoun, z.d.)
- Has a smooth surface, without irregularities, holes or streaks (Al Sadoun, z.d.)
- Are free of dust and other debris to avoid confusion with parasites.
- The surface should be as uniform as possible (Zulhainan et. al,2018).
- Is properly air dried and should be well fixated to the slide.

In practice

Preparing a thin smear is considered as the most difficult and error sensitive part of the whole preparation process (Brice, Bernice, Schallig). The four variables in thin smear preparation are the main influencers of the quality of the smear. Because the variable parameters must be applied in the correct way, experience and a well-developed technique are required for high quality thin smear preparation. Wrongly applying either one of the variable parameters will most likely result in poor quality smears. This is a frequently observed reason for poor quality thin smears in the field (Brice, Bernice, Schallig). The field setting makes it even more difficult to apply the variable parameters in the intended way, presumably making this aspect of thin smear preparation the most impactful on smear quality. Unfortunately, there is a lack of experienced staff, resulting in inexperienced staff preparing smears. The technical staff is not always competent enough to operate in the challenging field context. Meulah Brice says the following: 'Passing a smear preparation training doesn't mean you have experience yet. It can take a long time before some experience is gained'.

Figure 27 shows common mistakes and how to adjust the variable parameters for a better results.

	Correction of technique
Angle of spreader slide	• If the smear is too long, try to increase the spreader angle closer to 45° • If the smear is too short, try to decrease the spreader angle closer to 30°
Speed of spreader slide	• If the smear is too long, try to increase the speed of the spreader slide • If the smear is too short, try to decrease the speed of the spreader slide
Downward pressure of spreader slide	• If the smear appears streaky, apply less downward pressure while pushing the spreader slide forward • If the spreader slide feels unstable, apply more downward pressure and hold the spreader slide further down • NOTE — excessive downward pressure can result in slide breakages and the potential for injury
Hesitation	• Hesitation or stopping while creating the smear will result in horizontal lines across the smear, and an undesirable shape

Figure 27: Adjusting the angle, pressure and speed variable to increase the smear quality.

Appendix 11.8 shows a table with common errors that are being made and how to adjust the technique to improve the smear quality.

Because of wrongly applying the variable parameters smears often appear too thick, thin or short, leading to difficulties with diagnosis. Extremely thin smears may result in a too wide spread of RBCs and could make the RBCs appear as spherocytes, which is an indication to other diseases (Sysmex, 2013). A smear that is too thick will have overlapping RBCs, making diagnosis impossible, since it's required the RBCs lie next to each other.

Another observed problem in the field is the earlier mentioned unsterile environment and use of non-clean equipment. Especially for the thin smears, making use of clean equipment greatly enhances its quality, since use of e.g. a dirty spreader will result in contamination and irregularities in the smear.

Glass slides

The glass slides on which the thick and thin smear are prepared, play a fundamental role in ensuring high quality smears. Using high quality slides is of importance for efficient and effective smear preparation, however here is a lot of difference in slide quality. High quality slides do not fog or become opaque in tropical conditions. Poorer quality slides are cheaper, but deteriorate much faster in a hot, humid climate. The slides must have ground edges and a frosted end, so that the slide can be labelled. They must be made from corrosion resistant glass, other material, such as plastic is mostly not acceptable. Before usage, the slides must be washed, dried and wrapped. It is of importance that the slides are clean and scratch-free. Dirt and scratches on the slides will result in poorly prepared blood smears, which can compromise the quality and integrity of diagnosis. Even slides that are slightly scratched are considered unsuitable for blood smear preparation (Houwen, 2002).

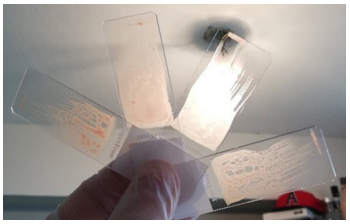


Figure 28: Unclean and non-sterile slides lead to poor quality smears.

To ensure that the staff has the correct materials, cleaned, wrapped slides, stains and other supplies are often prepared and provided from a central location. In some rural areas, however, laboratory staff must clean their own slides (Houwen, 2002). Cleaning slides must be done according to a procedure, for the procedure recommended by the WHO see appendix 11.9. This procedure is quite long and consists of multiple steps. However, cleaning slides this properly is frequently not done properly in the field setting (Korse). According to Korse, poor quality slides are frequently seen in the field. This is due to re-usage of slides and improper cleaning after use. Besides, it is challenging to store the slides in tropical and dusty conditions, without deteriorating quality. Poor quality or unclean slides greatly affect the ability of proper smear preparation. *Figure 28* shows the results of thin smears prepared on improperly cleaned slides.

Spreader slides

In practice, a second slide is often used as a spreader, and this is acceptable if the spreader slide has rounded or bevelled edges making the spreading width of the slide narrower than the actual slide width (*Figure 29*). The edge of a spreader should always be smooth to ensure even thickness for the entire width of the blood smear. Spreaders should be discarded after use, or cleaned thoroughly and dried before reuse. The presence of blood cells from a previous specimen on the spreader edge can cause significant carryover of blood cells, including malaria parasites in red blood cells and leukemic white blood cells, into the next blood smear (Houwen, 2002).

Figure 29: Types of slides. For smear preparation slides with bevelled edges should be used.

Air drying

Once the smears are prepared, the slides are laid in the open air to dry. Air drying both the thick and thin smear is needed to dry the blood and make it fix to the slide, which enables staining (TDR & WHO, 2016).

The duration or method of drying the slides may be varied but the parameters of the method used should be validated with respect to the equipment used, typical humidity of the environment and whether or not the blood is anti-coagulated to ensure that the thick smears are neither heat-fixed nor washed off. The duration of drying may need to be prolonged if the blood has been collected with an anti-coagulant such as (EDTA) (TDR & WHO, 2016). The typical air dry time is a couple of minutes for the thin smear, but can be up to 20 minutes for the thick smear.

Figure 30: Smears air drying in the open air. This leaves the smears exposed to e.g. dust and insects, which affects their quality.

The main problem during air drying of the smears is caused by environmental factors. While air drying, the slides often lay in the open air and are not covered (*Figure 30*). The smears are exposed to the open air for the duration it takes to dry, which is at least 10 minutes. There are drying boxes available on the market, but they are often not used by the field staff. Their reasoning for this is simply because they don't have it. Drying without cover results in flies, ants and small particles falling from trees settling on the smears, affecting its quality. Especially during the dry season, with lots of dust particles in the air, this is a problem (Brice).

Even though dust cannot totally ruin a smear, it does affect the readability of the slide, since these particles also colour during the staining process (Schallig, Brice). Having all kinds of small particles in the smears makes it difficult to make a distinction between contamination and actual parasites or other infections. Besides, Korse

stated that she saw smears dry for over two hours in the sun, while the staff was drinking coffee, this does greatly affect the smear quality. It appears that there is little understanding about the implications open air drying can bring. This could be due to the fact that the staff that prepares the smears doesn't necessarily read them under the microscope, meaning there is no quality feedback.

Fixation

Before staining, the thin smear should be fixed by quickly dipping the thin smear in pure methyl alcohol (Klassen-Fischer et al., z.d.). In general this is done by dipping the bottom of the slide (containing the thin smear) in a cup that contains the methanol (i*Figure 31*). Fixation is done to minimize cell damage and the production of artifacts and is essential for good staining and presentation of cellular detail (Brown, 2020). Optimal results are obtained by fixing and staining directly after the blood smear is completely air-dried (Houwen, 2002). The thick smear must not be fixed, as the cells of the thick smear must lyse. So close attention must be payed to not accidentally fixate the thick smear.

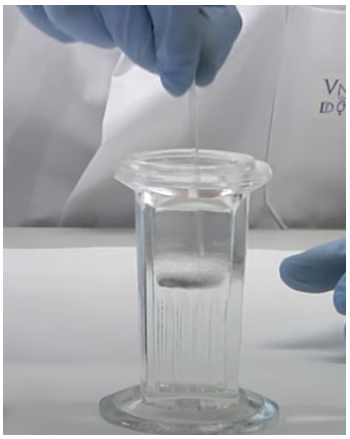


Figure 31: Fixation of the thin smear, by dipping the smear into 100% methanol.

Staining

After fixation the blood smears are stained, to distinguish the cells from each other. Staining is the colouring process of the thick and thin smear, which enhances contrast in samples, in order to allow examination of the various blood cells. A properly stained blood smear is critical for malaria diagnosis, especially for precise identification of malaria species (WHO, 2016a). The staining process 'reveals' the parasites that are present in the blood by colouring them, making them visible during microscopic assessment (*Figure 32*). The smear is covered with stain for approximately 10 minutes (*Figure 33*), then deluded with distilled water and placed on a drying rack. For the full Giemsa staining procedure according to the WHO, see appendix 11.10. In general, the use of Giemsa stain is recommended, since it's the most reliable for staining thick and thin blood smears (WHO, 2016a). Staining and the preparation of the stain itself is a precise process. Therefore, it is of importance that the making of the stain and applying the stain is performed accurately by a professional.

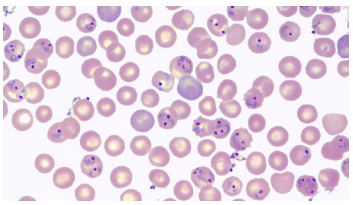


Figure 32: Stained RBCs revealing the parasites.

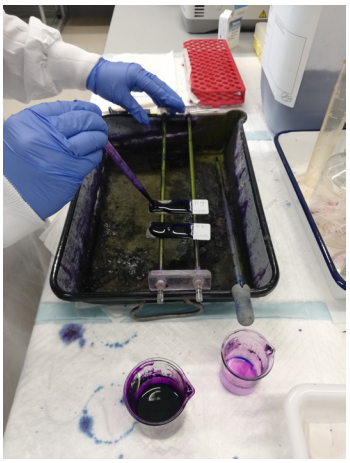


Figure 33: Covering the smears in stain at LUMC.

There are commonly seen mistakes in the staining process. One of the main mistakes is using weakened or 'exhausted' stain. In third world countries, using weakened stain frequently occurs due to the thought that it is a pity to throw it away and due to wrongly labelling the date of expiry (Brice). Stain solutions do weaken with repeated use, and prolonged exposure of the fixative to air at room temperature can result in impaired staining from formation of degradation products. How often the stain needs to be changed will depend on how many slides are put through it (WHO, 2016a).

Another problem is contamination. 'Stain contamination occurs when 'dirty' specimens such as faecal smears, skin cytology or material from abscesses are stained in the same solution as 'clean' blood smears. The easiest way to avoid this is to set up two staining stations, one for clean samples and one for dirty samples. Contamination of the fixative with

water can produce refractile 'water artifact' on slides. Slides should be completely dry before staining, slide holders should be dried before slides are loaded, and stains should be tightly covered when not in use. However, when staining is done in the field, it is difficult to avoid contamination of the stain, since it happens in the open air' (NEEL, 2017).

Preservation & storage

In principle, staining is a way to preserve blood smears. Once stained, the smears can be preserved and stored for a long period. However, it is well known that unstained blood smears are not easy to preserve. The stippling of RBCs degenerates within a few days and the malaria parasites become unrecognizable in a few weeks (Hosseini & Feng, 2012). Transport of blood smears can be risky, since the slides are vulnerable. Therefore storage of blood smears should be done in dedicated storage boxes (*Figure 34*). Besides, keeping the smears in a dark enclosed area is of importance to maintain it quality. In contradiction to drying boxes, these boxes are frequently used in the field for transportation purposes. It is of importance the smears are completely dry, if not, the blood might flow off the slide, which can lead to contamination of other slides.

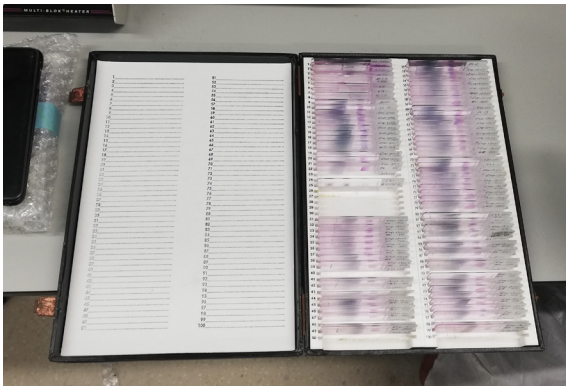


Figure 34: A slide preservation and transportation box.

Waste management

Waste materials resulting from malaria diagnostic testing can be infectious or environmentally damaging. There should an organized health-care waste management system to protect the staff, community and the environment. 'For an efficient, cost-effective waste management system, health personnel should be fully conversant with and trained in the segregation and disposal of different types of health care waste' (WHO, 2016b).

Some waste generated from RDTs can be infectious, vused sharps (lancets and needles) can cause serious injury or illness. If they are contaminated with blood or other body fluids, they can cause infection with hepatitis B, hepatitis C, HIV, and other infectious diseases. To protect health personnel, waste handlers, and the community against potential injury, all providers must establish safe, environmentally sound ways to handle and dispose of waste. Inappropriate handling

and disposal will have serious implications for everyone's health (PMI, z.d.)

WHO set strict guidelines for waste management of possible infectious waste, however as discussed in chapter 2.3 guidelines and procedure are not taken too seriously in the field context. This results in relatively poor waste management and could lead to hazardous situations for staff and participants.

Light microscopy with stained blood smears

Light microscopy (*Figure 35*) remains the gold standard, because of its accuracy and the amount of information that can be obtained through microscopy (Fançony et al., 2013). However, the sensitivity of light microscopy diagnosis is limited by the number of FOVs examined. When the WHO recommended procedure is followed, the theoretical detection limit is 5 parasites/µl of blood for a sensitive test. This, along with the possibility to obtain species and life stage information, are the main advantages of this diagnostic method. Furthermore, the relatively inexpensive equipment makes this the most cost-effective test currently available for highly endemic settings, even without taking into consideration that light microscopy equipment can also be used for the diagnosis of other diseases (Wilson, 2013). However, the preparation of the stained blood slides and the data interpretation are labour intensive and require highly trained experts (Tangpukdee et al., 2009).

Figure 35: A microscopist performing microscopic diagnosis.

A non-experienced microscopist can make many errors when interpreting the data; misidentification of the parasite species is common, as well as seriously underestimating the parasite count (OHRT & TANG, 1999). The applicability of this method in on-field settings is further limited by the required maintenance, the cost and the need for electricity of the microscopy equipment. To address this limitation, several simple, portable microscopes which are battery operated have been developed over the years. For example, Agbana et. al proposed that the wide-spread availability and advances in imaging capabilities of mobile phones could be leveraged on, by attaching an oil immersed ball lens onto the built-in camera, which effectively turns a mobile phone into a microscope (Wongsrichanalai et al., 1991).

Rapid diagnostic tests (RDTs)

In order to reduce diagnostic complexity and allow for point-of-care testing, RDTs were developed (*Figure 36, Figure 37*). RDTs are lateral flow immunochromatographic tests, that detect the antigens produced in human blood by the presence of malaria parasites. They consist of a strip of nitrocellulose, with some dye-labelled antibody specific for the target antigen on one end and a test line of antibody on the other end. A sample of the patient's blood is collected by a prick to finger, mixed with a buffer and applied to the dye-labelled antibody end. If malaria antigens are present in the blood, these labelled antibodies will attach to them, be carried over to the strip by the buffer and accumulate on the test line. This produces a visible line, which indicates a positive test result (Moody, 2002). Several WHO-qualified RDTs are available commercially and have been tested extensively in laboratory and field. The average cost per test in endemic settings is higher than that of light microscopy (Obeagu et al., 2018). The most commonly used ones can only detect P. falciparum or P. vivax, but tests that can detect multiple species and even distinguish between them are also available. A crude estimation of the parasiteamia can be made by the intensity of the test line. The main advantage of RDTs is that no expertise is required to administer and interpret them, which means they can even be used for self-diagnosis (Whitty CJM et al., 2000). Furthermore, they only take 15-30 minutes to process and require no electricity or additional equipment, making them uniquely suitable for in-field use. However, RDTs have several limitations. They are less sensitive (detection limit ± 100 parasites /µl), making them unsuitable for detecting early-stage infections. Some commonly used target antigens remain present in the blood beyond the clearance of the parasites, severely limiting the specificity of these tests (51 %) and making them unsuitable for disease monitoring or detecting repeated infections (Mbabazi et al., 2015). Besides, tropical doctor Henk Schallig states that a lot of information is lost with RDTs. An ill patient with a negative malaria diagnosis can be ill through an infection of another parasite, with microscopy this is diagnosable, with RDTs it's not. Besides, RDTs are not sensitive for malaria mutations that are currently on the rise, a test can be negative for a certain type of malaria, while the mutated version is present in the blood. Next to that Schallig states that due to the huge rise in use of RDTs, skills and experience is lost on the aspect of smear preparation and microscopy. This is problematic since, microscopy remains the gold standard in malaria diagnosis.

Figure 36: A rapid diagnostic test (RDT) for rapid malaria diagnosis.


Figure 37: A rapid diagnostic test done in the field.

2.5 CHAPTER CONCLUSION

From the step-by-step analysis of the smear preparation process in the field context multiple conclusions can be drawn.

Preparations are essential for the success of a field trip, especially gaining consent of the community leader is an important but sometimes challenging step. Having an understanding of the community resources and the amount of participants helps in smoothening the operation. It is noted that quite a variety of equipment that needs to be brought and the quality of the smears can be highly affected when certain essential equipment is forgotten. Minimizing the required amount of equipment for smear preparation would reduce this risk.

Contamination because of poor hygiene is a problem in the field context. However, due to the difficulty in assessing whether a smear is contaminated through not following hygienic precautions or by something else, it is difficult to make an estimate on the frequency this happens. According to tropical doctor Henk Schallig it's not a frequent occurring problem, however when it happens it highly affects the accuracy of diagnosis. De-risking contamination can be done through avoiding re-usage of materials, such as the spreader slide, and make use of disposable materials. When reusing materials it must be ensured the materials are thoroughly cleaned.

Blood collection involves critical steps. Sometimes there are difficulties in collecting the required volume of blood from a finger prick, that is needed for smear preparation. Especially with younger children this is a challenge, sometimes also due to their unwillingness to cooperate. Next to that, placing the required volume for each of the smears on the slide can be challenging. The blood volume for each of the smears affects the thickness of the smears, ensuring the right volume is used is important. Often the blood is placed straight from the finger on the slide, leading to different used blood volumes per slide, thus inconsistencies in the smears. Regulating the blood volume for both the thick and thin smear is an opportunity towards more consistency between slides.

The quality of the thick and thin smear is, obviously, most depending on how the actual smears are prepared. For both smears it is unwanted to have too thick or too thin smears. Too thick thick smears have trouble drying and trouble staining, it results in a lot of 'waste' making the parasites hard to see (Schallig). Too thin thick smears don't offer enough red blood cells and parasites to do diagnosis. Diagnosing thin smears requires the red blood cells to lay next to each other, not

on top of each other. Too thick thin smears cannot be read properly, as the stacked red blood cells appear as a blurry black mess under the microscope. Too thin thin smears cannot be properly diagnosed, because there are too little red blood cells per field of view and the RBC distribution is probably uneven. An uneven layer thickness for both smears result in an in-efficient workflow and difficulties with diagnosis. Ensuring the surface thickness of both smears is consistent in thickness and surface area, will increase the efficiency and accuracy of the diagnosis process. One of the main causes for poor quality thin smears is wrongly applying the variable parameters. Ensuring the parameters are applied in the correct way would increase the success rate.

For both, but especially the thin smear, experience is a key factor in being able to prepare high quality smears, especially in the challenging field setting. Due to a lack of experienced staff, inexperienced staff prepares smears, leading to poor quality smears. A design intervention that ensures inexperienced staff can make high quality smears is expected to have a high impact on the average smear quality.

Air drying presents a problem, since slides are frequently dried in the open air without coverage. This allows flies, ants, dust and other particles to settle on the slides while drying, affecting it's quality. Contamination from grease and dirt interferes with accurate visualization of the cells and organisms and should therefore be prevented. Ensuring the slides are covered while air-drying would take this problem away.

It can be concluded that the process of preparing and diagnosing thick and thin blood smears requires precision, experience and high quality equipment. The data gives insight in the global requirements that must be met during different stages of the process, in order to facilitate the preparation of high quality blood smears and accurate diagnosis. The smears can be seen as the core of malaria diagnosis - without good smears there is no accurate diagnosis. Because smear preparation is the beginning step of the process, it's critical that the smears meet the quality requirements. Poor quality smears will lead to misdiagnosis and eventually wrong treatment. This sketches the essence of assuring the quality of the smears. However, the challenging part is that the quality of blood smears is dependent on many factors; the competences of the staff, how well they follow the procedures and apply the right techniques, the quality of the equipment and the sterility of the environment. The influences of these factors need to be controlled in order to keep control over the smear quality.

2.6 CRITERIA & CHALLENGES

The criteria (cr) and challenges (ch) coming forth from the in-context procedural analysis are listed in this section. Throughout the report, additions to this list will be made.

1. **Context**
   - 1.1 (ch) There is inexperience among medical staff that prepares smears and performs diagnosis (2.3.4).
   - 1.2 (ch) The field context is non-sterile and unregulated (2.3.4).
   - 1.3 (ch) The climate is challenging and causes difficulties (2.3.4).
   - 1.4 (ch) The field is a low resource setting, while high smear quality relies on high quality resources (2.3.4).
2. **Material & equipment**
   - 2.1 (cr) Slides should be of the highest quality level, they must have ground edges and a frosted end (2.4.10).
   - 2.2 (cr) Slides must be clean and scratch-free (2.4.10).
   - 2.3 (cr) Spreader slides must have ground edges and a smooth surface without scratches (2.4.10).
   - 2.4 (cr) Slides must be thoroughly cleaned according to the WHO standards after use, when re-used (2.4.10).
   - 2.5 (cr) Infectious equipment must not be re-used for another patient (2.4.4)
   - 2.6 (ch) There often is a lack of resources in the field setting (2.3.4)
   - 2.7 (ch) Re-use is difficult to avoid in the field context, as there are limited available resources (2.3.4).
   - 2.8 (ch) Equipment is often not well cleaned or sterilised (2.3.4).
   - 2.9 (ch) Storing the slides in tropical and dusty conditions, without deteriorating quality (2.4.14).
3. **Blood collection**
   - 3.1 (cr) Blood must be collected using high quality equipment that facilitates accurate measurement and application of the blood volume (2.4.6).
   - 3.2 (ch) Accurately measuring and applying the required blood volume, as there is often no access to these tools (2.4.6).
   - 3.3 (ch) Obtaining sufficient blood from a finger prick, especially with children (2.4.6).
4. **Smear preparation**
   - 4.1 (cr) Both the thick and thin smear must be prepared on one slide (2.2)
   - 4.2 (cr) The thin smear must be prepared with the correct angle, pressure and speed variables (2.4.9).
   - 4.3 (ch) Correctly applying the angle, pressure and speed variables (2.4.9).
   - 4.4 (ch) Inexperience of the staff in the field setting (2.3.4).
5. **Thick smear quality**
   - 5.1 (cr) Must have a minimum of 200 readable field of views with multi-layered red blood cells (2.4.8).
   - 5.2 (cr) The letters of a newsprint must be barely readable through a wet thick smear (2.4.8).
   - 5.3 (cr) Requires 6 µL of blood and must have a diameter of 1,2 cm. (2.4.8).
   - 5.4 (cr) Are evenly spread, have 10-15 white blood cells per field of view at 1000x magnification (2.4.8).
   - 5.5 (cr) The red blood cells must be lysed (2.4.8).
6. **Thin smear quality**
   - 6.1 (cr) Must have a minimum of 300 readable field of views with mono-layered red blood cells (2.4.9).
   - 6.2 (cr) Must have a dense body and a blood thickness that decreases towards a 'feathered edge' at the end (2.4.9).
   - 6.3 (cr) Requires 2-3 µL of spread out blood and must take ½ to 2/3 of the slide's length (2.4.9).
   - 6.4 (cr) Must have a smooth surface, without irregularities, holes or streaks (2.4.9).

7. General smear quality

7.1 (cr) Smears must be free of dust and other debris (2.4.11).
7.2 (cr) The smear surface must be as uniform as possible (2.4.8).
7.3 (cr) The smears must be fully dried (2.4.8).
7.4 (ch) Meeting the quality requirements due to inexperience and poor facilities and equipment (2.4.9).
7.5 (ch) There is no quality feedback, the staff doesn't really know whether they've prepared a high or low quality smear (2.4.11).

8. General procedure

8.1 (cr) The lab must be set-up in an environment that is as sterile as possible (2.4.11).
8.2 (ch) The remote labs are often reliant on locally available resources. It frequently occurs the most basic ones are not there (2.4.2).
8.3 (cr) The hygiene protocol must be followed (2.4.4).
8.4 (ch) During the rainy season there often is not much space to set up the lab indoors (2.4.2).
8.5 (ch) Gaining the trust of the participants (2.4.3).

9. Labelling

9.1 (cr) Slides must be labelled with patient data (2.4.7).
9.2 (cr) The label must be resistant to the effects of fixing, staining, immersion oil and cleaning (2.4.7).
9.3 (ch) Unlabelled or wrongly labelled slides are frequently observed (2.4.7).

10. Fixation

10.1 (cr) The thin smear must be fixated in 100% methanol after drying (2.4.9).
10.2 (cr) The thin smear must be fixated directly after air drying (2.4.9).

11. Air drying

11.1 (cr) Both smears must be air dried for at least 10 minutes (2.4.11).
11.2 (cr) The smears must be air dried protectively (2.4.11).
11.3 (ch) Protective air drying is often not done, leaving the smears vulnerable to environmental contamination (2.4.11).
11.4 (ch) The relatively long drying time of the thick smear leads to long exposure of the smears in the open air (2.4.12).

12. Staining

12.1 (cr) Both smears must be stained according to the staining procedure (2.4.13).
12.2 (cr) High quality and non-expired stain must be used (2.4.13).
12.3 (ch) Contamination of the stain, due to exposure to the open air (2.4.13).

13. Storage

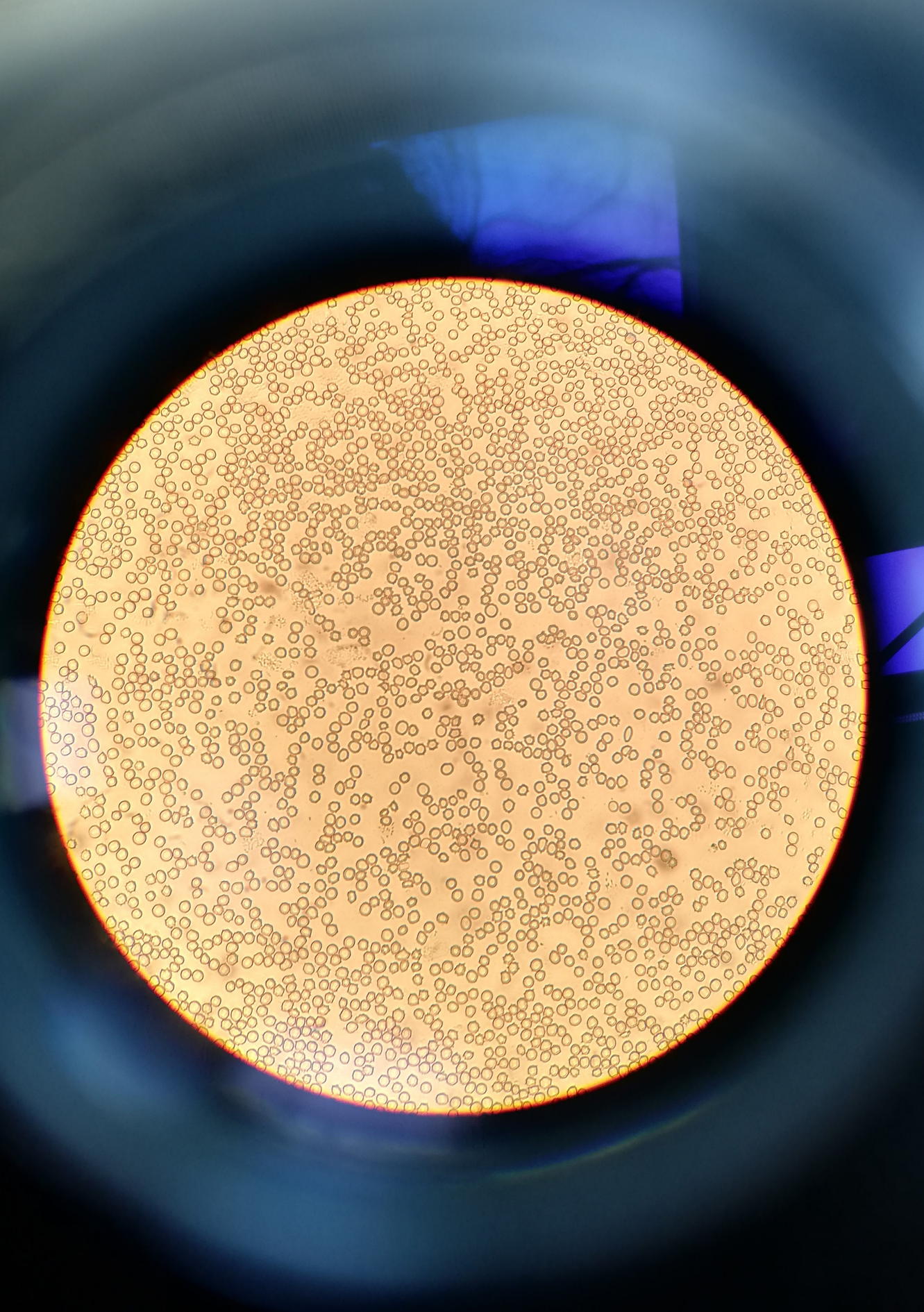
13.1 (cr) stained blood smears must be preserved in storage boxes, protecting them from light and dust (2.4.14).

14. Waste

14.1 (cr) The WHO guidelines must be followed regarding medical waste (2.4.15).

15. Diagnosis

15.1 (cr) Blood smears must be diagnosed through microscopy (2.4.16)
15.2 (cr) Diagnosis should be done by an educated and experienced individual (2.4.16)
15.3 (cr) Diagnosis of the thick and thin smear must be done according to the procedure (2.4.8 & 2.4.9).
15.4 (ch) Due to the rise of RDTs, microscopic diagnosis experience is decreasing (2.4.16).

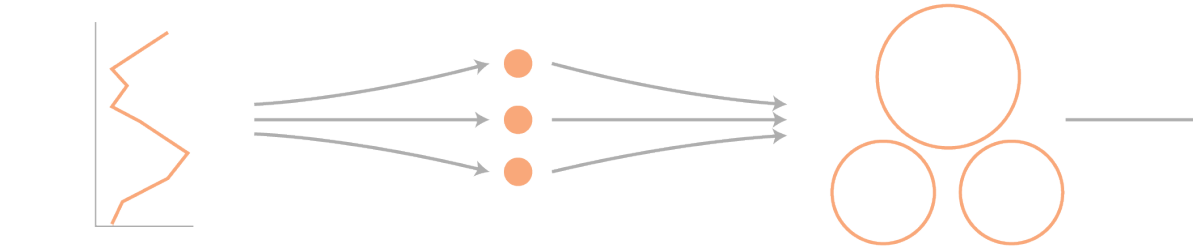


CHAPTER 3

SYNTHESIS

In the synthesis chapter the most important findings from the analysis phase are distilled. The information elements are combined to a shape a connected whole, where clear problem statements are made and eventually a design direction, solution space and vision are formulated. This shapes the foundation and start of the upcoming design process.

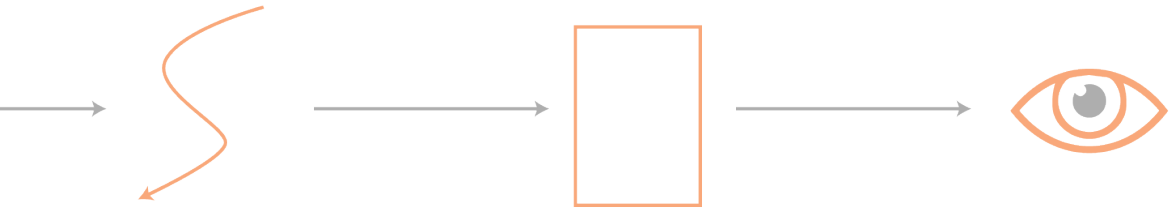
3.1 METHOD



Critical step impact

Problem definitions

Problem clusters



Design direction

Solution space

Vision

First, with the data from the analysis phase, the impact on the smear quality of each step in preparation process is mapped. The focus of the project is then set on the critical steps with the highest direct impact, which can be reduced by means of a design intervention. Within this 'problem space', clear problem statements are formulated regarding the current field situation. The overarching causes of these problems are then defined through clustering. Eventually, based on this information a design direction, solution space and future vision are formulated.

3.2 CRITICAL STEP IMPACT

The analysis chapter defines the current problems within the process' steps. It is important to know what the actual impact of the problems within these steps is on the quality of the smears. *Figure 38* visualises the relative impact per step on the smear quality. This data was obtained by interviewing experts from the field through an interactive online interview, this graph presents an average.

Three peaks are identified from the graph. The first peak at 'preparations' is due to the fact that collecting and bringing the required equipment in the right amount is key for a successful field trip. When essential equipment is forgotten or there is shortage, e.g. a micropipette, this highly affects the smear's quality. Besides this, other preparations such as having a plan, gathering the right permits, staff, informing the community all have effect on the efficiency and effectiveness of the process and indirectly on the quality of the smears. However, the impact of this step on the smear quality is indirect and interviewees stated that the frequency of occurrence of this problem is low.

For direct impact on the smear quality, the third and highest peak is most interesting too look at. The rising steps towards the peak regard blood collection and placement. They appear not to have major impact, but still they are direct influencers on the smear quality. The main peak concerns the actual preparation of the thick and thin smear. The errors occurring in these steps seem to have the most significant impact on the quality of the smear. The peak at the thick smear is lower compared to the thin smear, this can be explained due to the fact that the thin smear is more difficult to prepare and requires more skill and experience, more critical errors are made in this step. The process of air drying also appears to be problematic, the fact that smears often dry in open air without coverage has a high impact on the quality.

The third peak regards the staining process of the smears. In order to properly diagnose the smears, the colouring of the parasites must be done well. Staining is a relatively error sensitive process, since procedural guidelines need to be followed strictly and the quality of the stain influences the staining result.

The focus of this project should be on the impactful steps that have most direct impact on the quality of the smears. From this graph it can be concluded that this mainly regards the problems occurring in the blood collection and placement, the actual preparation of thick and thin smear and air drying.

It is decided to leave the staining process out of the project scope. Staining is a process on its own and it's frequently done back in the lab, which regards a totally different context and different timeframe. This makes it difficult to come up with a design intervention tackling both the problems in smear preparation and staining. Besides, the staining problems mainly regard not correctly following the procedural guidelines, where the smear preparation problems mainly regard experience, skills, the environment and resources. It is believed a design intervention is most effective in this spectrum. Besides, as previously stated, the smears are the core of the process. Greatly staining poor quality smears will still lead to a poor quality result.

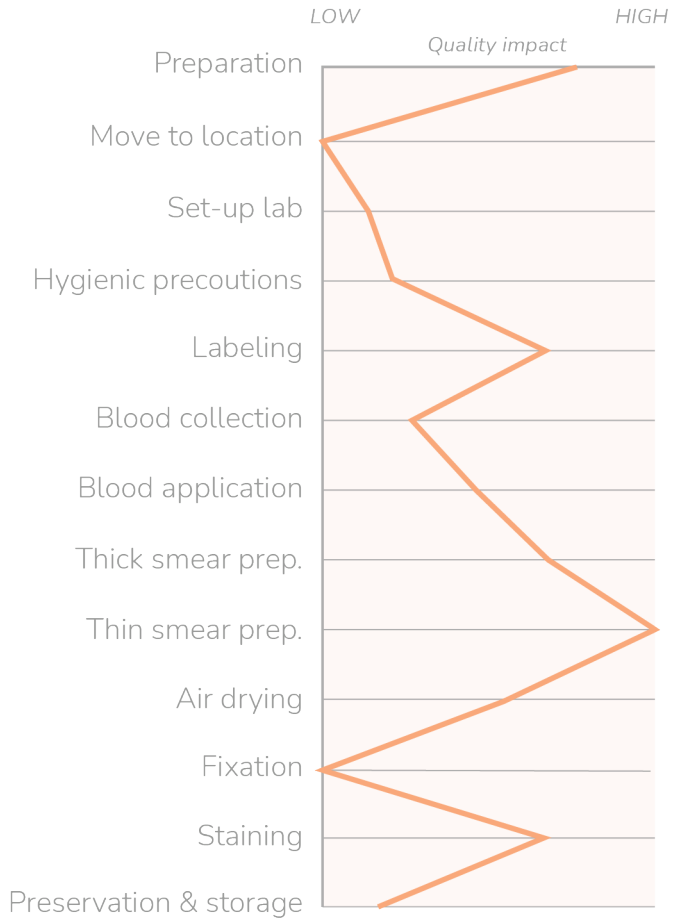


Figure 38: The relative impact on the smear quality per step within the preparation process.

3.3 PROBLEM DEFINITIONS

Based on the data from the analysis phase and the chosen problem space, four problem definitions are defined that are the main cause to poor quality smears.

1. Uncontrolled variables

Mainly in thin smear preparation, applying the right technique is of importance. In thin smear preparation there are four main variables that influence the quality of the smear: spreader push speed, spreader angle, downward force and the amount of blood used. These variables are one of the main causes to poor quality thin smears, since poorly applying one of these variables is easily done and leads to poor quality smears.

2. Lack of experienced staff members

Three of the four previously meant variables are skill dependent. Therefore experience is one of the key factors in preparing high quality thick and thin smears, especially in the challenging field context. However, due to a lack of experienced technical staff, smears are often prepared by inexperienced staff, resulting in low quality smears and inconsistencies in quality.

3. Degradation of the smears during air drying

Blood smears are left to dry in the open air most of the time. Since the minimum dry time is 10 minutes, the smears are exposed to dust, flies and all other particles during this time. This leads to contaminated smears, affecting the readability and accuracy of diagnosis of the smears.

4. Unavailability of proper and clean (local) resources and equipment

Since a limited amount of equipment can be brought to the field, the staff has to work with local resources. However, basic and necessary resources such as a table or chair are sometimes not available. Since a table or flat surface is essential in preparing quality smears, having no access to proper local resources generally lowers smear quality. Besides that, the quality, cleanliness and sterility of the equipment the staff is working with is often below acceptable. Using low quality and dirty equipment quickly leads to poor quality smears.

3.4 CLUSTER IMPACT

From the individual problems within the project scope, problem clusters are defined through interviews. The clusters give insight in the overarching cause of the problems and offer guidance in choosing a solution space. The three defined clusters are:

- Human influences – this regards the staff's experience, smear preparation technique and following the procedures.
- Environmental influences – this regards a non-sterile environment and factors like dust, flies and ants during air drying.
- Resources – this regards having access to the required resources and their quality.

Figure 39 visualizes the current impact of each cluster on the smear quality. The bigger the circle, the bigger the impact.

The human influences are considered as the most impactful cluster on the quality of the smears. Experience and skill are the most essential factor in the preparation process. Well educated and skilled people are able to prepare acceptable smears in a challenging context or with limited resources. However, this doesn't count for the opposite, unskilled people in a controlled and fully equipped environment, like a lab setting, won't be able to produce high quality smears (Korse, Schallig, Brice). The current lack of experienced staff in the field provides a problem and is the main reason for poor quality smears. Other than experience and technique, following the procedures, such as applying the required blood volume and using smear templates, is important for high quality results.

Environmental influences and resources are the other clusters with an identical impact on the smear quality. Environmental influences, like contamination caused by flies, dust and ants have a significant impact on the smear quality. Particles can be confused with parasites, affecting the accuracy of diagnosis (Brice, Schallig). Next to that, the field environment is challenging in terms of climate, (in)directly affecting the smear quality. Having access to sufficient and quality resources, such as microscopic slides, micropipettes is essential. Re-use of materials should in general be avoided to prevent sample contamination. However, it's frequently observed there is a lack of materials, or the materials are not clean (Korse). Using low quality and unclean materials impacts the quality of the smear.

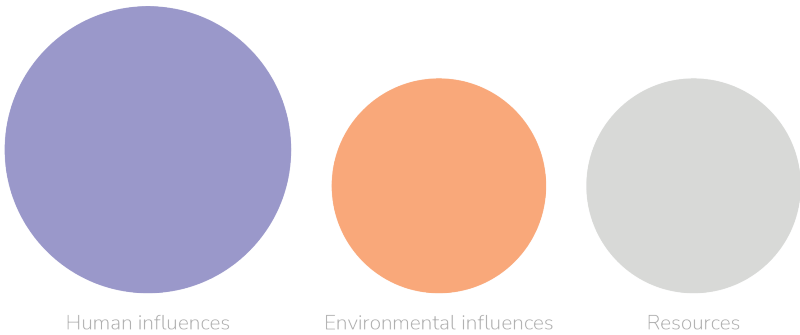


Figure 39: Cluster impact on the smear quality. The bigger the circle, the higher the impact.

3.5 SOLUTION SPACE

The solution space indicates the 'field' where potential solutions can be found. The triangle in *Figure 40* visualizes this field. The horizontal axis represents a gradient in complexity of the solution, the vertical axis represents how much human input and involvement is required. On the top left side, the solution has a low complexity and high human involvement, the right bottom side has a high complexity with low to none human involvement. The middle dot is a solution direction that is considered best of both worlds. A complex task can be solved through a relatively low complexity solution, without automating the process too much, the solution empowers the human.

Low complexity and high human involvement solutions is considered as the 'train' solution space. Think of re-education and writing context specific procedures, which could increase the staff's skill level and adjustability to a specific context. However, they don't take away the main cause for poor smear quality; human influences, they could only reduce the frequency of error occurrence. According to Chinonye, educating and training only has limited impact on the smear quality. She states that she sees a lot of 'trained' people preparing poor quality smears through simple mistakes. Henk Schallig confirms this problem, he states that everyone knows the procedure of smear preparation, but properly executing it is not self-evident. Taking this into account it is presumed that a solution in this direction has a relatively low impact on increasing the smear quality.

On the other end of the spectrum there's the 'take over' solution space. Solutions in this space will be a standalone product that fully takes over a human task (like a fully automated smear machine), requires little to none human input and can therefore be consistent in delivering quality output. However, since these products work autonomously it cannot adapt to a changing operating context, which is important for the changing field context. Besides, a complex product that is deployed in the field is unwanted, since a complex solution doesn't fit within the primitive context and its limited available resources (Schallig). Next to that, it is important to keep the process 'human'. As stated in chapter 2.4.3, there's distrust among participants in giving blood to the medical staff. In order to increase their comfort and trust it is necessary for them to actually see what's happening with their blood. Having a machine process their blood could make them suspicious, since they don't know what it is and what it does. Another problem occurring with this solution space is that the solution should not be a threat to the employment of the staff. If something is designed that requires fewer staff members, because of taking over their work, it will not be adopted (Schallig). Taking these aspects into account a solution in this space would not work in a field context.

The combined solution space is 'empower'. The ideal solution would solve the 'complex' task of smear preparation in a relatively 'simple' and 'human' manner. A solution in the 'empower' space can be seen as a tool assisting staff in their work. The tool requires human input, but doesn't totally take over the task. Such a tool can be designed to minimize the human and environmental influences on the smear's quality, while enhancing the workflow. However, the need for human input also means the human influences cannot be totally eliminated. On the one hand this is good, it enables the operator to adjust the

use of the product to the changing field context without needing the product to adjust, thus such a product can operate relatively context independently. Besides, it keeps the process 'human' and understandable. On the other hand there is still the possibility for human errors.

All interviewees mentioned that the 'empower' solution space would have most impact on the smear quality compared to the other spaces. It gives the opportunity to minimize the human influences, while leaving the human touch to the process. Besides, it enables the possibility for a more efficient and effective process and keep it low cost and simple.

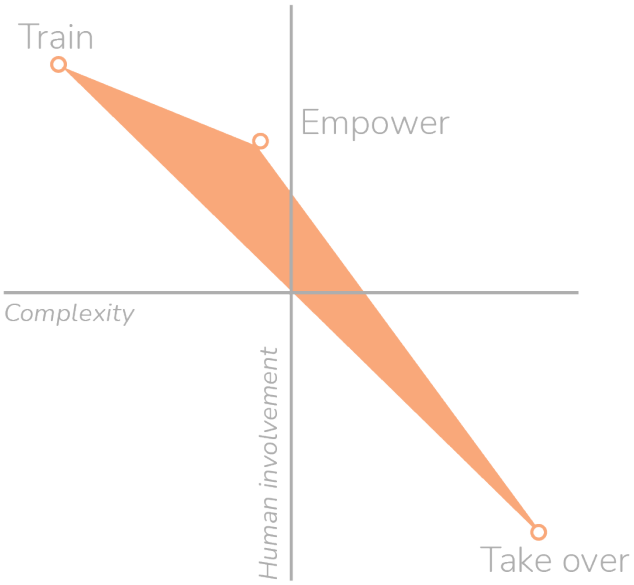


Figure 40: The solution space, indicating three optional directions.

3.6 VISION

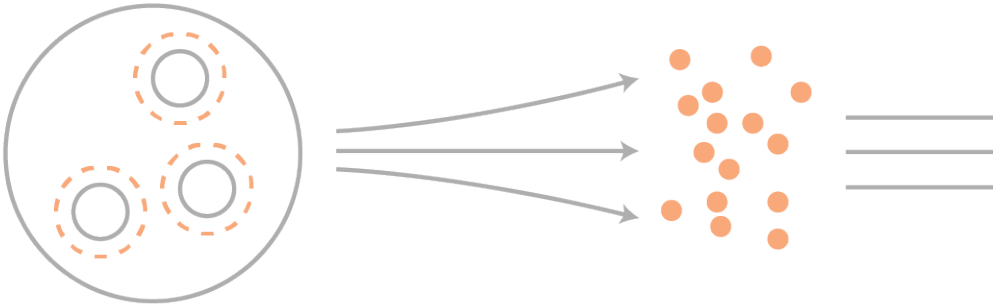
Through the development of a low-cost and field deployable tool, the malaria blood smear quality and quality consistency will be increased. This is done by minimizing the human- and environmental influences, taking into account the limited resources in the field context. The 'human empowering' tool enables preparation of a slide containing a thick and thin smear that meet the quality requirements, by an unexperienced individual. The product reduces the risk of human- and environmental contamination, through protective air drying.

CHAPTER 4

IDEATION

The analysis phase defines the current challenges and problems that are being faced during the smear preparation process in the field context. The ideation phase is the foundation for the development of the final product. During this phase ideas are 'invented' that provide potential solutions to one or multiple of the defined problems and challenges. Ideas from this phase are in a very preliminary stage of development and serve as a means to inspire and provide grip to further development of ideas into concepts (PL 2). Eventually, some of the most inspiring ideas are chosen to be rapidly prototyped, in order to test their working principles (PL 3).

4.1 METHOD



Abstract approach

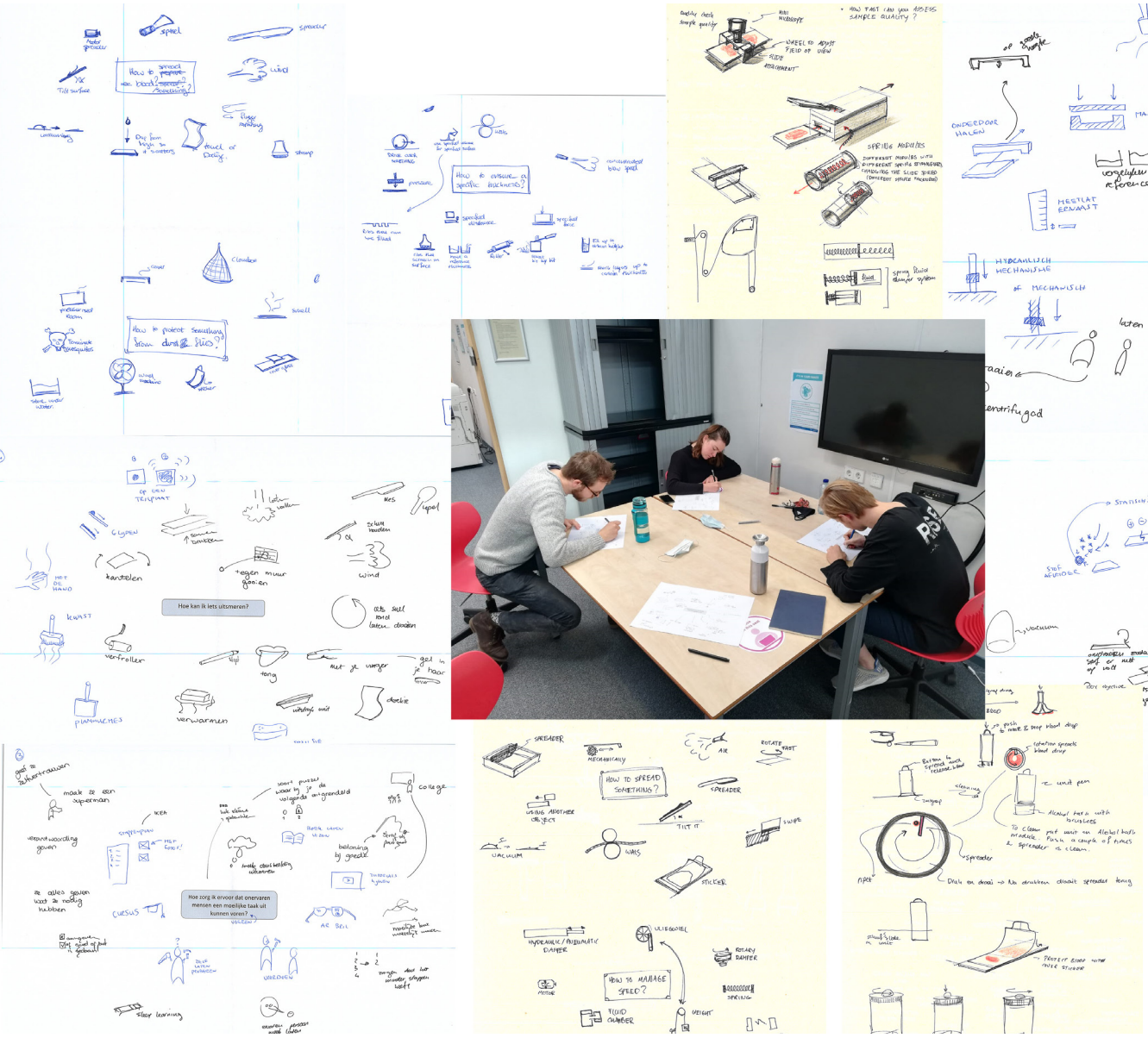
Brainstorming

Selecting Proof of concept Selecting

During idea generation it is preferred not to have too many limitations and restrictions. Having a wide variety of ideas inspires to innovative solutions, even crazy ideas can lead to great solutions. Therefore the problem statements in chapter 3.3 should be re-defined in more abstract terms, for the sake of stimulating the creative mind. For the reframed problem statements see appendix 11.12. The abstract problem definitions are the foundation to a good brainstorm session. Solo and multi-participant brainstorm sessions are held in order to come up with new innovative ideas. After idea generation, the ideas are clustered (grouped) into overarching solution directions. This method is used to organise the large amount of ideas by categorising them. This creates the opportunity to find and create new contexts and connections among themes. After clustering the ideas are ranked on multiple criteria, leaving a selection of the ideas with most potential. As a means to test and assess some of the developed ideas, rapid prototypes are built. These prototypes are made for quick tangible results, to get a feeling for the idea's working principles.

4.2 BRAINSTORMING

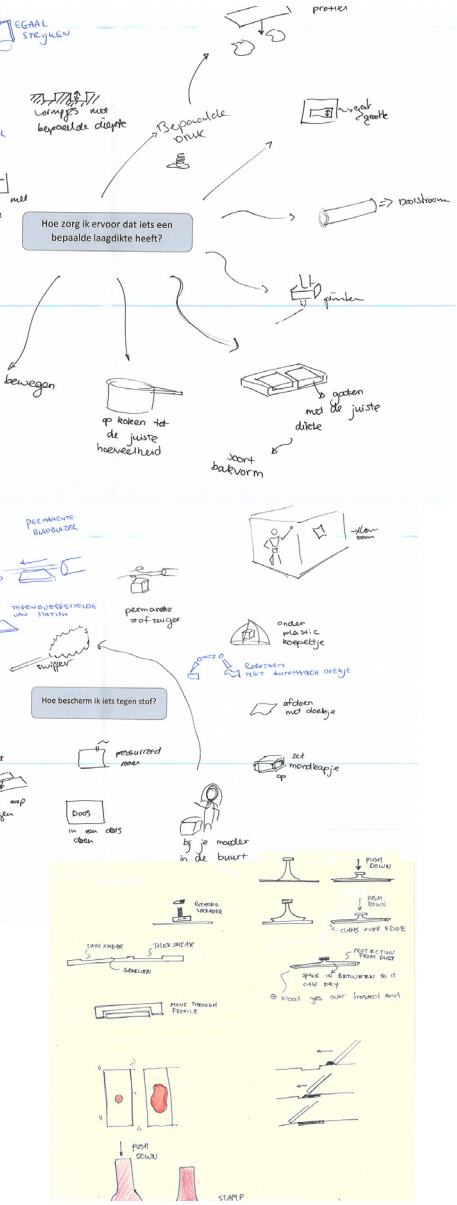
Multiple brainstorm sessions were done, solo sessions as well as sessions with various participants. The image below shows some of the results coming from the sessions.



4.3 SELECTING

Due to the large volume of ideas, a preliminary selection of good ideas is made based on rational thinking and a bit of gut feeling. After this preliminary selection a further selection was made based on the following criteria *Figure 41*:

- Simplicity (use)
- Feasibility (context, resources)
- Presumed potential smear quality



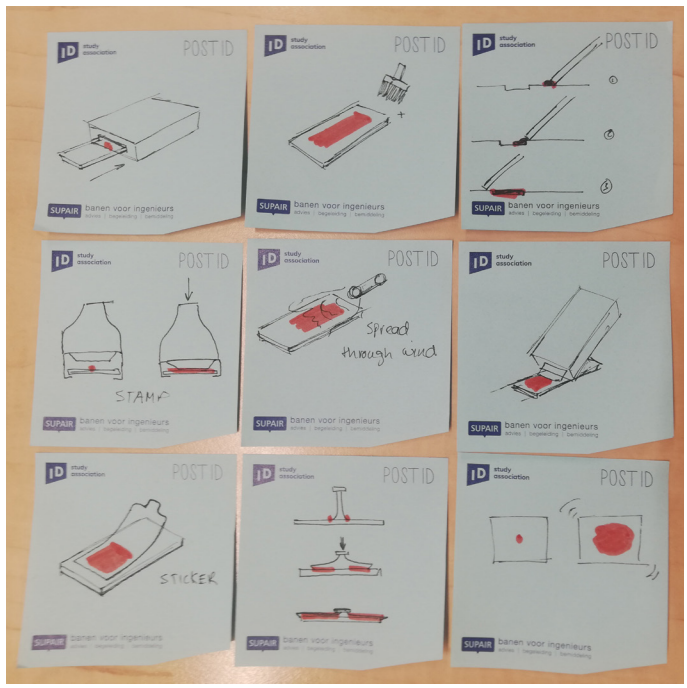


Figure 41: A preliminary selection of ideas.

4.4 WORKING PRINCIPLES

During the ideation phase many ideas were generated, some of them tackling the core problems in blood smear preparation. It was decided to directly test and assess some of the most promising ideas on their working principles, use and effectiveness of smear preparation – learn by doing. Contrary to ideas on paper, rapid prototyping gives fast tangible results, creating insights that cannot be gathered through paper sketching. The main focus of the prototypes lies on the preparation of thin smears, since this appears to be the most challenging and error sensitive part of the process (chapter 3.2). It is believed that once an effective working principle is found for thin smear preparation, this principle can be translated, adapted and applied to the preparation of thick smears. This chapter showcases the prototypes and evaluates them one by one. From the prototyping and testing insights are gained, which are defined as criteria and challenges that can be used as requirements and guidelines for upcoming prototypes and designs. Finally, a choice is made on which idea and prototype to continue with for further development.

Side note: Most of the prototypes are assessed with one single test, since at this stage my own blood was used. Therefore rough estimations on the effectiveness of the prototypes were made, but no definitive conclusions were drawn.

Figure 42: Bags with prototypes

4.4.1 SLIDER

The prototype

The prototype is a 3D printed multi-part slide mechanism (*Figure 43)*. 3D printing is chosen as prototyping technique, since it's an accessible and fast way to relatively accurately create an envisioned tangible product.

Goal

The prototype is meant as a quick and dirty prototype, to test if the manual operation of thin blood smear preparation can be done by using a tool. Besides, it is desired to know whether a 3D printed spreader mechanism offers potential for creating a high quality blood smear.

Can a (3D printed) tool take over the manual tasks of thin smear preparation and ensure high quality smears?

Working principle

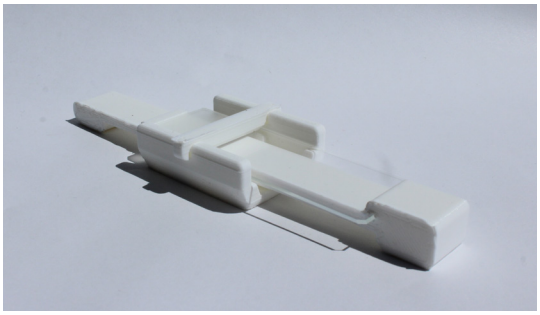
The drop of blood for the thin smear is placed on the slide, while the tool is in its initial position. With one hand the spreader and spreader holder are held in position (*Figure 45*). With the other hand the slide holder, with the slide inserted, is moved forward. This movement ensures the spread of the drop of blood by the spreader (*Figure 46*). The slide holder has limited points of contact with the slide in order to reduce friction and increase the smoothness of the movement.

Testing & conclusion

Figure 47 shows the result of a single test that was performed with the mechanism. Even though the movement was smooth and the speed was well controlled, the result is a very low quality smear. The smear is too compressed, thick and irregular. This could be due to the spreader, which is a loosely attached component and needs to be held in position by hand. This leads to an inconstant downward pressure and poor contact between the spreader and slide, resulting in a uneven and very poor surface quality.

Criteria

- The spreader must be fixated to the tool, in order to apply an evenly distributed pressure.
- Contact between the spreader and slide must be ensured at all times while the smear is prepared.
- The product must have as few components as possible, since holding and operating many components at the same time is not user friendly and results in lower quality smears.



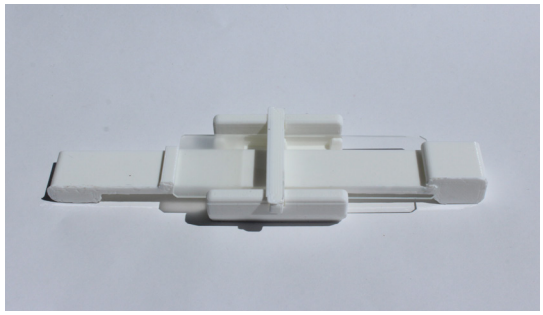


Figure 43: The slider prototype.

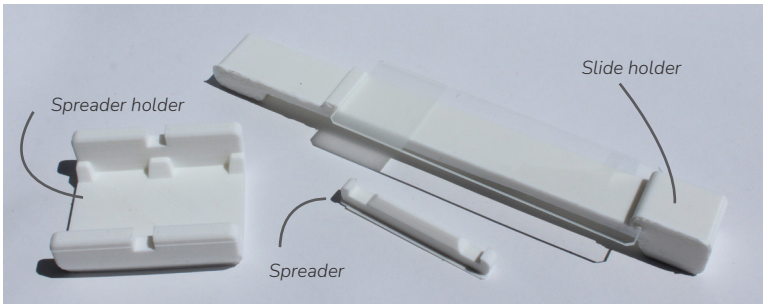


Figure 44: The prototype components.

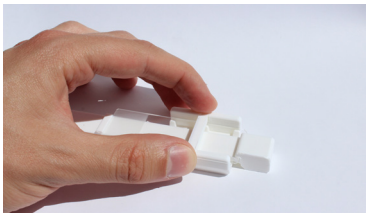
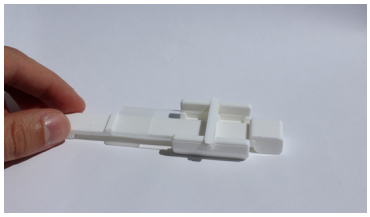


Figure 45: Holding the spreader in postion.



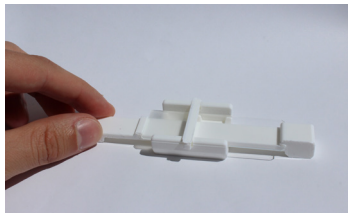


Figure 46: Moving the slide holder forward to spread the blood.

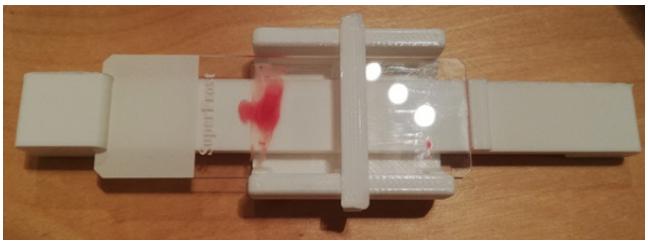


Figure 47: Test result using the prototype.

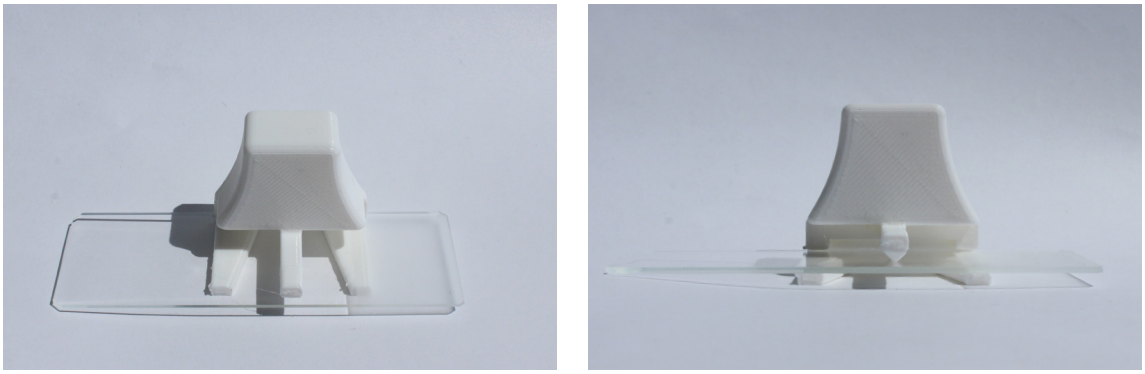


Figure 48: The credit card swiper, a single part mechanism with integrated spreader.

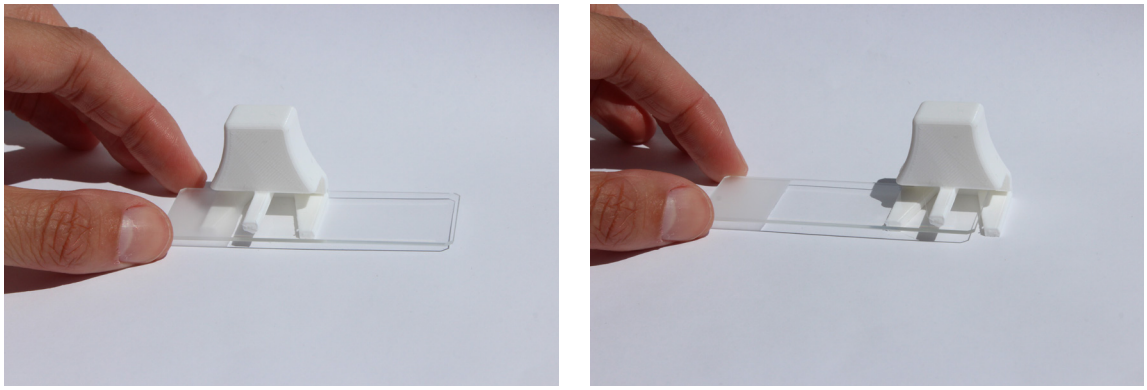


Figure 49: Using the prototype. The prototype itself should be held with the other hand.

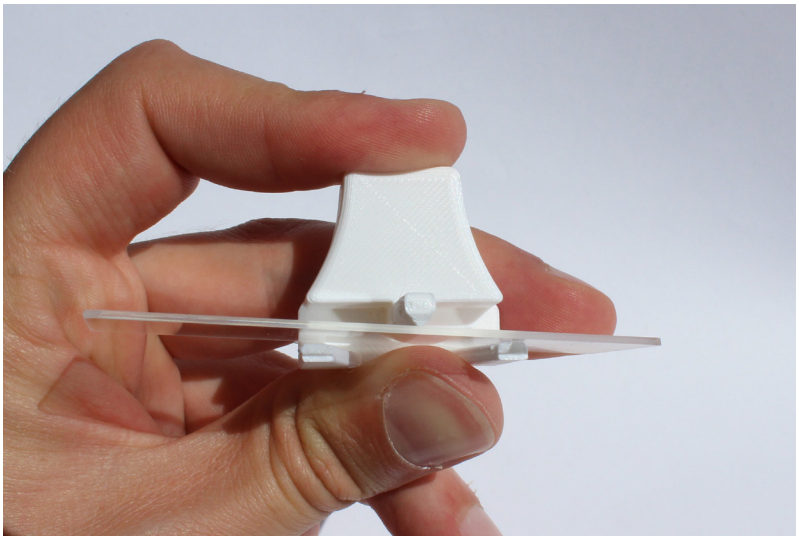


Figure 50: Too little clamping results in the slide to tilt, this is unwanted.

4.4.2 CREDIT CARD SWIPER

The prototype

The swipe movement of a credit card through a credit card dock is used as an inspirational reference movement. Taking the criteria from the previous prototype into account, this prototype is a 3D printed single part mechanism, with a spreader that is integrated with the product (*Figure 48*).

Goal

Does the credit card swipe movement offer potential for thin smear preparation?

Does an integrated spreader apply a better evenly distributed pressure?

Working principle

First, the slide is placed inside the clamping rim, with the spreader located just in front of the frosted end (*Figure 49*). Then the drop of blood is placed on the slide, after which the slide is moved backwards touching ensuring the drop of blood touches the spreader, spreading the blood along its edge. Next, the slide is 'swiped' through the spreader in a smooth movement, spreading the blood (*Figure 49*). Contrary to the vertical swipe movement of a credit card, the swipe orientation is horizontal, in order to prevent blood flowing off the slide.

Testing & conclusion

Multiple prototype iterations were made in order to test different types of dimensioning. In the initial prototype there was too little clamping force, making it easy for the slide to tilt (*Figure 50*). Therefore an extended version was printed to avoid this problem. However, due to less space to hold the slide, the extended version made movement more difficult. The movement shows potential in terms of well spreading the blood, however not in terms of ease of use. Eventually it was decided to not 'waste' any blood on this prototype, since the same error occurred in this prototype as in the Slider prototype (4.4.1); the spreader was not well connected to the slide, which would definitely have led to poor quality smears. It appears that integrating a fixed spreader does not result in a better connection to the slide, there always is little space between the two due to tolerances. This suggests that integrating the spreader with a spring system is required, in order to ensure contact between the spreader and slide.

Criteria

- The slide needs to be clamped on all sides to prevent tilting.
- The clamping must be minimal in terms of friction, but enough to ensure stability of the slide in the product.
- A spring system for the thin smear spreader is required, in order to ensure contact between the spreader and slide and apply evenly distributed pressure.

4.4.3 CASSETTE

The prototype

The prototype is a 3D printed single part mechanism, with a fixed integrated spreader (*Figure 51*). Based on the criteria from the previous prototype, the second iteration of this prototype was provided with an integrated spring system that ensures contact between spreader and slide (*Figure 52*).

Goal

The cassette was built in order to test the functioning of a cassette system with an integrated springy spreader. The cassette ensures a more clamped slide compared to previous prototypes, predicted to result in a straighter movement and better smear result. Additionally, the cassette offers the opportunity to serve as a shield while air drying.

Does a spreader spring mechanism result in better connection between spreader and slide and increases the smear quality?

Does clamping the slide at all sides result in a straighter movement?

Working principle

The slide is inserted in the cassette, after which the drop of blood is placed on the slide. Next, contact between the drop of blood and spreader is required by pushing in the slide just far enough (*Figure 53*). Once the blood is spread across the edge of the spreader, the slide can be pushed 'through' the cassette, spreading the blood (*Figure 53*).

Testing & conclusion

Figure 54 shows the result of the test with the cassette. After assessing video footage, the poor result is suspected to be due to a too fast movement of the slide through the cassette. A too high velocity results in short smears with inconsistencies in its surface. Next to that, the poor quality smear is also likely to be the result of the 3D printed spreader. The layers of the 3D print result in irregularities in the smears' surface, therefore 3D printing of the spreader does not suffice for good quality smears. The surface of the spreader needs to be sharp and smooth, or low quality smears are guaranteed. Another downside of the design is the fact that the spreader is integrated in the cassette and therefore not visible to the user. For the sake of user experience and smear quality it is desired to visually see when the drop of blood touches the spreader and see if the blood spreads along its edge.

Even though the smear quality results were still poor, there were some significant improvements compared to previous prototypes. Because of the spring system the spreader is in good contact with the slide, while maintaining smooth movement of the slide through the cassette without much resistance. The spring system works very well in terms

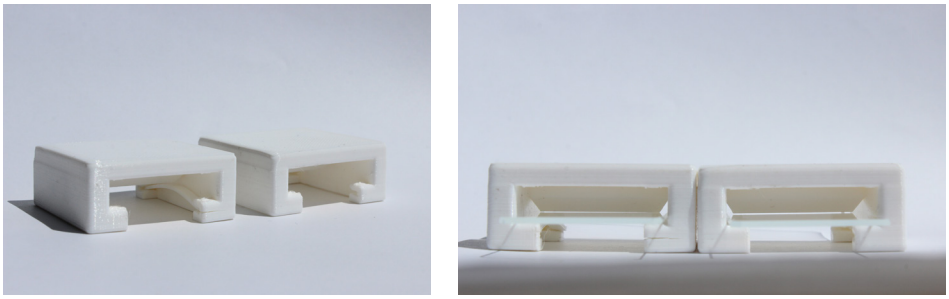


Figure 51: Two prototypes of the cassette.

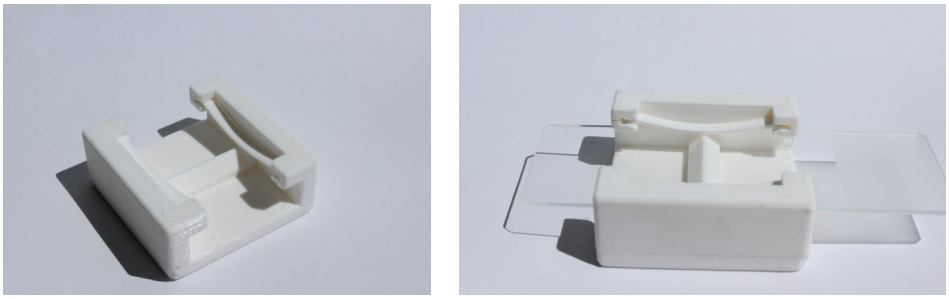


Figure 52: Integrated spring system, to ensure contact between the spreader and slide.

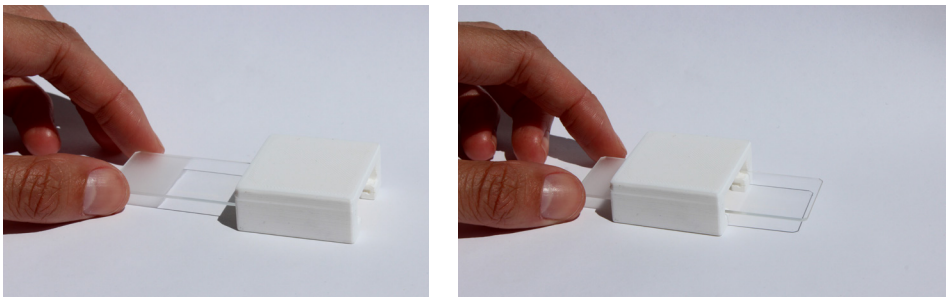


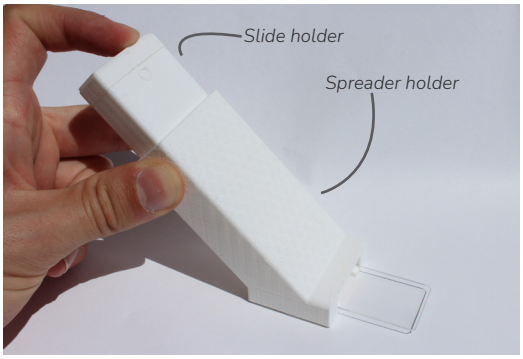
Figure 53: Use of the cassette. Pushing the slide through the device spreads the blood.

of connecting the surfaces of the spreader and slide. However, further research is needed to determine the exact spring pressure and its effect on the smear quality. Additionally, the spring system did successfully enable eliminating one of the three variables in thin smear preparation; applying a (constant) downward pressure. Having the parameter of this variable fixed is thought to result in fewer errors in thin smear preparation. Next to that, with correct dimensioning, the cassette offers potential to protect the slide from external contamination, e.g. dust and flies, while air drying, since the slide can dry inside the cassette.

Figure 54: Test result using the cassette.

Criteria

- The surface of the spreader must be sharp and smooth, 3D printed spreaders give poor smear results.
- It must be visible to the user when the drop of blood touches the spreader and when the blood has spread along its edge.



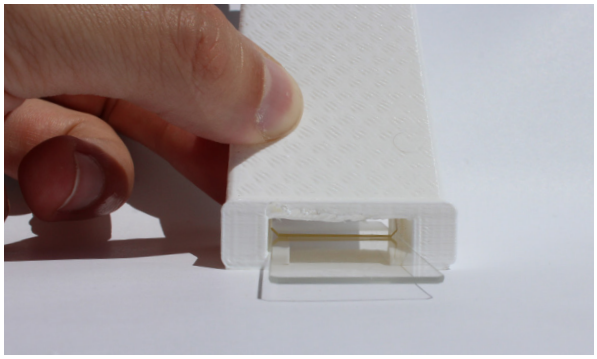
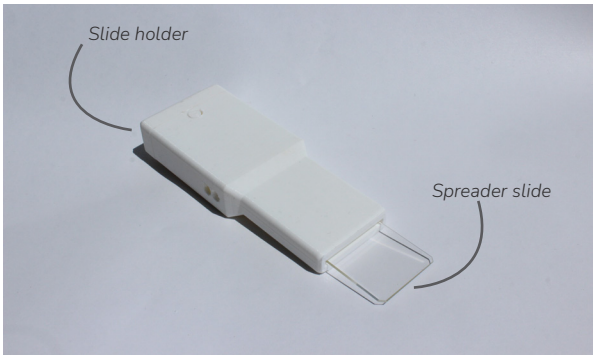


Figure 55: The Mimicker.



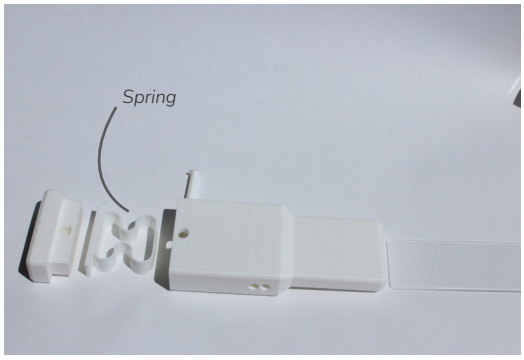


Figure 56: The slide holder of the Mimicker with an integrated spring.

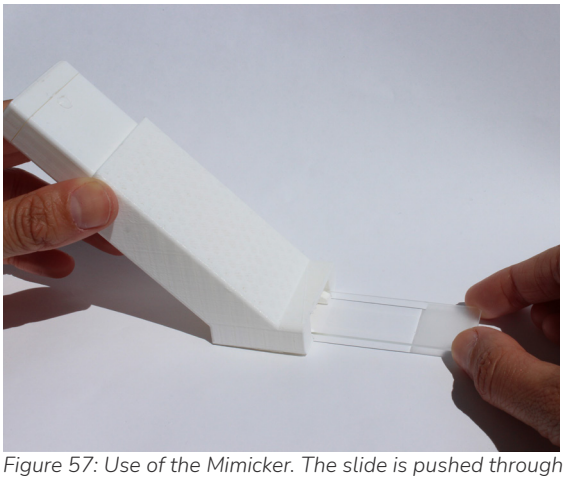
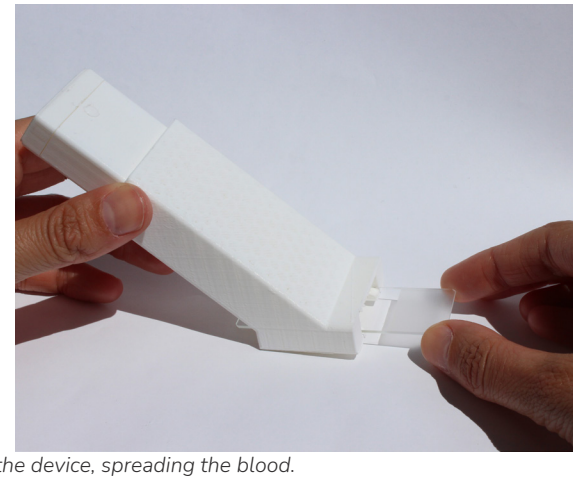
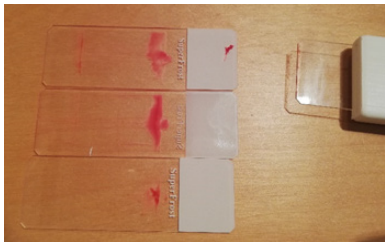



Figure 57: Use of the Mimicker. The slide is pushed through the device, spreading the blood.



4.4.4 MIMICKER

The prototype

The prototype is a multi-component 3D printed tool (*Figure 55*). Since the criteria from chapter 4.4.3 states that a 3D printed spreader does not suffice, a glass slide is used as spreader.

Goal

Since previous prototypes gave poor results, this prototype is used to test if a tool that mimics the conventional manual method is actually capable of producing high quality smears. The goal of this prototype is simulate the manual operation while fixating the angle and downward force parameters.

Can the manual operation be mimicked with a tool, while obtaining high quality results?

Working principle

First, the spreader slide is inserted in its holder. The holder is equipped with a 3D printed spring, which ensures a constant force and contact between the spreader and slide (*Figure 56)*. Next a slide is placed in the 'smear slide holder', after which the 'spreader holder' is inserted in the smear slide holder. Now that the spreader and slide are in contact with each other, the drop of blood is placed on the slide. After touching the drop with the spreader, the slide is moved 'through' the Mimicker, spreading the blood (*Figure 57*).

Testing & conclusion

Figure 57 shows the results of tests done with the mimicker. The results are not promising, the smears are too short and show too much inconsistencies in surface quality. This is most likely due to a too high downward pressure caused by the spring, resulting in short smears with gaps and streaks. Besides, the product was not easy to handle. It was quite a struggle to put the blood on the slide and move the slide forward, while maintaining pressure. Overall, the whole experience was not very smooth. In a next prototype it is desired to have an adjustable pressure, to find out which pressure parameter produces the best result. Also, the product should be more user friendly in use, easier to hold and move. So far, high quality results mimicking the manual operation using a tool cannot be obtained.

Criteria

- The downward pressure must be controllable.
- The product must be compact, easy to hold and move. It must be easy to operate the product by one person with two hands. Discomfort in use quickly results in poor quality smears.

4.4.5 MIMICKER+

The prototype

The initial Mimicker prototype didn't show good results. However, I felt that it must be possible to prepare high quality smears using this principle, since it basically mimics the manual operation. The Mimicker+ prototype is an upgraded version of the initial Mimicker. The slide is now being held by a 'bottom slide holder' (*Figure 59*), this makes the slide easier to hold and enables a smoother spreading movement. The black screws ensure the spreader holder is fixed in its holder, something that had to be done manually in the previous prototype, resulting in discomfort. The screw on top of the spreader holder (*Figure 59, Figure 60*) is used to adjust the spring tension, enabling different downward spreader pressures and setting the pressure that results in the best smear quality.

Goal

The goal of an upgraded version of the mimicker is to confirm the working principle and show that it is possible to create high quality smears using a manually operated tool like this.

Can the actual manual operation be mimicked with a tool, while having high quality results?

Working principle

The working principle remains the same compared to the previous mimicker version, see chapter 4.4.4 and *Figure 61*.

Testing & conclusion

Figure 58 show the results of tests done with the mimicker +. It shows a much better result compared to the previous version. The smears are stretched longer and have a better surface quality and thickness. Still the smears appear slightly too thick, but this is most likely due to the relative high amount of blood that was used. The results show that there is potential in creating high quality smears using a human controlled tool. With better controlled blood volume and movement speed it is believed consistently preparing high quality smears using a simple tool is feasible. The positive indication about the possibilities for creating high quality smears using this principle, give confidence in translating the working principles of this prototype to other prototypes. Meaning if the current set criteria are met, high quality smears can be prepared, disregarding the type of prototype.

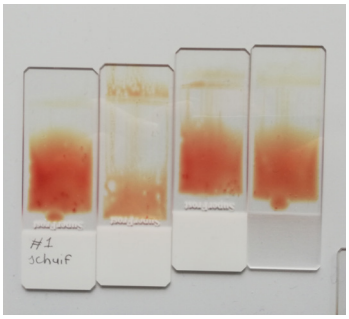


Figure 58: Test results using the Mimicker+.

The actual downward pressure that can be adjusted by the top screw cannot be measured, the same accounts for the movement velocity. Further research is required on the variable's parameter values that generate the best result.

Criteria

- Blood volume must to be applied in a controlled manner, so that the required blood volume is used.
- The variable parameters must be applied in a controlled manner, so that the highest quality results can consistently be obtained.

Figure 59: The Mimicker+.

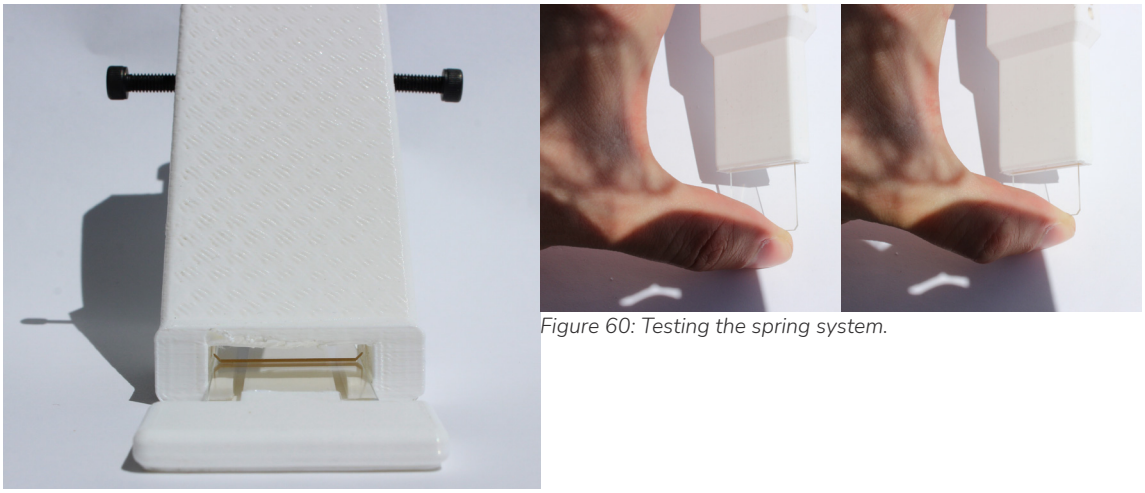


Figure 60: Testing the spring system.

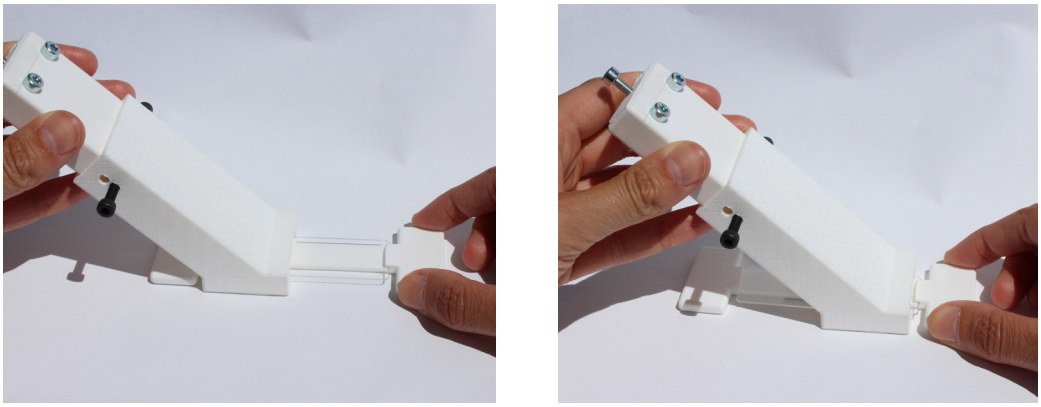


Figure 61: Using the Mimicker+.

Figure 62: A laser cutting, 'engraving' the notches in the plexiglass sheet.

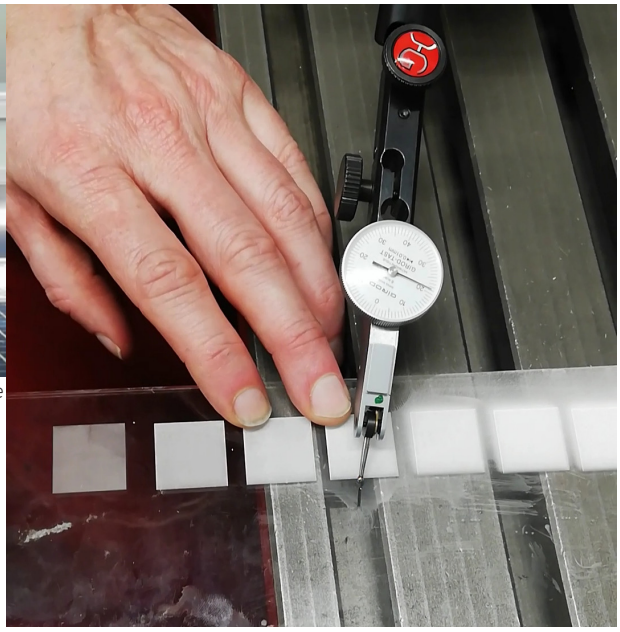


Figure 63: Assessing the notch depth.

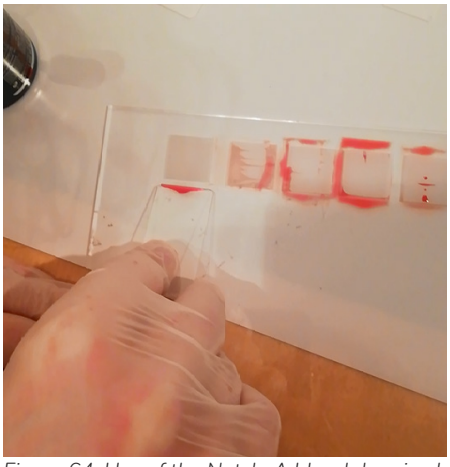


Figure 64: Use of the Notch. A blood drop is placed in front of the notch, after which it is spread.

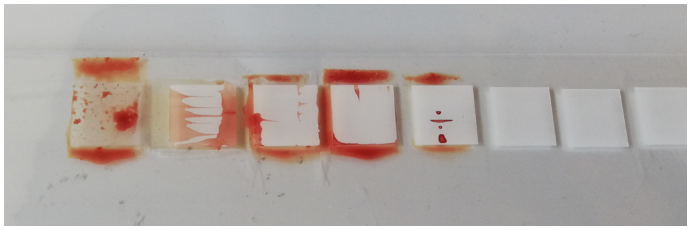


Figure 65: Test result using the Notch.

4.4.6 NOTCH

The prototype

The prototype is made from transparent plexiglass, with multiple laser cut notches (*Figure 62)*. Since it's not possible to set the engraving depth with a laser cutter, the power of the laser was adjusted for each notch. This resulted in notches with varying depths. With a highly sensitive depth meter (*Figure 63*), the depth of the notches were determined. The depth ranges from 10 - 60 µm.

Goal

The goal of the notch prototype is to see whether it is possible to produce thin smears by pushing a drop of blood in a controlled notch depth, ensuring a controlled and consistent thickness blood layer with a predefined surface area.

Working principle

The notch has a very simple working principle, the blood is pushed in a very shallow notch. The notch depth matches the perfect thickness of the thin smear, which is 1 RBC thick (3-6 µm). The blood drop is placed in front of the notch, after which the drop is pushed in the notch with a spreader slide (*Figure 64*). Because the notch depth is fixed, it potentially eliminates varying smear thickness. Besides, the size of the notch can be dimensioned in such a way that it matches with the required amount of field of views for diagnosis.

Testing & conclusion

Figure 65 shows the results of several tests with the notch prototype. The notch principle didn't work as expected, the blood tended to flow alongside the edges of the notch and not inside the notch. This is probably due to the viscosity of blood. The test shows that the notch principle in its current state doesn't show potential to prepare high quality thin smears.

4.5 CHAPTER CONCLUSION

It is concluded that the cassette is the concept that provides most opportunities to continue with. Even though the cassette didn't show good results in its initial test, it does show potential on other aspects. Besides, with the single test that was performed, no conclusions can be drawn on effectiveness yet. Also, not all the criteria that were set later on were integrated in the cassette, which would most likely have improved its working principles. The cassette is a compact, single part device, which makes it a good fit for the field setting. A multi-part product brings an increased risk in terms of component loss and failure. The simplicity in use to prepare a smear by just inserting the slide in the cassette, offers potential to minimize the required experience. Besides, the simplicity of the design creates potential to produce the product at a low cost, one of the main drivers for adoption in the field context. Next to that, the cassette offers the opportunity to protectively dry the slides inside it's body. The cassette can protect the smear from external contamination, increasing the quality of the smear. Based on the generated insights and data, and a little bit of designer intuition, the cassette is believed to be the concept that has most potential to significantly increase quality consistency of blood smears in the field.

The prototypes served as a means to quickly make the ideas from the ideation phase tangible and assess them on their working principles. Through the development of the prototypes many insights were gained that resulted in criteria, which must be met in next steps in the design phase. The prototypes cannot really be compared with each other in terms of delivered smear quality, since each subsequent prototype has incorporated features coming from criteria from the previous prototypes. However, it can be stated that the prototype evolvement shows potential to prepare high quality smears using an assisting and 'empowering' tool. It is assumed that when applying the found criteria in this and previous chapters and integrating them with any of the models, each model has potential to prepare high quality smears. Therefore, the decision with which model is continued towards the conceptualisation phase, is not as much based on test results quality-wise, but on the user experience and additional features that can be integrated in the model.

4.6 CRITERIA & CHALLENGES

16. Prototype

16.1 (cr) 3D printing of the spreaders must not be done, as it generates low quality smears (4.4.3).

16.2 (ch) The applied downward pressure must be controllable (4.4.4).

17. Product

17.1 (cr) The thin smear spreader must be fixated to the tool, in order to apply an evenly distributed pressure (4.4.1).

17.2 (cr) Contact between the spreader and slide must be ensured at all times while the smear is prepared (4.4.1).

17.3 (ch) The product must have as few components as possible (4.4.1).

17.4 (cr) The slide needs to be clamped on all sides to prevent tilting (4.4.2).

17.5 (cr) A spring system for the thin smear spreader is required (4.4.2).

17.6 (ch) The product must be compact (4.4.4).

17.7 (ch) The clamping must be minimal in terms of friction, but enough to ensure stability of the slide in the product (4.4.2).

17.8 (cr) The product must enable application of the required volume of blood (4.4.5).

17.9 (ch) The variable parameters must be applied in a controlled and measurable manner (4.4.5).

17.10 (cr) The edge of the spreader must be sharp and smooth. (4.4.3)

18. Product use

18.1 (cr) It must be visible to the user when the drop of blood touches the spreader and when the blood has spread along its edge (4.4.3).

18.2 (ch) It must be easy to operate the product with two hands (4.4.4).

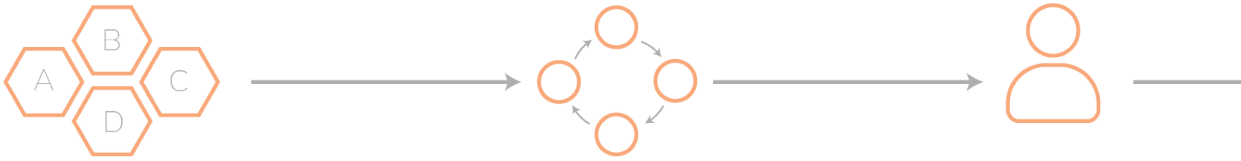
CHAPTER 5

CONCEPTUALISATION

From this chapter on, the 'cassette' will be referred to as 'Malaria Blood Sampling System' (MBSS).

During conceptualisation the MBSS will develop from PL 3 to PL 4, meaning the envisioned use and technology of the concept will be formulated more in depth. This chapter goes deeper on the working principles of the device, after which the working principles are tested and assessed. Next to that, the variable parameter values are defined through multiple test setups, so that these parameters can eventually be integrated in the design.

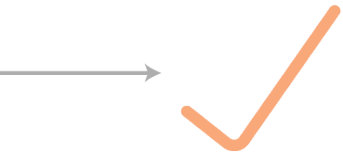
5.1 METHOD



Fundamental considerations

Defining working principles

User scenario



Proof of concept

The conceptualisation part is split up in three parts. First some fundamental considerations are made. These considerations influence the working principles and use of the design, therefore it helps to define them at the start of the conceptualisation phase. Once defined, the product's working principles and the accompanying technology are further developed, based on the found criteria, resulting in a more advanced analytical concept. Eventually, the analytical concept is prototyped and tested on its performance, in order to proof or deny the working principles of the concept.

5.2 FUNDAMENTAL CONSIDERATIONS

Some fundamental considerations need to be made about how the product is used and what features it must include. These considerations have effect on the eventual design and use of the product, therefore it is of importance to discuss these considerations upfront.

5.2.1 DISPOSE VS. RE-USE

Re-use of products requires that they are cleaned before use. Since the MBSS concerns a medical product, it is of importance that it is cleaned according to medical guidelines ensuring a sterile product. However, challenge 2.8 states that the available equipment in the operating context is often not sterile or clean at all. This is concerning, since the preparation of high quality smears requires high quality, clean and sterile equipment. Re-usage of the device would result in losing control over the quality of the smears, due to the above mentioned reasons. The main goal of implementation of the device is to increase and secure consistent high quality smears. Therefore it is decided that within this context, making the MBSS a single use product disposable product is the best option to ensure smear quality.

However, disposables do have downsides, especially regarding waste. The main question that should be asked is: does the increase in quality consistency of the smears and therefore the increase in diagnosis accuracy by using a disposable device outweigh the generated waste? The answer is yes. Take the high increase in use of disposable RDTs as an example. It shows that increasing the chances of correct and fast diagnosis outweighs the amount waste that it generates. It also shows that third world countries are able to manage the required infrastructure of this waste stream. The reward is higher than the problems that it brings. It is believed that the same accounts for a disposable MBSS.

Disposable products are in general more costly, since each individual product can only be used once. Of course in a non-disposable scenario, the washing and maintaining the quality of the device also adds to the costs. Since low costs are one of the main drivers for adopting a product in this context, it is of importance to keep the costs as low as possible. This indicates that the cost price of a disposable medical product should be as low as possible, in order to keep it affordable for third world countries.

5.2.2 RECYCLING

With the device being a disposable, it is interesting to assess the opportunities for possible recycling of the material. However, since it regards medical waste there are strict guidelines, because of potential infectious or toxic material. Recycling of medical waste has been a widely known problem within the healthcare industry (*Figure 66*). 'It's difficult to think about sustainability when we have to weigh that up against the safety of a patient' (Ryan Ko). 'Healthcare workers on the front line emphasise that there is a legitimate need for single-use plastics, primarily to prevent infectious diseases from taking hold and spreading – and Covid-19 is a perfect example of this need. While no one would argue that safely disposing of hazardous used personal protection equipment is essential when it comes to infectious diseases like Covid-19, only 15% of healthcare waste is actually classed as hazardous' (Hope NGO, 2020). This suggests that further research is required on which components might fall under that 15%. However, the likeliness of the possibility for recycling is low, since the product comes in contact with possibly infectious blood.

Figure 66: The majority of the medical waste is incinse

5.2.3 INCLUDING THE SLIDE

The previous chapter stated the concern about frequent unclean and low quality equipment used in the field context, this also concerns the glass slides. Keeping control over the quality and cleanliness of the slides is of great importance for the quality of the smear. Unclean slides result in poor quality smears quickly (*Figure 67*). Keeping the main goal of the MBSS in mind, it is necessary to secure the quality of the slides, in order to guarantee high quality smears. This suggests that in order to keep in control, it is necessary the device is delivered including a slide. This guarantees the quality, sterility and cleanliness of the slide. This mean that the slide is considered, just like the MBSS, a single use disposable product. Given the example of the RDTs in the previous chapter, it is believed that this impact is outweighed by the assurance of providing high quality, sterile and clean equipment that facilitate the preparation of high quality smears.

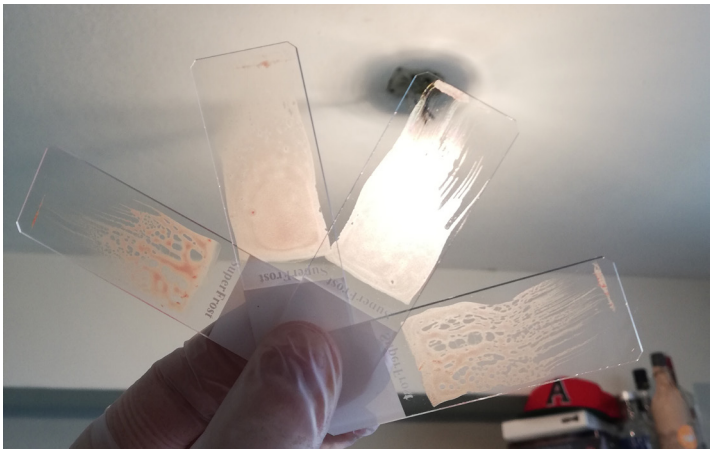


Figure 67: Unclean slides lead to poor quality smears.

5.3 DEFINING THE WORKING PRINCIPLES

The ideation and working principles chapter (4) cover the MBSS' working principles in a very basic way. This subchapter discusses how the working principles are further developed and established, accompanied with argumentation for the made choices. It shows the step by step advancement of a 'simple box' into a more advanced product. This was an iterative process, some adaptions were made because of insights that were obtained later, for the sake of clarity they are not presented chronologically.

5.3.1 THE BASICS

The basics describe fundamental, but obvious features of the device and why they are implemented the way they are.

Holding the slide

The slide must be inserted and held by the MBSS device. In order to smoothly push the slide inwards, it must be guided and supported at all sides (criteria 4.4.2). A C-profile, that matches the width dimension of the slide, including a small tolerance, enables this stability and smooth insertion (*Figure 68*). An advantage of the C-profile compared to an enclosed O-profile is that it is more flexible, this flexibility enhances the smoothness of insertion of the slide. Besides, the C-profile has a smaller contact area with the slide, lowering the friction during insertion. The width dimensions of the bottom ends are kept as small as possible, so that it creates enough support, but friction and material use are minimized.

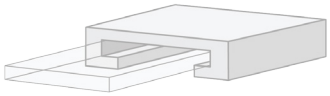


Figure 68: MBSS device: C-profile, ensuring stability of the slide.

Dimensioning

The most prominent influencer of the size of the MBSS is the slide. The device must be able to hold the entire slide. This is of importance for air drying, since the smears must be protected against environmental contamination by the cassette during this process. Therefore, the length of the device is stretched as long as the length of a slide (*Figure 69*). The 'ceiling' of the MBSS is slightly moved upwards (*Figure 70*), since it is unwanted that the drop of blood and smears touch the ceiling while the slide is inserted. Besides, there must be room for the spreaders to apply downward pressure from the top, which is not possible without any space to do so. The open space at the front and back of the device, caused by lifting the ceiling, is assumed not to be a significant threat for contamination through dust or other particles.

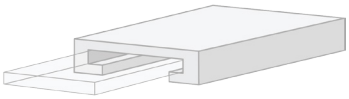


Figure 69: MBSS device: the lenght of the device is adjusted to the length of the slide.

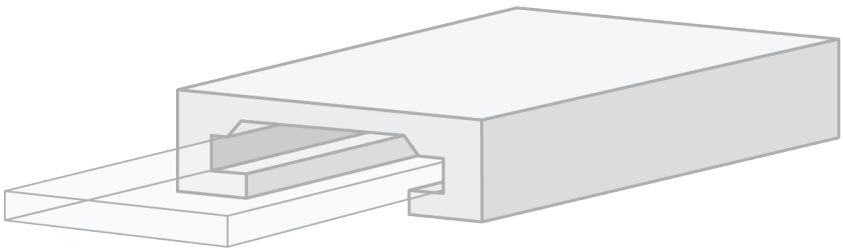


Figure 70: MBSS device: ceiling is moved upwards.

5.3.2 SMEAR PREPARATION

To ensure efficiency of the process and to make smear preparation as simple as possible, both smears must be prepared on one slide at the same time (criteria 2.2). The initial idea and prototype of the device (chapter 4.4.3) facilitated preparation of the thin smear, this principle needs to be translated to the preparation of both smears on the same slide.

Smear layout

The conventional smear layout (back-to-back) in manual smear preparation, where the smears lie underneath each other, is difficult to realise with the MBSS device. This is due to the fact that both smears are prepared while the slide enters the device and would result in unwanted overlap of the smears. *Figure 71* shows a more convenient layout that enables preparation of the both smears in one push movement, without overlap. This side-to-side layout results in narrower, but lengthier smears, in order to obtain the required surface area for both smears. Taking into account the fact that in back-to-back formation the thin smear takes about 2/3rd of the length of the slide, means that that in side-to-side formation the smear must be about 14mm wide, in order to obtain a similar surface area. These custom smear dimensions require custom spreaders, which will be discussed next.

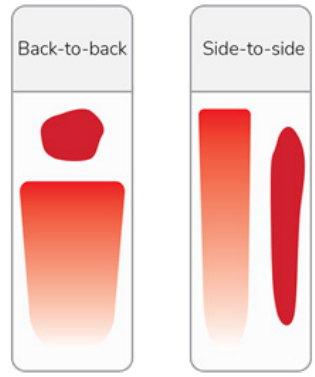


Figure 71: Conventional and MBSS smear lay-out.

Thick smear

Applying the side-to-side smear layout requires a narrow thin smear spreader in order to achieve a narrow and long stretched smear. The designed spreader is in contact with the slide and facilitates spreading of the blood over the full length of the slide, but with a limited width (*Figure 73*). Since the slide will dry inside the device, it is best to allocate the spreader as far up front as possible, to minimize the total length of the device, decreasing material use and minimizing storage space.

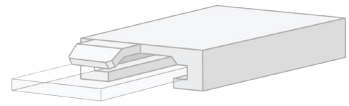


Figure 72: MBSS device: thin smear spreader is added.

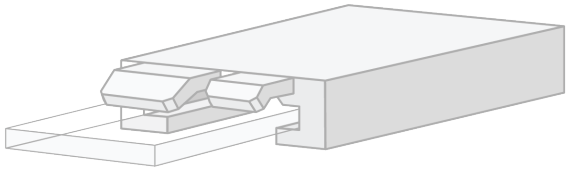


Figure 73: MBSS device: thick smear spreader is added.

5.3.3 ELIMINATING THE VARIABLES

The angle, pressure and speed variables cause inconsistencies in the quality of thin smears (2.4.8). In order to increase the quality consistency of the thin smears, the impact of these variables needs to be minimized. This chapter discusses how the design eliminates/ minimizes these variables.

Pressure

As stated, generating a monolayer requires a certain downward pressure by the thin smear spreader on the slide. Criteria 17.5 states that a spring system in the spreader is required, to establish an evenly distributed and constant downward pressure, to enhance the quality of the thin smear. This spring system is integrated in the spreader, by making the spreader have the same characteristics as a beam that can be deformed (*Figure 74*). The point mass at the end of the beam results in internal forces, deflecting the beam. Translating this principle to the spreader, so that it deflects and leaves a downward pressure, results in elongating the spreader and fixating it at one end (*Figure 75*). Without the slide inserted, the spreader is 'rest' position, when the slide is inserted, the spreader is pushed up, resulting in a downward force (*Figure 76*). Playing with the beam dimensions and material properties, the required pressure can be generated. The exact pressure parameter is determined in chapter 6.

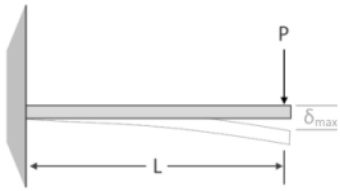


Figure 74: Beam deformation by pressure. This principle is used for the thin smear spreader to apply a downward force.

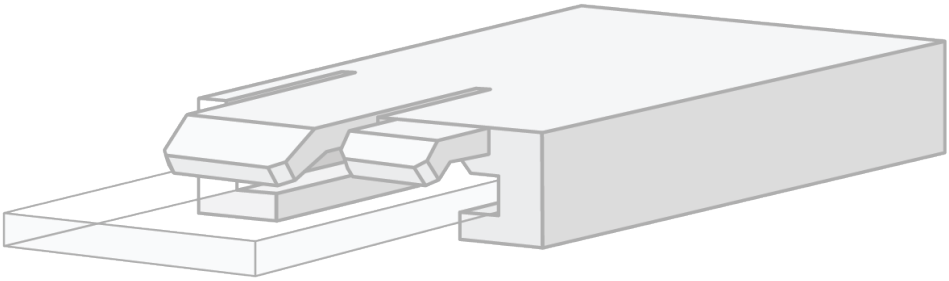


Figure 75: MBSS device: thin smear spreader is elongated so that downward pressure can be applied.

Angle

Having the spreader integrated in the MBSS offers the opportunity to fixate the spreader angle. This enables application of a consistent angle for every prepared slide, resulting in more quality consistent smears. Similar to the pressure parameter value, the angle parameter is determined in chapter 6. Once the parameter is found, the angle can be applied in the design. Something to keep in mind is the fact that angle in 'rest' position is different compared to the 'tense position', this difference must be taken into account when integrating the angle parameter.

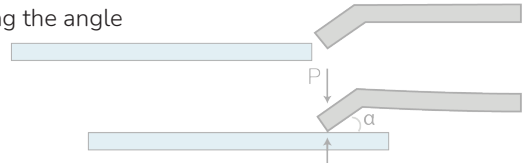


Figure 76: Insertion of the slide causes the spreader to move from 'rest' position in 'tense' position.

Velocity

Regulating or even automating the velocity with which the slide moves inside the device is a challenge. This requires mechanical spring components or even motorised components. The essence of the product is to keep it simple in use, in order to minimize the required experience to operate. Besides, additional and more complex components will be more likely to fail in a challenging context such as the field. In order to make a decision, the impact of the speed on the smear quality needs to be determined, this is done in chapter 6. Once the velocity range of high quality smears is defined, it can be decided whether to keep the speed manual or automate it.

Blood volume

For both smears specific blood volumes are required. The current way blood drops are applied is often not very accurate. Therefore it is required that there is some kind of blood volume regulation included with the MBSS, which ensures application of the required blood volume per smear. The product should somehow facilitate this. There are two main options, through an integrated feature in the device or through providing an additional (such as a capillary) that is supplied with it.

From an idea from the ideation phase, a concept is developed for the integration of a capillary tube in the device (*Figure 77*). A capillary tube can extract and drop liquids very accurately. In front of both spreaders a small capillary tube is placed, each facilitating the required blood volume for each smear. The idea behind this system is that the participant can drop a drop of blood straight from his finger in the capillary tube (*Figure 78*), while the capillary tube fills up with the required blood volume. Once the tubes are filled, the tubes are pushed downward (*Figure 79*), dropping the blood on the slide. The working principles of this system still need to be validated.

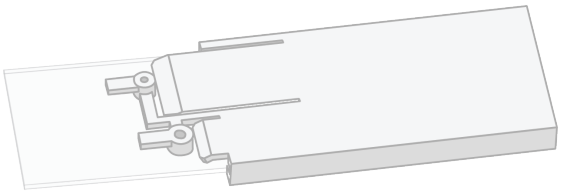


Figure 77: Integration of two capillary tubes to apply blood.

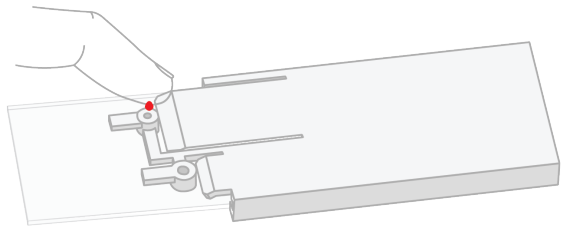


Figure 78: Applying blood from the finger to the tube.

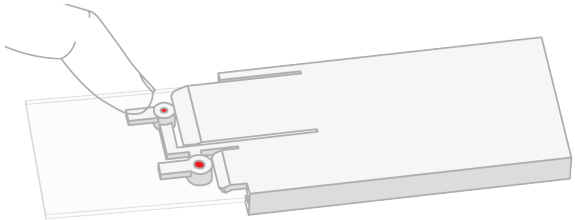


Figure 79: Pushing the tube down to apply the blood to the slide.

5.3.4 CONCUSION

According to the set requirements in the previous chapters, the MBSS' working principles and use have been further defined, resulting in a more detailed concept. *Figure 80* shows the envisioned concept at this stage of the design process. The smear lay-out has been optimized to the use of the device, the MBSS facilitates the side-to-side layout instead of the conventional back-to-back layout. This layout and the fact that the working principle of the thin smear is translated to the thick smear, enables preparation of both smears on the same slide during insertion of the slide. The integrated thin smear spreader, with fixed angle and pressure, eliminate these skill based variables from the preparation process. The same counts for the thick smear spreader, which has a fixed distance from the slide, ensuring a consistent thickness. The blood volume variable can potentially be eliminated by the integration of a capillary system in the device. The impact of the velocity on the smear quality requires further research, in order to make a decision to keep this a manual operation or that it should be automated.

The current design has potential to eliminate three of four variables, presumably ensuring much more consistency in smear quality. The fact these variables are potentially eliminated, increase the accessibility and ease of smear preparation, requiring less skill and experience as an operator.

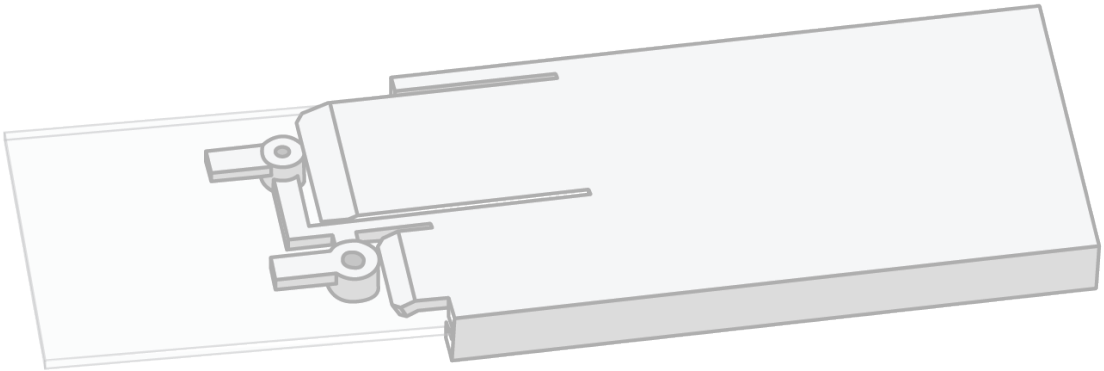


Figure 80: The analytical concept of the MBSS device.

5.4 USER SCENARIO

The working principles of the MBSS have been developed and tested, this chapter discusses the envisioned use of the device in the field context. Step by step it discusses how to operate the product and prepare a the thick and thin smear. The device must be designed in such a way that it minimizes the risk of errors in use, in order to maximize the smear quality. Therefore, thorough analysis on how the product is envisioned to be used is important, in order to find potential critical elements in the design, that can have a negative impact on the smear quality. Based on the found critical elements, new criteria are formulated, which are used for further development.

5.4.1 CONCLUSION

A couple of main topics come forward from analysing the envisioned use of the MBSS. Ensuring the product is used in the intended way and ensuring the product cannot be used in the wrong way are two topics that come forward, and no, they are not the same. Ensuring intended use is about providing the user with the right use-cues, giving the user feedback on multiple aspects to let him know he's doing the right thing. For example; giving the user visual feedback on the quality of the smears, or providing the user with a click sound when the slide is fully pushed inside the device. Ensuring wrong use of the product cannot occur is about making the product fool proof, through adding features that make unintended impossible. This should be done in situations that can really harm the quality of the slide, for example; ensuring the slide cannot be retracted once it's pushed inside the device, since this will ruin the smear. These situations must be prevented through adding design features.

Another reoccurring topic is waste management. How and where the waste should be disposed is of importance, since it concerns medical and hazardous waste. Hazardous waste needs to be disposed in separated containers (*Figure 81*). This is assumed not to be a problem, since in the current situation the medical staff already has to deal with this type of waste. Meaning the waste infrastructure is already there, but presumably with the use of the MBSS its capacity must be scaled up.

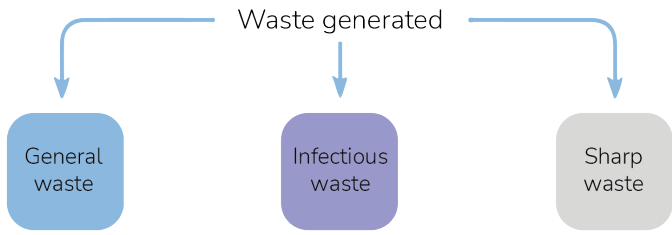


Figure 81: Waste disposable bins.

5.5 PROOF OF CONCEPT

Now that the use case and working principles of the MBSS are defined, it is time to test and assess the device on its functionality. A prototype has been made through 3D printing, to validate use case and analytical working. Multiple tests have been performed, preparing thick and thin smears and assessing their quality. This subchapter ends with new criteria, for the next concept iteration.

5.5.1 METHOD

A prototype of the concept coming from the conceptualisation phase is modelled and 3D printed. Next, a test setup is designed, so that the tests can be executed in a similar fashion, in a controlled manner. Eventually, the quality of the prepared smears are assessed, a new systematic method on how this can done visually and fast has been designed. The first two tests are about testing the thick and thin smear individually, in order to validate the one is not affected by the other. During this test, variations in movement speed of the slide are applied, in order to make preliminary statements about its impact on the quality. In the third test both smears are prepared in one simultaneous motion. Finally, a last test has been performed in order to test the working principles of the capillary tube system in practice.

5.5.2 PROTOTYPE

The tests are performed with a FDM 3D printed prototype that is based on the concept described in chapter 5.3.4 (*Figure 82*). The prototype looks and works slightly different from the theoretically defined concept, however its fundamentals and smear preparation mechanism are the same. The difference between the prototype and concept is due to the discussed limitations of 3D printed prototypes (chapter 4.4) - 3D printed spreaders lead to poor quality smears. In order to approximate the characteristics of glass slides, the spreaders are laser cut from a Plexiglas sheet and inserted in the device. The high pulse rate of the laser ensures a sharp and smooth spreader edge (criteria 17.10).

Once the spreaders are inserted in the device, it is ready to be used. The wide spreader is used for thin smear preparation, the spreader edge is in contact with the slide, enabling preparation of a monolayer. The narrow spreader is used for thick smear preparation. Leaving about 0,4mm space between the slide and spreader ensures a thicker spread layer of blood (*Figure 82*).

The front of the MBSS is equipped with a rough approximation of the capillary tube system that is designed to facilitate the application of the required blood volumes on the slide (*Figure 82*). A small tube cut from a micropipette functions as capillary, holding the blood.

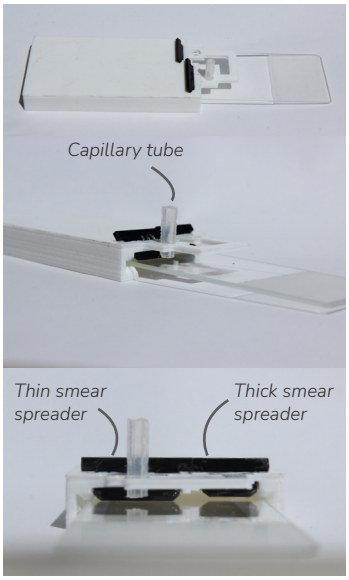


Figure 82: The initial MBSS prototype.

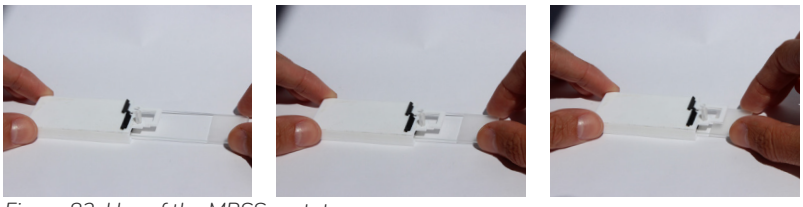


Figure 83: Use of the MBSS prototype.

5.5.3 TEST SETUP & USE

Contrary to the envisioned use, where the slide is inserted in the device, the device itself is now the moving object, moving over the slide. Because of the 'loosely' inserted spreaders, this method works more conveniently and is assumed not to make a difference in the results. For the sake of convenience while testing, the slide is held by a slide holder, stabilizing the slide in the preparation process. The use of the prototype is as follows (*Figure 84*):

1. The slide is inserted in the holder.
2. The device is slid over the matte end of the slide.
3. Using a micropipette, the required volume of blood for each smear is dropped on the slide, in front of the spreaders.
4. The device is moved forward, until the spreaders touch the drop of blood. A short moment is waited, until the blood has spread along the edge of the spreader.
5. Once the blood has spread along the spreader's edge, the MBSS is ready to be moved forward. This movement results in spreading the blood.
6. The slide is removed from the holder.

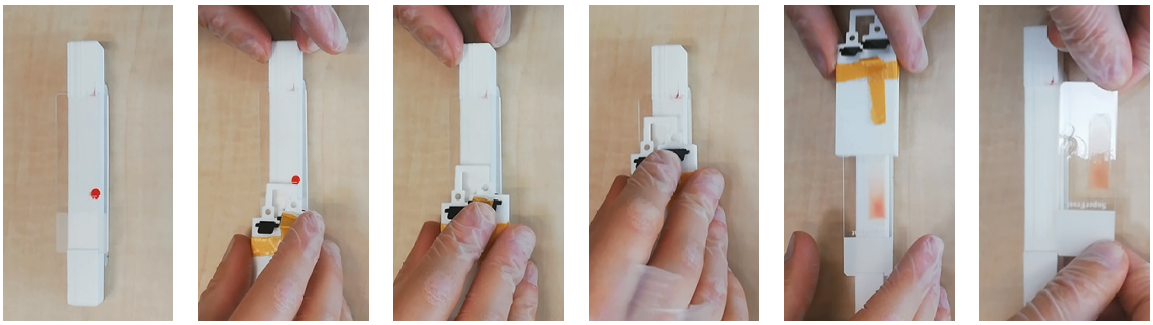


Figure 84: Test procedure using the MBSS.

5.5.4 SMEAR ASSESSMENT

In order to assess the smears on their quality, each individual smear needs to be assessed in a standardized way. Chapter 2.6 defines the quality requirements for high quality thick and thin smears, these requirements are used as benchmark in assessment. Because of the high volume of smears that are prepared during testing, a new method that enables quick and visual assessment of smears is developed. Through the use of a developed template, assessment can be done with the bare eye and does not require a microscope (*Figure 85*). A prepared smear is put on top of the template, it can be visually assessed whether the smear matches the required length and width (indicated in green). Whenever the smear only meets the red surface area, it indicates the surface area is insufficient and the smear is of low quality. When the smear meets the red, orange and green surface area, the smear meets the quality surface area quality requirements. The thickness of the thin smear is assessed by holding the smear into a light source, the area with a 'rainbow effect' indicates the area of the monolayer. The thick smear is of proper thickness when the 'can you read me' text on the template can be barely read (criteria 5.2). After assessment, the smear is categorized in one of the four quality categories. For the full assessment procedure see appendix 11.12.

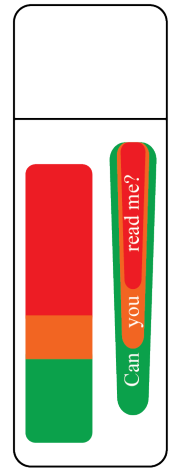


Figure 85: A developed assessment template to assess smears by the bare eye.

5.5.5 THIN SMEAR

This first test is about examining the possibility to prepare high quality thin smears using the MBSS device. A total of 17 tests have been done, using different movement velocities. This was done in order to experimentally find out what the potential influence of the velocity is and with which velocity the best smears are obtained. As seen from *Figure 86*, the spreader is located 'behind' the drop of blood, after which the drop is pushed forward. Normally the spreader is located in front of the drop and the drop is dragged forward. Both methods were tested and it appears it makes no difference in quality.

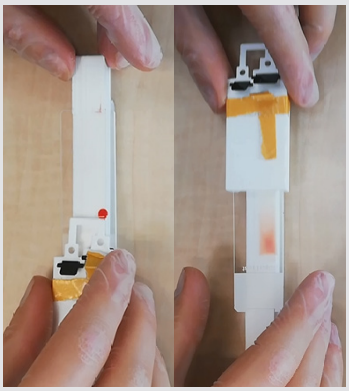
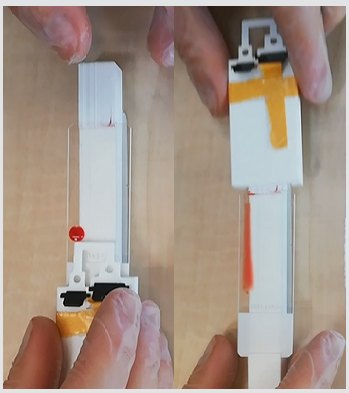


Figure 86: Thin smear preparation.

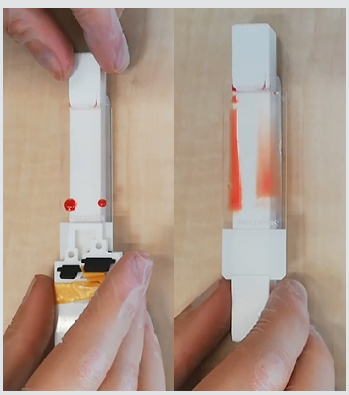
5.5.6 THICK SMEAR

Using the same working principle as for the thin smear, the thick smear is prepared and assessed on its quality. A total of 5 thick smears have been prepared. Because of the observations during the thin smear test, all thick smears are prepared applying manual downward pressure.

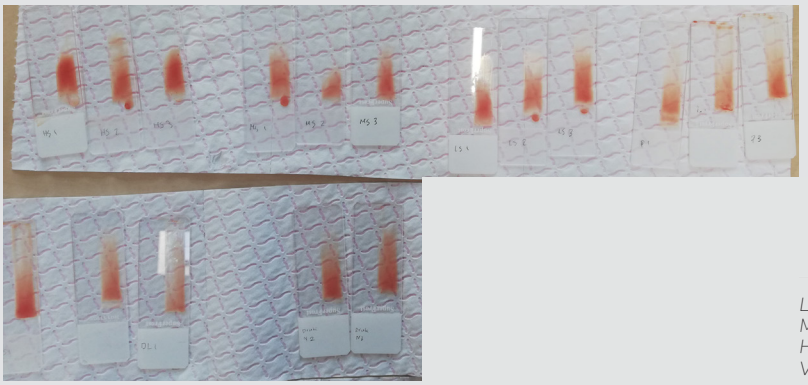


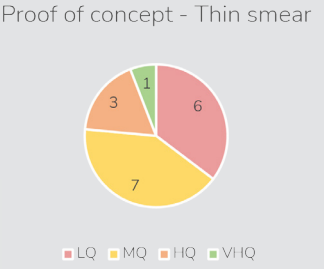
5.5.7 COMBINED SMEARS

The previous two tests show that using the MBSS it is possible to prepare high quality thick and thin smears that meet the quality requirements, when preparing them solely. This test is about preparing both the thick and thin smear on one slide, in the same movement, to see whether the same quality results can be obtained compared to the individual tests. A total of 5 tests have been performed.



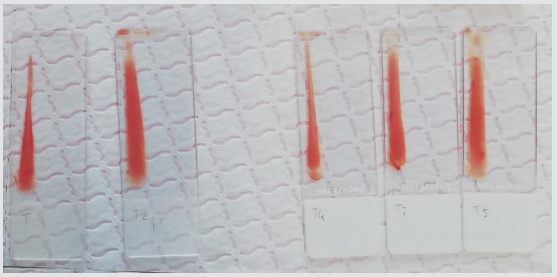
Results

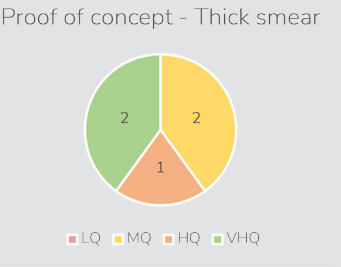




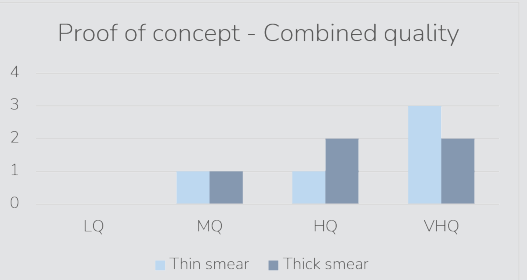
LQ = Low quality (not diagnosable)
MQ = Medium quality (not diagnosable)
HQ = High quality (diagnosable)
VHQ = Very high quality (diagnosable)

Results





Results



Thin smear observations

- Higher velocities result in shorter smears. In general these smears have a less smooth surface area.
- Lower velocities result in longer stretched smears, often with a significantly larger monolayer surface area compared to higher velocities. Also the surface smoothness tends to be better using lower velocities.
- Because the spreaders are 'loosely' inserted in the device, it was difficult to apply an even pressure. Therefore, after noticing this, manual pressure was applied on top of the spreader, so that the spreader cannot move upwards during preparation. This ensured much more consistency in downward pressure. The smears where this pressure was applied were of much higher quality and more consistent in quality.
- The amount of blood highly influences thickness of smears.

Discussion & conclusion

Most of the prepared thin smears do not meet the quality requirements and are allocated in the medium and low quality category. This can be attributed to several factors; firstly, the amount of blood used highly

Thick smear observations

- The smears become decreasingly wide at the end of the smear.
- The surface of the smears look uniform, without any holes or streaks.
- The quality of the thick smear is less dependent on speed than the thin smear.
- The thickness of the smears is similar over different tests. Using the template, the letters were barely readable, which indicates a correct thickness.
- The amount of blood used mainly influences the length of the smear and its surface area, but not the thickness.

Discussion & conclusion

With more than half of the smears categorised in the 'high quality' category or higher, the results are promising. The medium quality ones lack surface area, which is presumed to be due to using too little blood. Similarly to the thin smear test, the blood volume was difficult to regulate, resulting in differences in volume between the tests. The smears where the correct volume was applied, show a good thickness

Combined smears observations

- The smears don't affect each other, in terms of overlapping or fusion of the blood drop. There even is quite some space between the smears.
- Blood volume influences the thickness and length of both smears.
- Movement velocity influences the surface area of both smears.
- A feathered edge is visible in all thin smears

Discussion & conclusion

With more than half of the smears categorised in the 'high quality' category or higher, the results are promising. The medium quality ones lack surface area, which is presumed to be due to using too little blood. Similarly to the thin smear test, the blood volume was difficult to regulate, resulting in differences in volume between the tests. The smears where the correct volume was applied, show a good thickness and surface area. This suggests that leaving 0,4 mm space between the spreader and slide results in thick smears with a good thickness. Also, the body of the smear seems to show a surface with few irregularities.

impacts the thickness of the smear. The micropipette that was used to place the blood on the slide was not accurate enough, resulting in different used blood volumes between smears. Too much blood in combination with a high velocity often resulted in too thick and short smears. Another inconsistent factor is the fact that the spreader is loosely inserted in the device, meaning the downward pressure is not consistent over different smears. However, after applying manual downward pressure to the spreader, the results were better and the smears showed fewer irregularities and a larger monolayer surface area. Another influencing factor is the velocity that was used to spread the blood. It appears that using a high velocity results in short smears that are in general too thick. Using a low velocity results in longer stretched and thinner smears, which are of higher quality. This indicates that using low speeds is preferred for thin smear preparation using the device.

Nevertheless, some of the smears do meet the quality requirements and show a smooth surface with a sufficient monolayer surface area. This is especially the case when the downward pressure is applied to the spreader, in combination with a low velocity. These smears are proof that it is possible to create high quality thin smears using the MBSS device.

and surface area. This suggests that leaving 0,4 mm space between the spreader and slide results in thick smears with a good thickness. Also, the body of the smear seems to show a surface with few irregularities.

The decreasingly wide body of the smear can be explained due to the decreasing blood volume the longer the smear gets. This phenomenon does not influence the smear quality, it actually shows proof for the fact that the smear has a consistent thickness over its length.

Compared to manually prepared thick smears, these smears seem to show more consistency in in thickness and evenness of the surface. It can be concluded that this method of thick smear preparation has potential to create higher quality smears compared to the manual method.

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Compared to manually prepared thick smears, these smears seem to show more consistency in in thickness and evenness of the surface. It can be concluded that this method of thick smear preparation has potential to create higher quality smears compared to the manual method.

5.5.8 CAPILLARY SYSTEM

The concept of the capillary tube system facilitates criteria 17.8, which states that is required to accurately apply the required volume of blood. It was first tested (with coffee), whether the principle of a capillary tube and dropping a drop of blood on the slide when touching it works . Another important aspect of the system is that the drop of blood should be spread along the edge of the thin smear spreader, in order to achieve the width of the smear, this worked (*Figure 87*). It turns out that when the capillary is placed close enough to the spreader, the liquid will spread along the spreader's edge. However, the closer the tube is placed in front of the spreader, the more difficult it becomes to push the capillary down to make it touch the slide and drop the drop of blood.

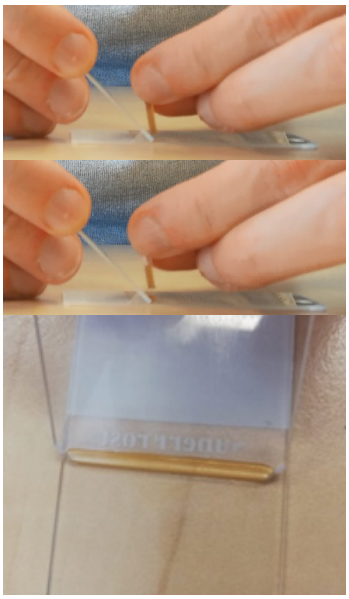


Figure 87: Testing whether the liquid spreads along the spreader's edge, using a capillary tube.

Next, the entire capillary system was tested with coffee as well. However, due to the preliminary and inaccurate prototype of the capillary system, the system did not perform as expected. It was observed that when adding liquid to the top of the tube, the drop moves down to below the bottom of the tube. This results in a drop hanging below the tube (*Figure 88*). The idea behind the tube is that it holds a fixed volume of liquid, however with the drop hanging below it this volume becomes inaccurate.

It was observed that the blood that is dropped at the top of the tube, tends to flow downwards and form a 'drop' underneath the tube (*Figure 88)*. The capillary tends to extract the entire blood drop, even though this goes beyond its volume. This results in the capillary holding more blood than required, also dropping too much blood on the slide.

It turns out that this system still has too many inaccuracies and inconsistencies to make a claim about its effectiveness. Therefore, multiple advancements and iterations on the system have been developed and made (*Figure 90*). However, thus far, the system is considered not to function as envisioned, mainly due to the inaccuracies of the prototyping method. For these reasons, it is decided to not continue with further development of the capillary system, as it is not the core and the priority of the project. Nevertheless, it is believed that further development of this system after this thesis, has the potential to result in a system that enables accurate blood volume application.

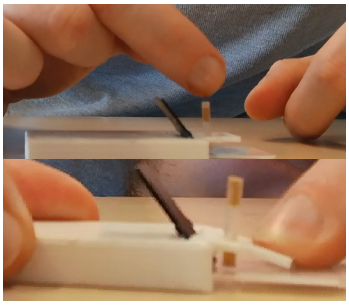


Figure 88: Testing of the capillary system with the prototype. It was difficult to make the tube touch the slide.

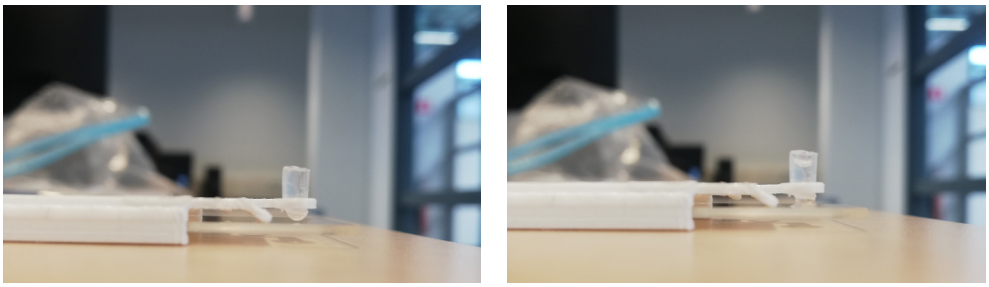


Figure 89: The drop tends to flow 'fall out' of the tube, resulting in dropping more blood than desired.

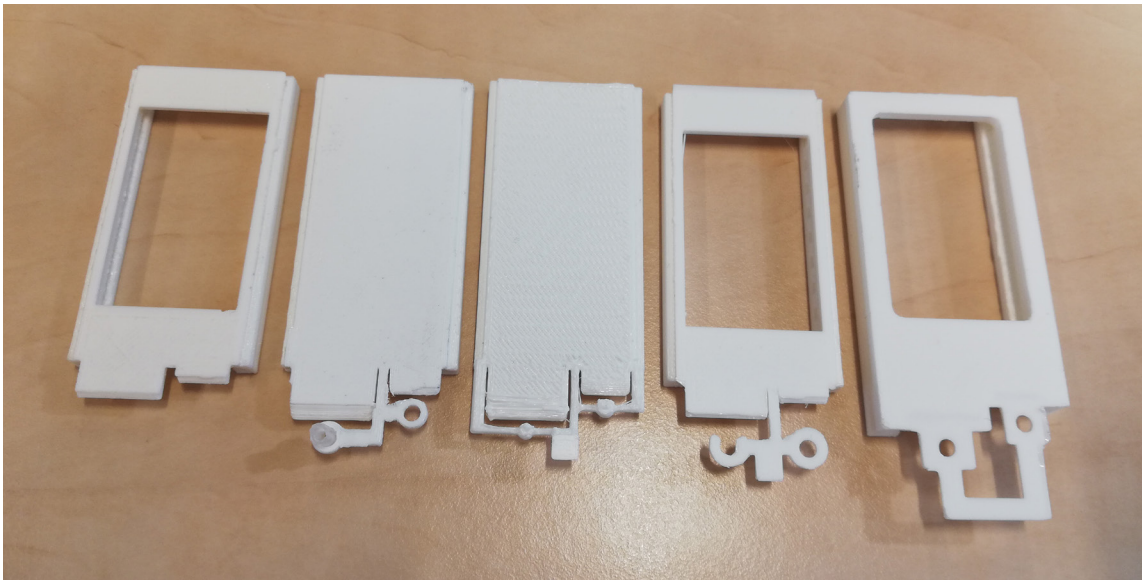


Figure 90: Multiple prototypes with different capillary tube systems.

5.6 CHAPTER CONCLUSION

It can be concluded that high quality thick and thin smears can be prepared using the MBSS. The device shows promising results on the simultaneous preparation of the smears. The separate thin smears show very much potential, the bodies of some of the smears were stretched over the full length of the slide, having more than sufficient monolayer surface area. Even though a small part of the thin smears were categorised in the 'very high' and 'high' quality category, these smears do show proof for successful usage of the tool. The separate thick smears show very promising results as well, their thickness was very consistent, just like their surface quality with very few irregularities. The fact that none of the prepared thick smears was categorised in the 'low quality' category shows its potential. The thick smears were in general of good quality, covering enough surface of the slide, enabling proper diagnosis. The combined smears actually show the best overall results. With no smears located in the 'low quality' category and most smears in the 'very high' quality category, shows the potential of this tool. It shows that it is possible to simultaneously prepare both the thick and thin smear in one movement. Where both smears need to be separately prepared in the conventional, manual method, this is presumed to enhance the ease and efficiency of the smear preparation process. About the impact of the variables on the smear quality, no concrete conclusion can be drawn yet. This is due to the fact that the pressure and speed were not fixated with this prototype and it is unknown which exact parameter values were used. However, it can be stated that in general a low speed resulted in the highest quality smears. The smears were best stretched over the full surface and appeared thinnest. In order to make specific statements about the impact of the variables, further research is required.

The capillary system did not work as expected. The blood that is dropped at the top of the tube, tends to flow downwards and form a 'drop' underneath the tube. The capillary tends to extract the entire blood drop, even though this goes beyond its volume. This results in the capillary holding more blood than required, also dropping too much blood on the slide. On the other hand, the capillary system does show a user advantage in terms of usage. In manual smear preparation the blood is often applied straight from the finger onto the slide, through the capillary system this principle can be maintained. It works more convenient than separately extracting the blood with a capillary tube and then applying it to the slide. It is believed this system has potential, however, within the project's timeframe it's expected be unfeasible to fully develop this system in combination with fully developing the working principles of the MBSS. Therefore the decision is made to leave the capillary system out of the product. This means that another solution to the blood volume application is required. It is decided that adding a plastic capillary tube to the MBSS package is currently the best feasible and viable option. With the capillary tube the blood volume can accurately be measured and applied to the slide.

All in all, the MBSS shows promising results on delivering high quality thick and thin smears and in easy facilitation of the process of blood smear preparation.

5.7 CRITERIA & CHALLENGES

19. Product functionality

19.1 (cr) The distance between the spreader and slide should be 0,4mm for optimal thick smear thickness (5.8.6).

19.2 (cr) The slide that is stored internally in the device, must be held in position by the device and ensure it cannot fall out (5.4).

19.3 (ch) It must be ensured that the slide is pulled out far enough, but not too far (5.4).

19.4 (ch) It must be ensured that the slide is fully inserted in the device, but not too far (5.4).

19.5 (ch) It must be ensured that the slide cannot be retracted once the smear is prepared and the slide is stored inside the device (5.4).

19.6 (cr) The product must be transparent (5.4).

20. Product use

20.1 (ch) It must be ensured the slide can be pulled out and pushed in with a smooth movement (5.4).

20.2 (ch) It must be ensured the spreaders are not touched by the operator's fingers (5.4).

20.3 (ch) The user must receive feedback on whether the smears succeeded. A quality indication must be received (5.4).

21. Transport and packaging

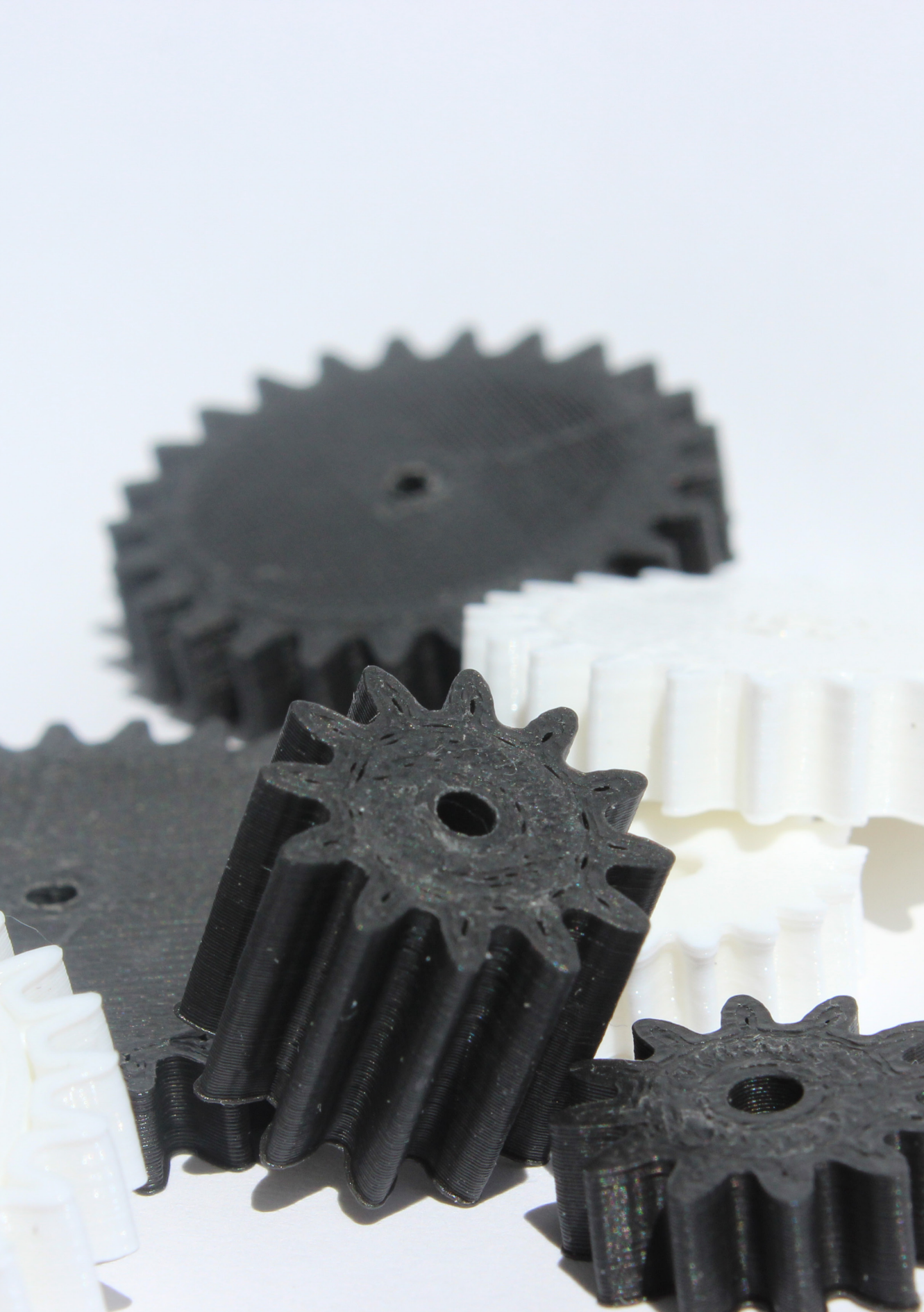
21.1 (ch) While opening the packaging, it must be ensured no components can suddenly fall out of the packaging (5.4).

21.2 (ch) The device must be packaged in such a way so that it can be safely and efficiently transported, without components breaking down (5.4).

22. Product

22.1 (cr) The MBSS device must include a slide (5.2.3).

22.2 (cr) The MBSS device must contain a capillary tube (5.5.8).



CHAPTER 6

PARAMETER DETERMINATION

The three skill-based variables angle, pressure and speed highly influence the quality of the thin smear. Previous tests show that in order to consistently prepare high quality smears, it is of importance to use fixed parameter values for these variables (challenge 17.9). This subchapter presents and discusses multiple test-setups, with which the variable parameters values are found that produce the highest quality smears. The found parameters are eventually translated and integrated in the final design of the MBSS.

6.1 METHOD

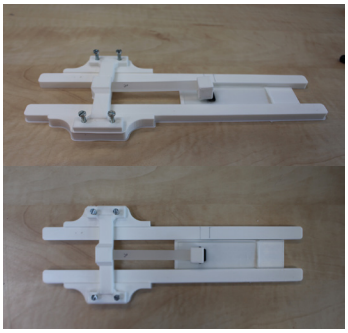
Parameter determination

Parameter validation & speed range

A first parameter test, assessing the influence of the three variables, was performed using the conventional, manual method of smear preparation. However, it appeared that this test was not accurate in terms of finding the best parameter. Besides, the results were difficult to translate to the design of the device, as the spreader dimensions were not the same. This test can be found in appendix 11.13. Therefore, another test setup that has been designed to produce more accurate results. Eventually the parameters that are found through the test setups are validated using an automated test setup. This setup prepares multiple smears using the exact same parameter values, so that the quality consistency can be assessed and conclusions can be drawn on the effectiveness and influence of the parameters. In addition, the automated setup enables determination of the speed range.

6.2 MANUAL TEST - BEAM DISPLACEMENT

Based on the insights obtained from the initial test (appendix 11.13), a test setup has been designed and built, which facilitates more accurate application of the parameter values. This test setup makes use of the thin smear dimensions that are envisioned for the MBSS, this enables more accurate translation and integration of the parameters from the test setup to the device's design.



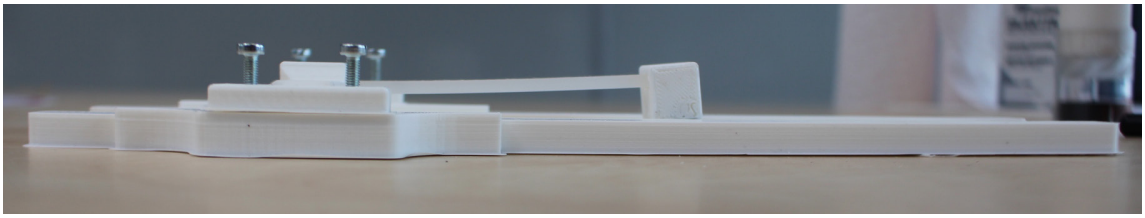


Figure 91: Test setup that was used to determine the best parameters for the angle, pressure and speed variables.

Method

A total of 50 tests have been conducted, in which the applied speed, angle and pressure parameters varied (for the test order and parameter value combination see appendix 11.14). A more accurate micropipette is used, to apply the blood volume in a more controlled way. *Figure 91* shows the test setup.

Procedure

The procedure is as follows (*Figure 92)*:

1. Put a slide in the slide holder.
2. Move the slide holder forward, until the spreader is applying pressure at the beginning of the slide.
3. Add a drop of blood on the slide, in front of the spreader.
4. Move the slide holder slightly forward, until the spreader touches the drop of blood.
5. Now the smear is ready to be prepared, move the slide holder forward with a constant speed to create a thin smear.
6. Get the slide out.

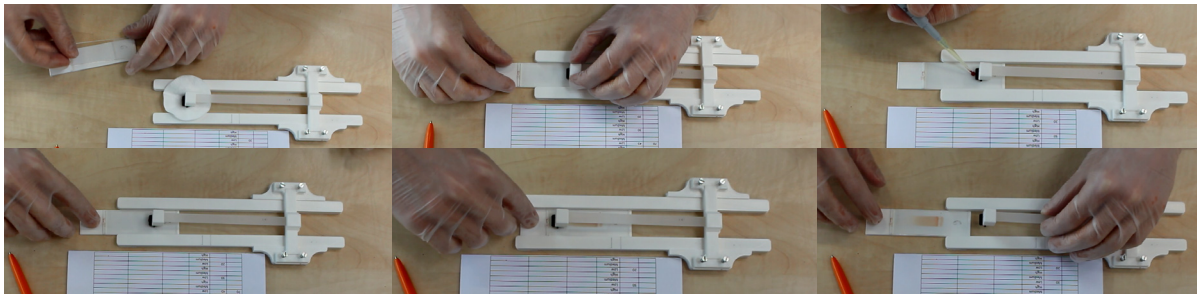


Figure 92: Test procedure using the test setup.

6.2.1 CHANGING THE PARAMETER VALUES

The three variables speed, angle and pressure can be adjusted in the following manner:

Speed

Three different speed 'levels' are manually applied; low (±0,05 m/s), medium (± 0,1m/s) and high speed (±0,15 m/s). Even though the speed is manually controlled, it's fairly easy to apply similar velocities between tests. Manually applying the velocity doesn't give an exact number in m/s that should be used to prepare the best quality smears, instead it gives an indication and approximation on the speed range that results in high quality smears.

Angle

The angle is determined by the angle of the spreader corresponding to the slide. The spreader is attached in the 'spreader holder' (*Figure 93*). The spreader holder has an integrated angle in which the spreader can be attached. Three spreader holders were 3D printed, each with a different angle; 20, 30 and 45 degrees.

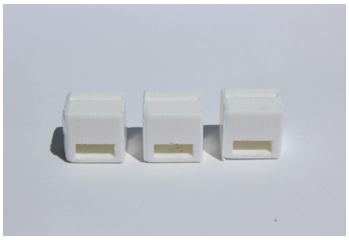
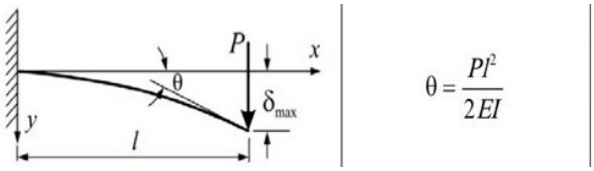


Figure 93: Spreader holder with different integrated angles.

Pressure

The downward pressure is applied using the beam displacement principle. The beam, to which the spreader holder is attached, is bent upwards when the glass slide is in contact with the spreader. This results in a downward spring force from the spreader on the glass. The actual force is dependent on the material properties and the dimensions of the beam. With the formula from *Figure 94*, the downward pressure can be calculated. Five different beam lengths (*Figure 95*) were used, each applying a different pressure.

Figure 95: Different beam lengths to apply different pressures.



$$\theta = \frac{Pl^2}{2EI}$$

Figure 94: Beam principle, with this formula the applied pressure can be calculated.

6.2.2 DATA ANALYSIS

The quality of the smears is assessed and categorized using the assessment template. Eventually, the influence of each parameter value is 'measured' and compared through a visual graph, enabling concluding which parameter value facilitates the best results.

6.2.3 RESULTS

*Figure 96*shows the results of the prepared smears.

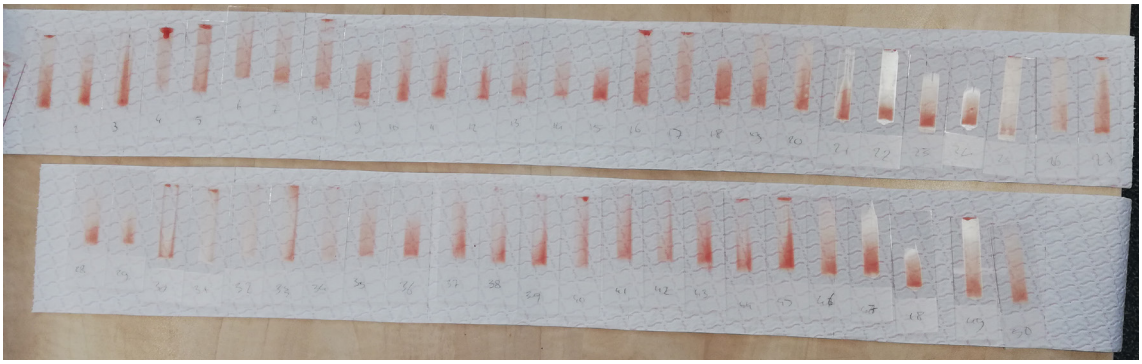


Figure 96: Test results of 50 prepared thin smears.

Using the template and assessment procedure, *Figure 97* shows the quality categorisation of the smears.

Figure 97: Quality categorisation of the smears per applied variable parameter.

The graph explained:

- The first three columns show the used velocity with the corresponding amount of the different quality smears.
- Column 4-6 show the used angle with the corresponding amount of the different quality smears.
- Column 7-12 show the used pressure with the corresponding amount of the different quality smears. The number after 'P' relates to the length of the beam used in mm.

6.2.4 DISCUSSION

Speed

Figure 98 shows the influence of the speed on the quality of the smears. It appears that when using a high speed, the amount of low quality smears is much higher compared to medium- and low speed. In general the smears created with high velocity were too short and didn't have sufficient surface areas with monolayers. The lower the speed, the higher the percentage of high quality slides. The 'low speed' even didn't result in a single low quality smear. This suggests that the lower the speed, the better the quality of the smears.

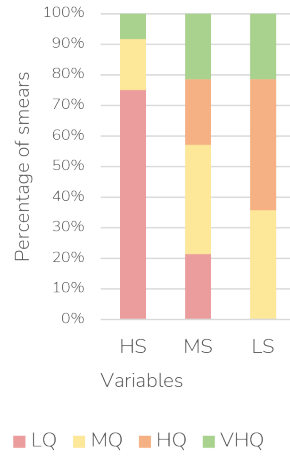


Figure 98: Quality categorisation of the smears prepared with different speed parameters.

Angle

Figure 99 shows the influence of the angle on the quality of the smears. The results show that the higher the angle, the higher the amount of low and medium quality smears and vice versa. A higher angle often resulted in more holes and streaks, also the smears appeared shorter compared to lower angle smears. At a lower angle the smears appeared smoother and longer, resulting in a better surface quality and a larger monolayer surface area.

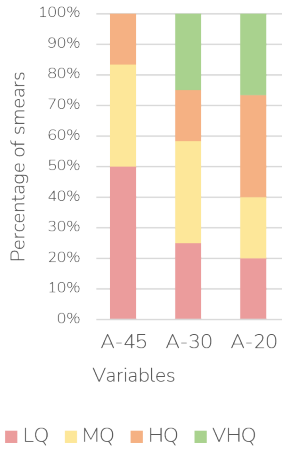


Figure 99: Quality categorisation of the smears prepared with different angle parameters.

Pressure

Figure 100 shows the influence of pressure on the quality of the smears. The number behind P indicates the length of the beam, the longer the beam the lower the pressure. With pressures P-150 & P-130 a relatively high percentage of low- and medium quality smears is present. Many of these smears appeared with holes and streaks, presumably because of too little downward pressure. P-70 even shows worse results, with only low- and medium quality smears. Due to the high pressure the smears were too short and the movement was not smooth, also holes and streaks were frequently present. The highest percentage of high quality smears is in the 'medium' pressure range with pressure P-110. The smear surface was the smoothest, had a good length while having a smooth forward movement.

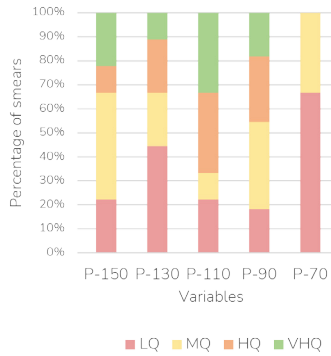


Figure 100: Quality categorisation of the smears prepared with different pressure parameters.

General

Low velocities tend to give the best results, however the lower the velocity, the more difficult it becomes to keep it a smooth movement. A non-smooth movement could result in lines and streaks in the smear. On the other hand, the results didn't show a frequent occurrence of lines or streaks when applying a low speed. Since the speed was manually applied, there are differences in applied velocities within the same velocity category (low-, medium- and high speed). This results that no quantitative conclusions can be drawn on the influence of the speed on the quality of the smears. However, it can be stated that in general, a low speed gives the best results. Further research that defines the specific velocity values is required. Besides, the velocity range that can be used to produce high quality smears needs to be defined, in order to conclude whether to keep this a manual or automated operation.

Even with a more accurate pipette, the blood volume still slightly varied per slide. This resulted in slight differences in thickness of the smears. However, this had relatively no impact on the quality of the smears, since the difference in thickness is mainly in the first part of the smear, while the monolayer area remains nearly the same.

Literature states that different angles should be used with different viscosities of blood (2.4.8), when fixating the angle this won't be possible anymore. The impact of fixating the angle related to different blood viscosities requires further research.

6.2.5 PRESSURE CALCULATION

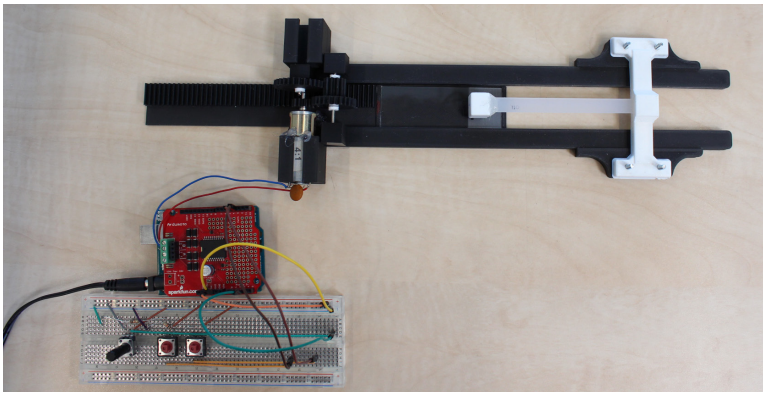
Since P-110 results in the largest amount of high quality smears, the corresponding pressure value is calculated using the formula from *Figure 94*. The pressure is calculated using mathematic software 'Maple'. The result shows that a pressure of 0,63N is the required pressure that leads to the best results. For the full calculation see appendix 11.13.

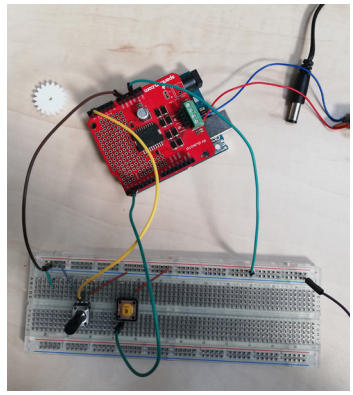
6.2.6 CONCLUSION

The data from *Figure 97* gives a clear overview of the influence of each parameter value on the quality of the smear. They show that a combination of a low movement speed (± 0,05 m/s), an angle of 20 degrees and a pressure of 0,63N (64g) result in the largest amount of high quality thin smears. This combination of parameter values ensures a lengthy smear with enough monolayers field of views for diagnosis and a smooth surface. The fact that these parameter values are now known, is a step towards increasing and ensuring the consistency of the thin smear quality. Eventually the values found with this test can be translated and integrated to the design of the MBSS, so that the device can apply fixed parameter values for each smear that it prepares.

The chosen parameter values require further research, to assess the consistency in quality when multiple smears are prepared using the exact same parameters per slide. Only then a conclusion can be drawn about the consistency in quality these parameter values provide.

The previous test setup determination of the angle and pressure parameter values and an estimation of the speed range. However, one last test and validation of the parameters is desired, to validate whether the chosen parameters truly show consistency in preparing high quality thin smears and to determine the speed range. The outcome is decisive for the choice to automatically or manually operate the speed. Therefore a final test setup is built, which is an automated advancement of the setup from the previous chapter (*Figure 101*). A speed configurable motor is added to the setup, which now enables smear preparation repetition using the exact same speed parameter values. The automated setup also enables determination of the speed range in which high quality thin smears are prepared.





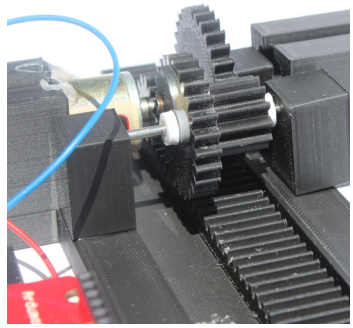


Figure 101: Automated advancement of the test setup that was used in chapter 6.2.

6.3.1 METHOD

Validation

10 tests have been performed, using the parameter values defined in chapter 6.2.6. The 10 tests are repeated with the exact same parameters under the same conditions. Since the manual test did not result in a specific velocity parameter, a couple of calibration tests are executed within the 'low' velocity range. Eventually one velocity value is chosen that results in high quality smears. Eventually the smears are assessed on their quality using the assessment template. Also the cell distribution was assessed with a microscope, in order to validate the cell distribution of the thin smears.

Speed range

The speed range is determined by preparing smears at various speeds, while keeping the angle and pressure parameter as defined. This test consists of three stages: starting at high speed and lowering the speed up to the point where high quality smears are prepared, setting the high boundary. Once this point is reached, the speed is lowered up to the point where quality starts to decrease, setting the low boundary. Once this point is reached the speed is increased again, to validate the initial high boundary. This process is recorded and analysed afterwards with motion analysis software 'Tracker', to find the exact speed boundaries in m/s.

Figure 102: The test set-up.

6.3.2 RESULTS

Validation

Figure 103 shows the results of the 10 performed tests.

Using the template and assessment procedure, the pie chart in *Figure 104* shows the quality categorisation of the smears.

Speed range

Figure 105 shows the results of the smears that were prepared using the different speeds.

After smear assessment using the template, the high and low boundary are chosen. The corresponding velocities were calculated using the tracking software .

High boundary: <0,093 m/s = 0,34 km/h

Low boundary: >0,01 m/s = 0,037 km/h

6.3.3 DISCUSSION & CONCLUSION

It can be concluded that when applying the determined parameters from chapter 6.2.6, consistent high quality thin smears can be prepared. The smears are well stretched over the full length of the slide and meet mono layer surface area requirements. The surface of the smears show few irregularities, meaning the surface is of good quality. One inconsistency that is noticed, is the fact that some of the smears end sooner at the right top side. This is assumed not to be due to any of the parameters, but most likely because of slightly uneven pressure distribution of the spreader on the slide. Another observation are the horizontal lines in the smears. When assessing under the microscope, these lines do not impact the quality of the smear (*Figure 107*). It is assumed the lines are a result of the gearbox, due to small spaces between the gears the movement is not super smooth.

Regarding the speed range it can be concluded that the range in which high quality smears are produced is wide. It appears that there is basically no lower boundary, since applying an extremely low velocity of 0,01 m/s still results in a high quality smear. On the other hand, the high boundary of 0,093 m/s, must not be exceeded, since this results in lower quality smears. Even though the high boundary is not high, it is believed that with the proper instructions the user's movement speed can be kept within the speed range fairly easy. Therefore, it is decided to keep the speed a manually controlled variable in smear preparation using the MBSS. This comes with multiple advantages; it keeps the design simple, easy to use and low cost. Adding automated speed control components would increase the complexity of the product, making it more sensitive to failure and more challenging to use in the context. Next to that, manually controlling the speed lets the human touch to the process and product interaction remain. This is of importance for the cooperation and trust of the participants and the feeling of control of the user.

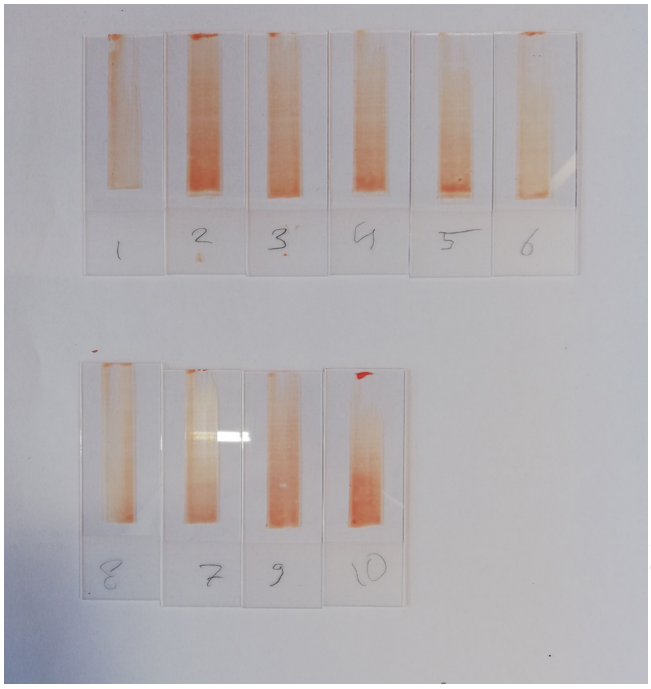


Figure 103: Test results of 10 perfomed tests, using identical variable parameters.

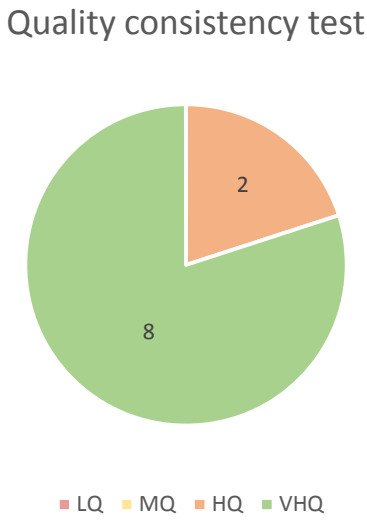


Figure 104: Pie chart showing the quality categorisation of the 10 smears.

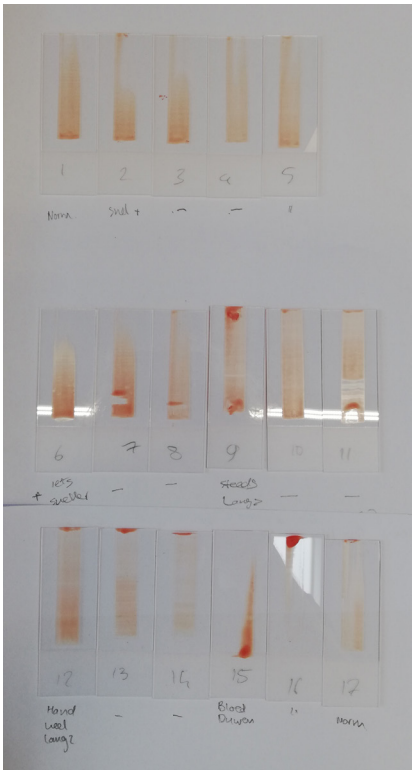


Figure 105: Prepared smears in the 'speed range determination' test.

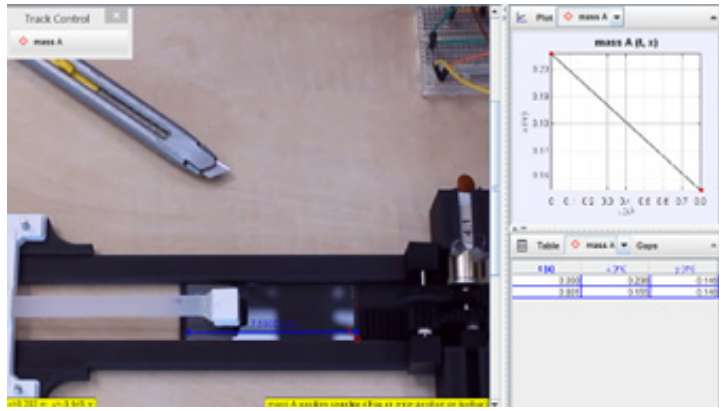


Figure 106: Using motion tracking software, the speed range parameters were determined.

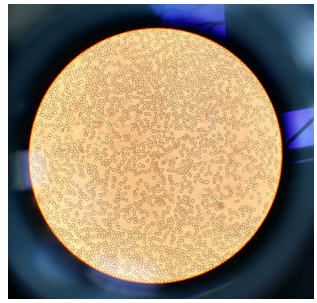
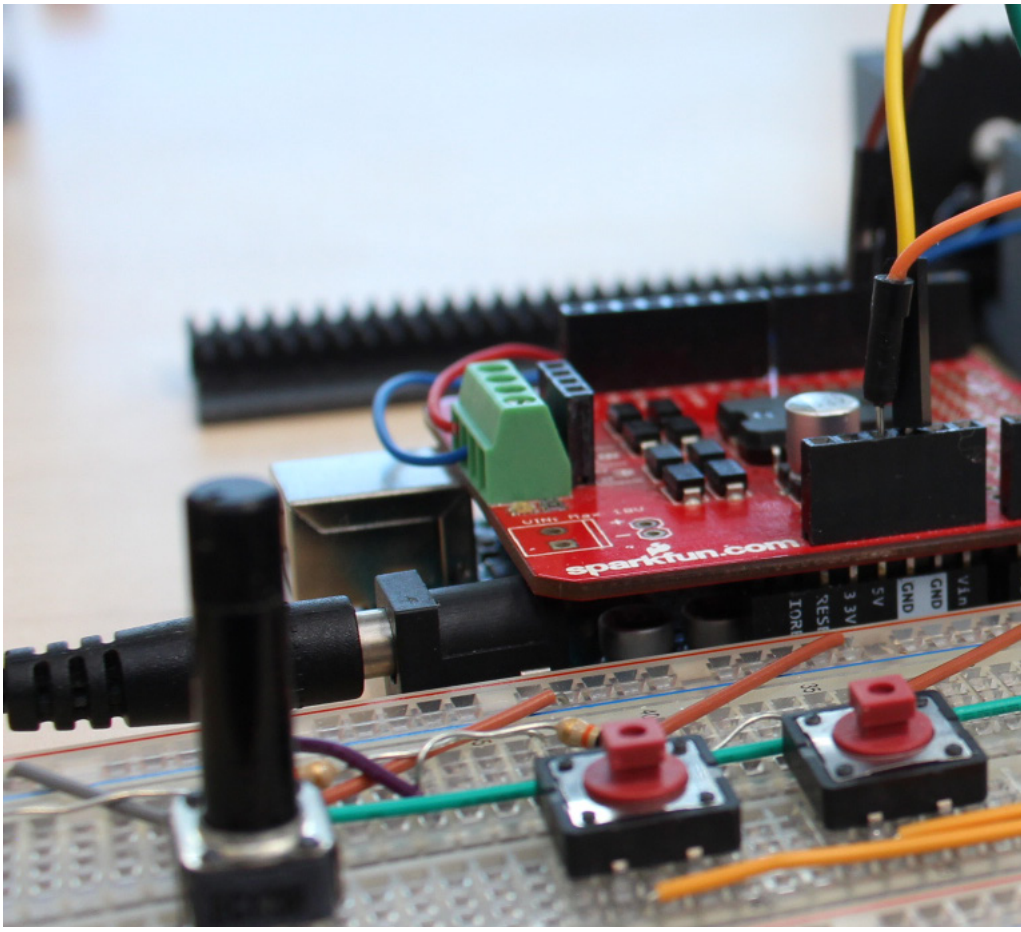


Figure 107: Assessing the smears under the microscope shows a good RBC distribution

6.4 CHAPTER CONCLUSION

Chapter 2 discusses that the skill based variables speed, angle and pressure in thin smear preparation are one of the most impactful factors on the smear's quality. Applying a wrong parameter on either one of these variables will result in a poor quality smear. Therefore, for the effectiveness of the device, the impact of these variables must be minimized or where possible eliminated. Now that through excessive testing the combination of speed, angle and pressure parameter values have been defined, a higher thick and thin smear quality and more quality consistency can be guaranteed when applying these parameters. Knowing and eventually implementing the parameter values in the MBSS design is a good step towards taking away the human errors in smear preparation. Since correctly applying the parameters in manual smear preparation is skill and experience dependent, the integration of the parameter values in the MBSS will take away the need for experience on this aspect in smear preparation. Integrating the parameter values makes smear preparation much simpler and accessible.



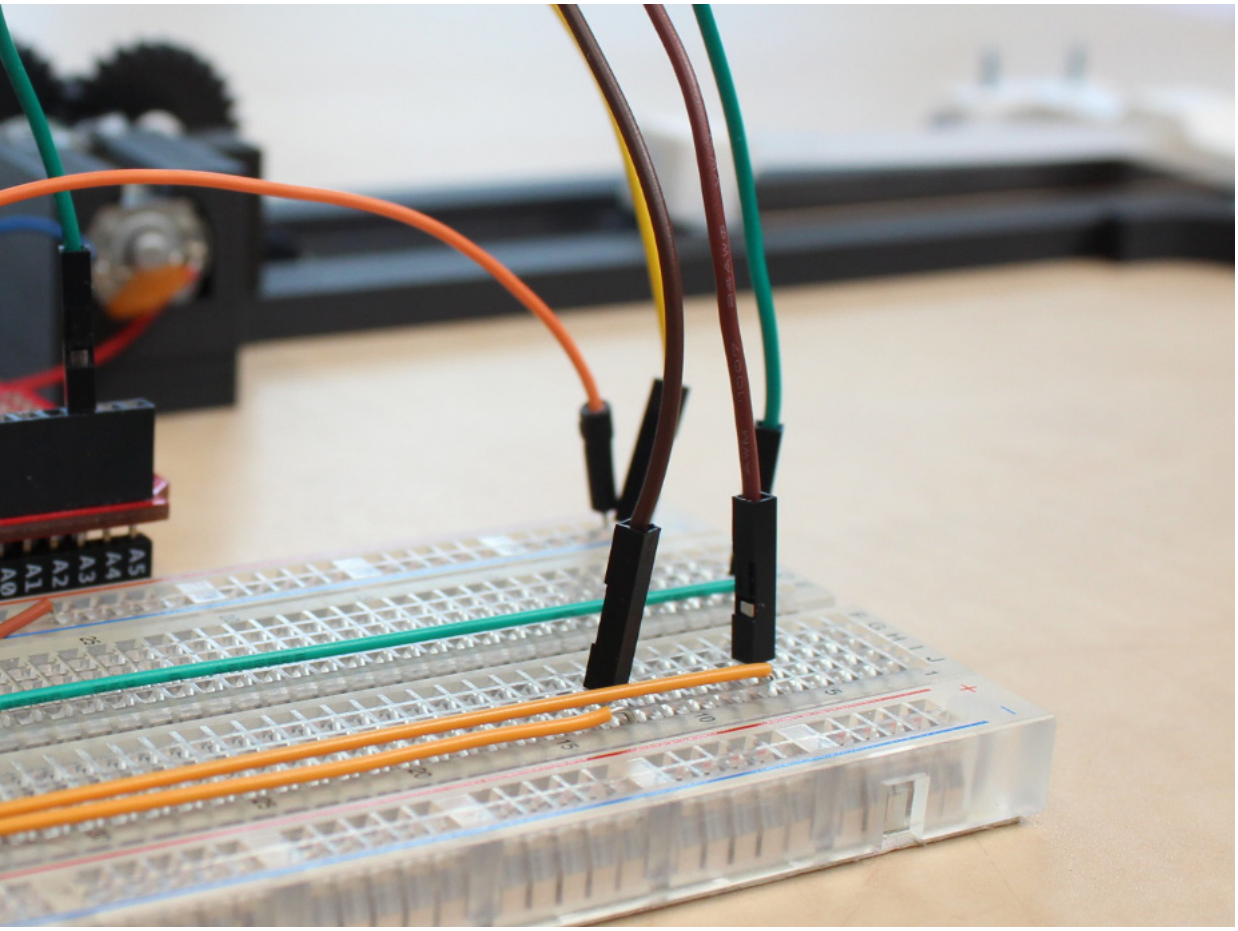
23. Product functionality

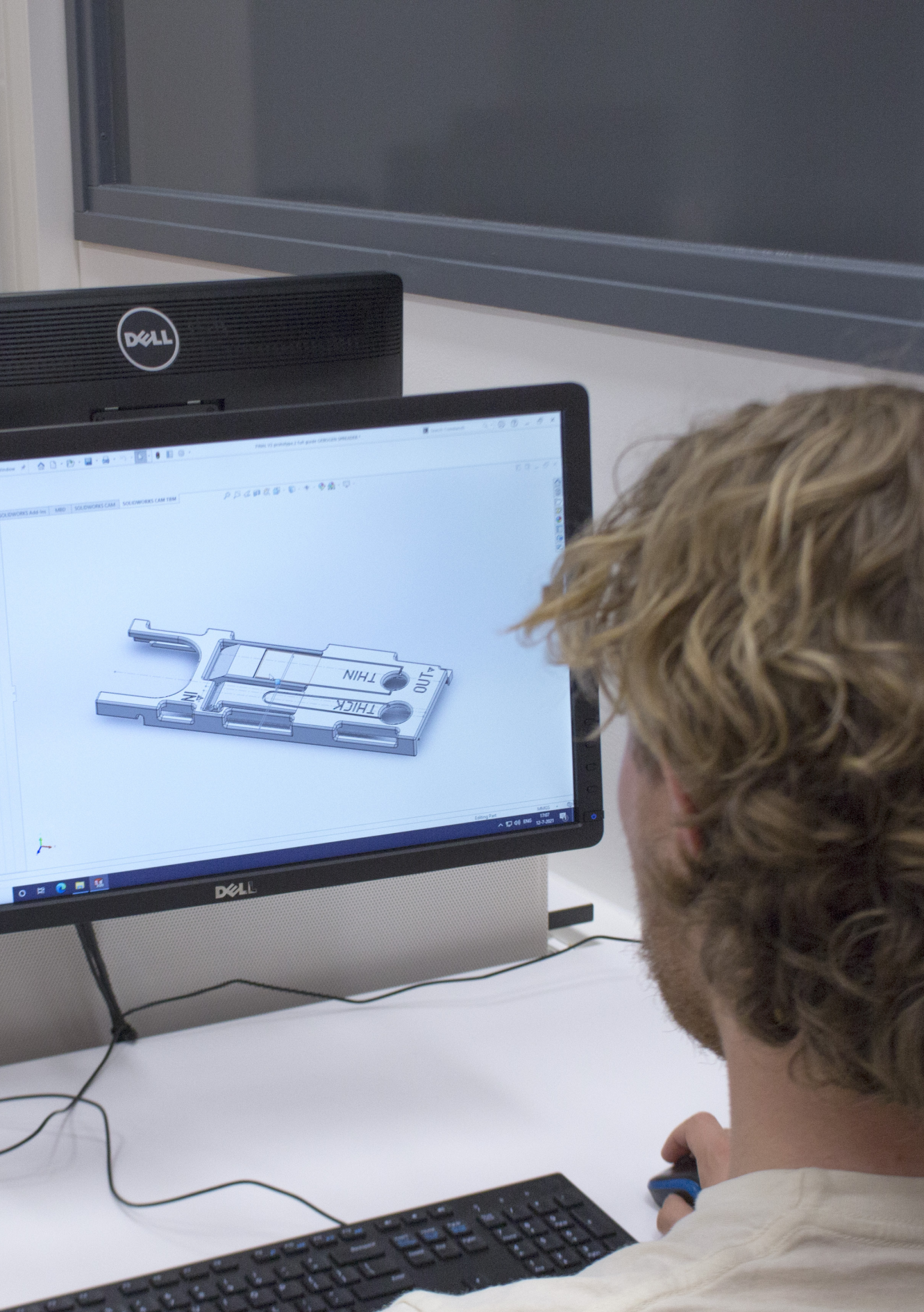
23.1 (cr) The thin smear spreader must have a 20 degree angle compared to the slide (6.2).

23.2 (cr) The thin smear spreader must produce a 0,6N downward force on the slide during spreading (6.2).

23.3 (cr) The minimum applicable speed of inserting the slide must be 0,01 m/s (6.3).

23.4 (cr) The maximum applicable speed of inserting the slide must be 0,093 m/s (6.4).




DELL
THIN
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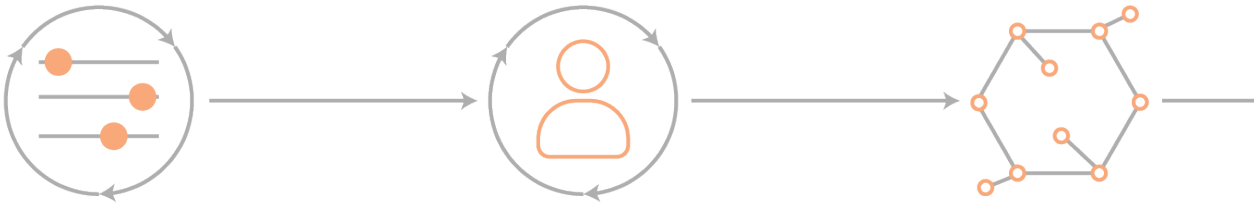
CHAPTER 7

IMPLEMENTATION

This chapter is about implementing the gathered knowledge from the user scenario and parameter tests in the design MBSS. This chapter will show the advancement of the current MBSS design into a state-of-the-art product. It must be noted that this process was iterative and many variations and designs have been developed that lead to the design presented in this chapter.

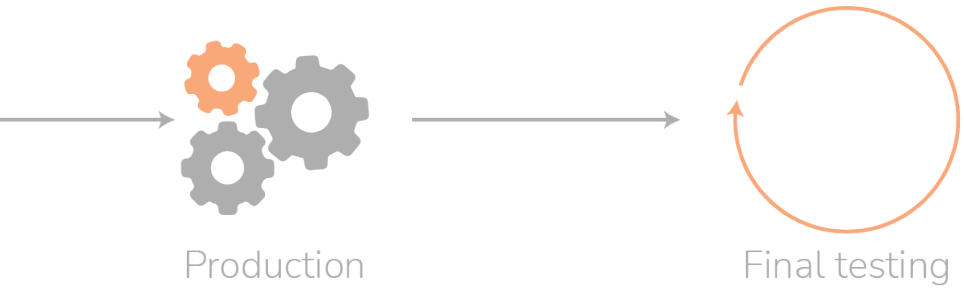
7.1 METHOD



Implementing parameters

Implementing user scenario features

Materialisation



First, the defined parameters are implemented in the design, so that the device can consistently deliver high quality smears, by eliminating the angle and pressure variable. Next, the insights from the user scenario are implemented in the product. After implementation of all the features, the materialisation and production of the MBSS will be discussed. Eventually, a prototype of the envisioned concept is made, which is tested on its performance. Based on this test, final criteria are set, which are used to develop the final design.

7.2 IMPLEMENTING PARAMETERS

The angle and pressure parameter need to be integrated, since the decision is made keep the movement speed a manually controlled variable. This can be done relatively easily.

7.2.1 PRESSURE

The calculated pressure of 0,6N (6.2), needs to be integrated in the MBSS, so that its spreader applies this exact pressure to the slide. 5.3 discusses how the spreader applies pressure to the slide, through an integrated spring mechanism. Now that the required pressure and the spreader dimensions are known, the required displacement to apply this pressure can be calculated. The displacement is calculated at a value of 0,6mm (see appendix 11.14 for the calculation), meaning the spreader must be bent upwards by the slide with 0,6mm (*Figure 108)*. The material properties of a Formlabs resin are used, since this material is used for a final prototype.

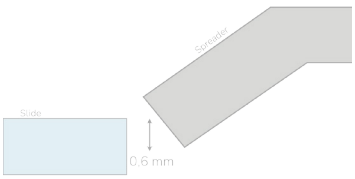


Figure 108: Required displacement of 0,6mm to apply a downward pressure of 0,6N.

7.2.2 ANGLE

By changing the model's angle compared to the slide in the 3D CAD software, the 20 degree angle can easily be integrated in the design. However, what must be taken into account is that due to the applied pressure, the spreader bends up 0,6mm when the slide is inserted, changing the angle. The angle with which the spreader bends needs to be added to the angle of the spreader, to ensure the 20 degree angle when the slide is inserted (*Figure 109*).

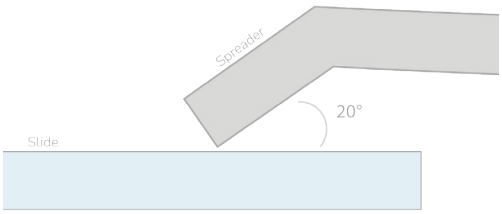


Figure 109: Integrated angle of 20 degrees.

7.3 INTEGRATION USER SCENARIO CRITERIA

Figure 110 shows the conceptual design as defined in chapter 5. The capillary system is removed, since it is replaced for a capillary tube that is added to the package. Now that the parameters are integrated in the design, the criteria coming from the user scenario must be integrated. The envisioned user scenario from chapter 5.4 resulted in several criteria that must be met, in order to ensure the product is used in the intended way and to ensure the product cannot be used in the wrong way. This chapter shows the integration of new features to meet these criteria.

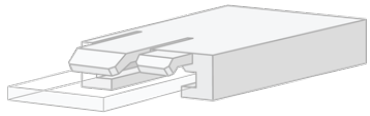


Figure 110: Conceptual design as defined in chapter 5.

It is unwanted that during the insertion movement the device's spreaders are touched by the operator's fingers (challenge 20.2). Therefore the area in front of the spreaders is protected by adding another section (*Figure 111*). The holes in front of the spreaders enable application of the blood drops from the capillary tube. The hole in front of the thick smear spreader is larger, since more blood is required. This new section also ensures covering up and protecting the front, frosted, end of the slide. However, this disables easy insertion of the slide in the device, since the operator's fingers will touch this front section before the slide is fully pushed inside. Therefore, a circular cut at the front of the device is made (*Figure 112*), so that the slide can be fully pushed inside the device's conveniently (challenge 19.4).

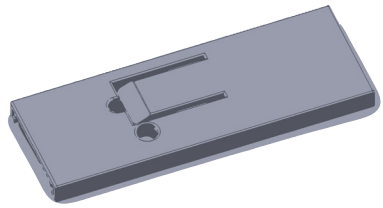


Figure 111: Front section is added, to protect the spreaders from being touched.

Ensuring the slide is held inside the device's body is done automatically by the thin smear spreader. The downward force produced by the spreader results in enough friction to hold the slide in place. However, when pulling out the slide from the device, it must be ensured that the slide is not pulled out too far (challenge 19.3). It is challenging to make a 'stop' system for this, as a solution to this would most likely require modification of the slide, something that is not envisioned. Therefore it is decided to give the user visual feedback until where the slide must be pulled out, by means of an indication line. Besides, it can be visually seen whether the slide is pulled out far enough, at the moment the drops of blood touch the spreaders.

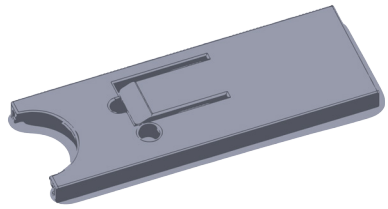


Figure 112: Circular cut in the front section, in order to enable the user to fully insert the slide.

Next, a snap connection is added to the front and back of the device (*Figure 113*). The front snap connection ensures that when the slide is fully pushed in the device, the slide cannot be pulled out of the device again, since this would ruin the smears (challenge 19.5). Another snap connection is added to the end of the device. This ensures that the slide is stopped when pushed inwards (challenge 19.4). The shape of this snap connection is different compared to the front snap, as it is required that the slide can exit the end of the device. The combination of the snap connections ensures that the slide is securely stored in the device. Besides, the snap connections give the user auditory feedback, as the front snap makes a 'click' sound when the slide is pushed in far enough. As a reminder the slide should not be pulled back once inserted, but pushed out on the other side, 'In' and 'Out' is textually added (*Figure 114*).

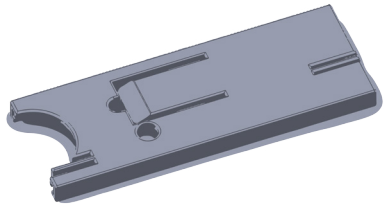


Figure 113: Added snap connections, to ensure the slide is held in position and cannot be pulled out at the wrong end once prepared.

Challenge 20.3 states that the user must receive feedback whether the smear succeeded or not, a quality indication must be given so that the it can be confirmed that the smear is of actual high quality and suitable for diagnosis. Even though it is envisioned the MBSS device will make sure the smears are of consistent high quality, it is desired that this can

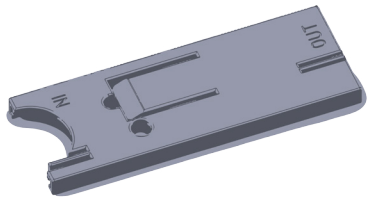


Figure 114: Added 'In' and 'Out' text.

be checked and confirmed by the operator. The main requirements of the smear quality is sufficient surface area, the thickness of the smears and having a smooth surface. Since the smears are prepared inside the MBSS and not visible to the user, it is decided to make the device of a transparent material, so that the user can observe and check the smear quality. In order to assess if the smears have the required surface area the top of the device is equipped with indication lines. The lines at the indicate the required length and width of the thick and thin smear (*Figure 115*). It can be visually assessed by the operator whether the prepared smear meets the dimensions of the indication lines. If this is the case, it means the smear's surface area is sufficient. The transparent body of the MBSS enables a double check when there is doubt about the thickness of the thin smear. The smear can be held into a light source and checked whether a 'rainbow effect' appears, this indicates the monolayer.

Figure 116 shows renders of the currently envisioned device.

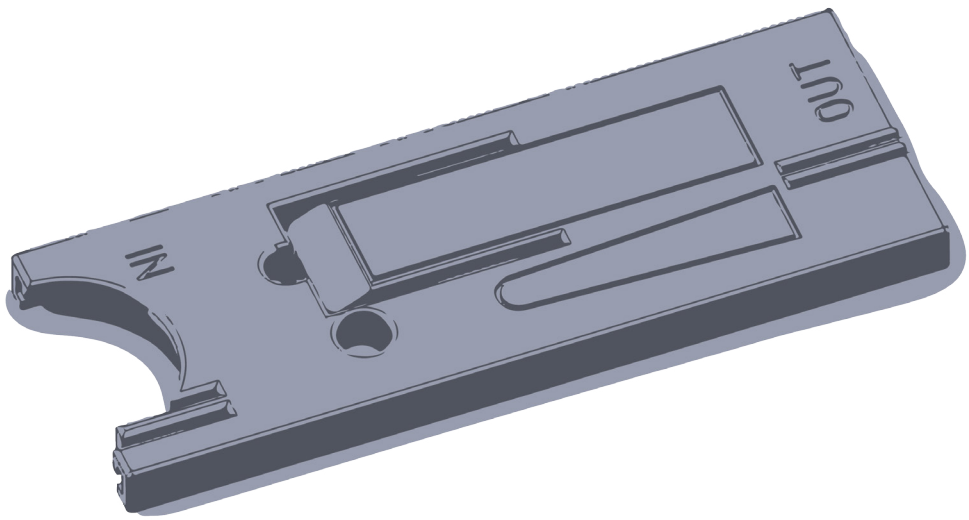


Figure 115: Lines that indicate the required length and width of the thick and thin smear are added to the top of the device, as quality feedback for the user. If the smears are stretched outside these lines, it indicates the smears meet the surface area requirements.

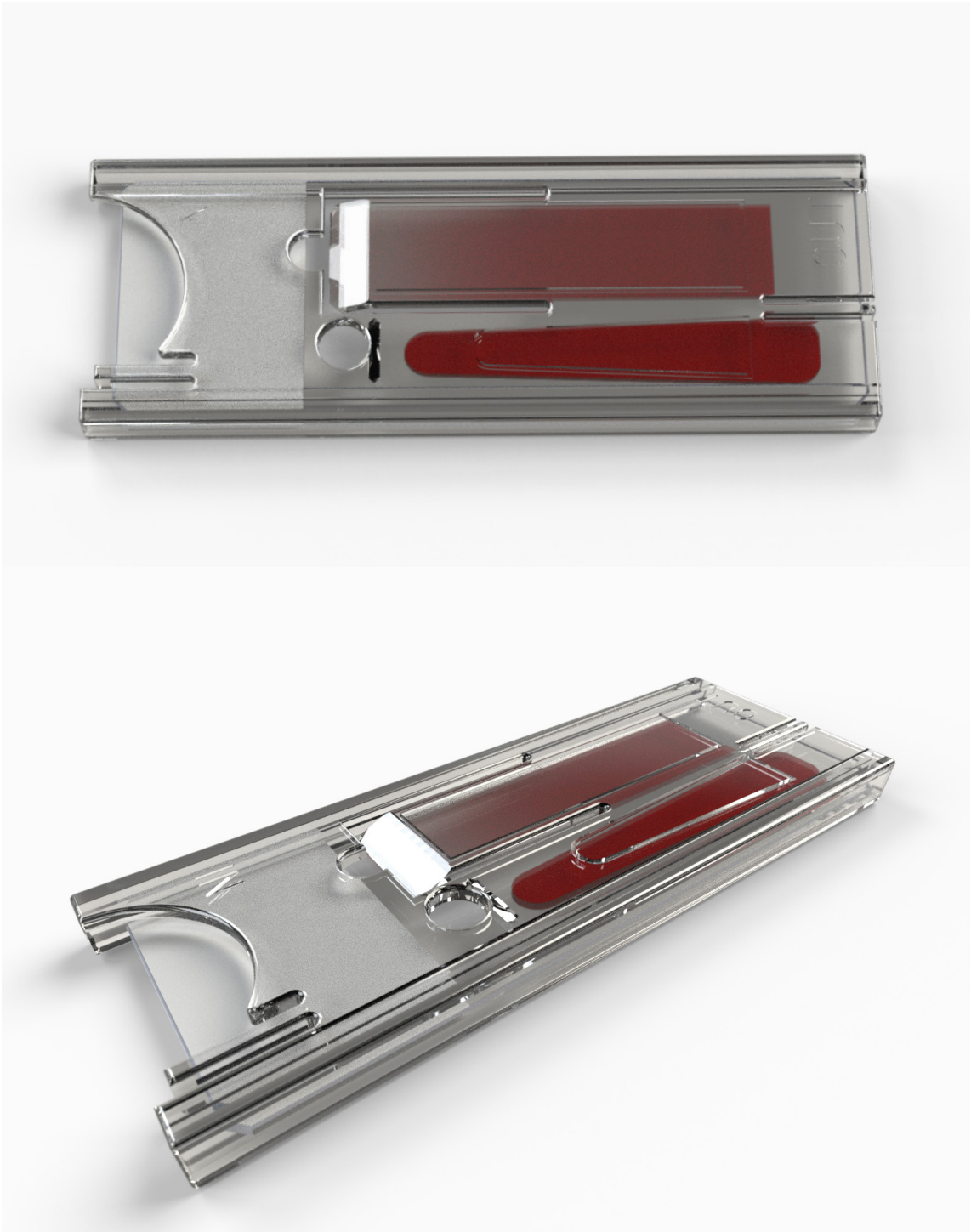


Figure 116: Renders of the envisioned concept thus far.

7.4 MATERIALISATION

A material analysis was conducted using material database software CES.

The device must be transparent (criteria 19.6), in order to enable visual assessment of the prepared smears. Plotting material transparency over price in CES, creates *Figure 117*. 'Transparent' or 'optical quality' is the desired transparency level. Looking at the figure, it shows that PET, PS, PP, and PVC are the most suitable options. Although, PS and PET are the only materials that are standard transparent. The other materials are processed differently to make them transparent. ABS would be an option, but the price is relatively high. PET shows good characteristics in this figure, since it is the most transparent and cheapest material.

It is desired that the product is strong and stiff, so that it cannot be too easily deformed. Even though the exact required strength and stiffness of the material is unknown, in general better properties are desired in products like the MBSS. This diagram Young's modulus over Yield strength (*Figure 118*) with the is mainly informative and not decisive in the material selection. It appears that PP performs worst compared to the other materials. In general, the other materials lay in a similar characteristic range, with PVC and PET scoring high on average.

Since the temperature in the field can rise high, the product can be exposed to direct sunlight. Therefore it is of importance the product can withstand such high temperatures and long sun exposure. Since the MBSS is will be injection molded (7.5), the maximum service temperature is plotted over injection moldability (*Figure 119*). It appears that PET and PVC are not as temperature resistant compared to the others, with a maximum service temperature lower boundary of 50-60 degrees Celsius, this might become a risk. However, when looking in practice, PET bottles do not melt when exposed to direct sunlight, indicating it is likely PET is still a suitable material. PET and PVC are not excellent to injection molding, this is due to the fact that the flow of these materials is more difficult, in general requiring a higher pressure. This is not problematic, however, the molds for injection molding these materials will be more expensive as they need to withstand higher pressures.

7.4.1 CONCLUSION

The analysis shows that there are multiple suitable materials. However, when looking at one of the most important aspects, costs, PET scores significantly better compared to the other materials. Besides, PET has good mechanical properties and is a very transparent material by nature and transparency of the device of importance for the device's performance. Although PET is not as easy to injection mold as the other materials, it is believed that this is outweighed by its low cost and good optical properties. An alternative material would be PS, which has similar mechanical properties as PET, is easier to injection mold, but is higher in costs. This analysis provides material suggestions, however, based on the final design (also considering further development after this project) and required properties a more in depth analysis is required.

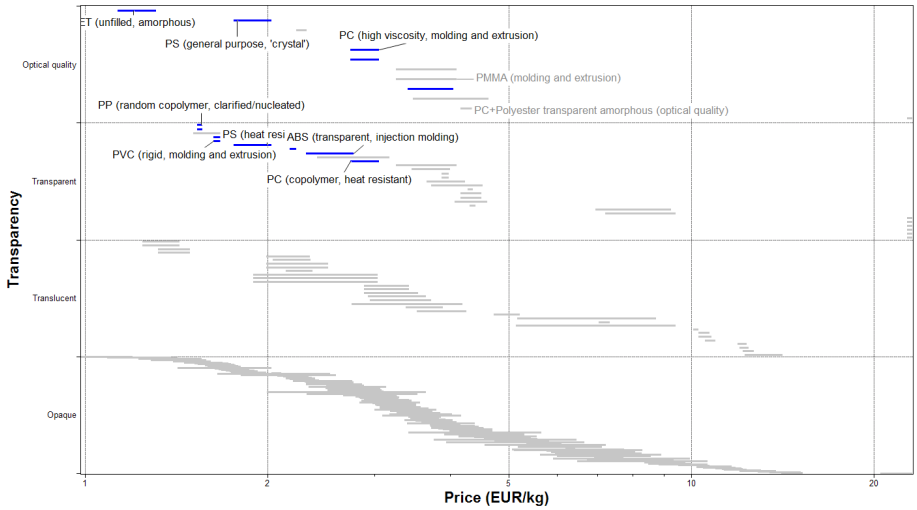


Figure 117: Transparancy over price material plot in CES.

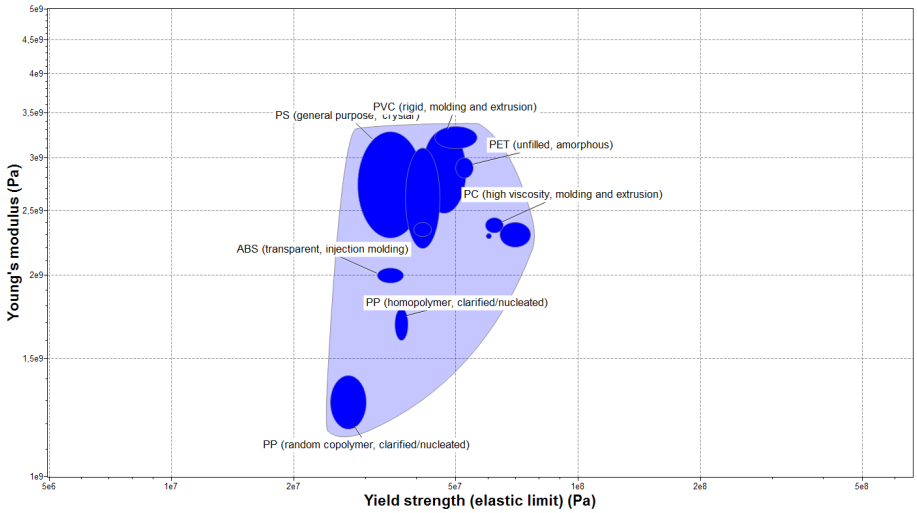


Figure 118: Young's modulus over Yield strength material plot in CES.

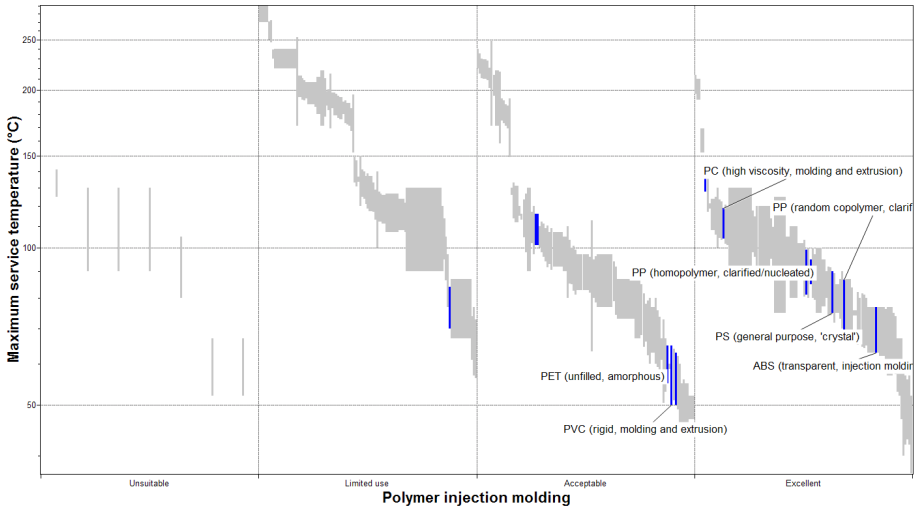


Figure 119: Maximum service temperature over injection molding abilities material plot in CES.

7.5 PRODUCTION

The fact that a new MBSS device is used for every prepared thick and thin smear, indicates that mass production of the product will be likely. Mass producing such a product is the fastest and cheapest through injection molding. Injection molding is a manufacturing process where molten material is injected into a mold under a high pressure. The current design of the MBSS requires further optimisation for successful production through injection molding. The mold needs to be able to open and close in a specified orientation, the logical orientation for the MBSS is vertically. However, the device's current C-profile makes it impossible to open the mold again after injection, since the mold will be stuck behind the bottom guide (*Figure 122*). Opening the mold will result in breaking of the bottom guides. In order to maintain the C-profile and its functionality and enable proper opening and closing of the mold, the design of the device is optimized. This is done through adding gaps in the ceiling (*Figure 120*). These gaps enable production of the bottom guides, while maintaining proper opening and closing of the mold. *Figure 123* shows this principle; the upper mold is able to go 'through' the ceiling of the device. In combination with the lower mold, the bottom guides can be made at the places where the holes in the ceiling are located. This required the ceiling guides to move towards the centre of the product, as can be seen on the image. The ceiling guides ensure protection against dust or insects coming through the ceiling holes. Similarly, gaps in the bottom guides (*Figure 121*) enable enclosure of the ceiling (*Figure 124*).

Additionally, injection molding requires similar wall thicknesses throughout the product, in order to have a steady injection flow, drying and cooling times. Also, draft angles must be applied, in order for the mold to properly open and close.

Figure 125 shows a render of the design.

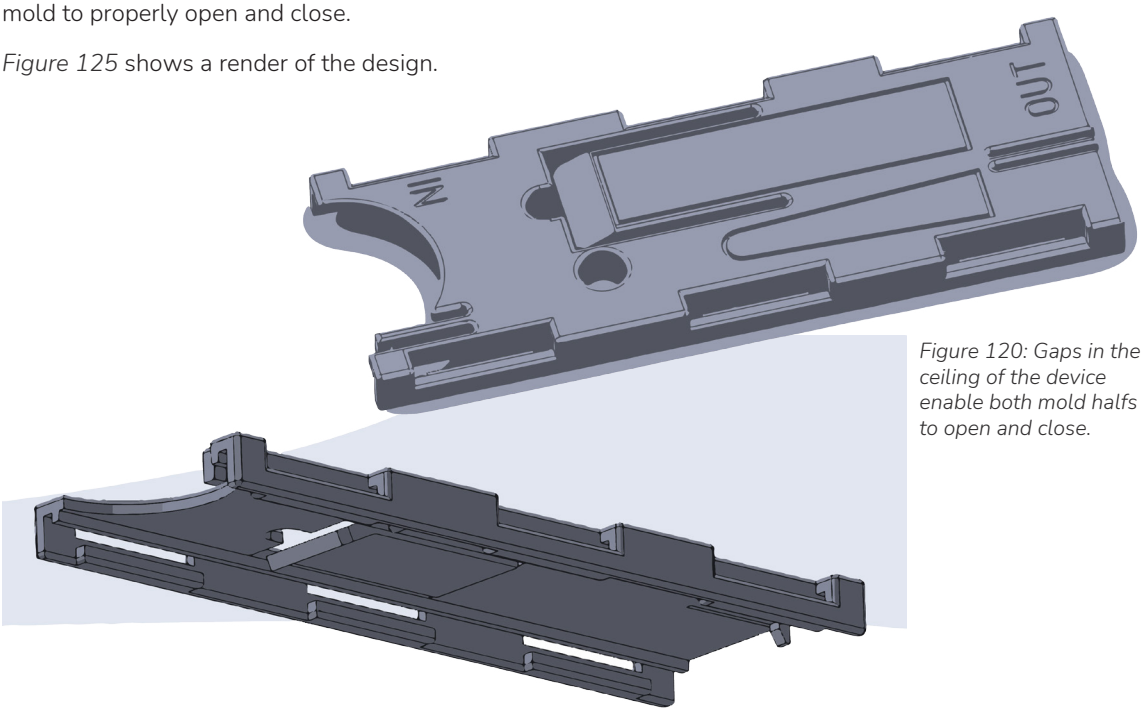


Figure 120: Gaps in the ceiling of the device enable both mold halfs to open and close.

Figure 121: Gaps in the bottom guides enable enclosure of the ceiling.

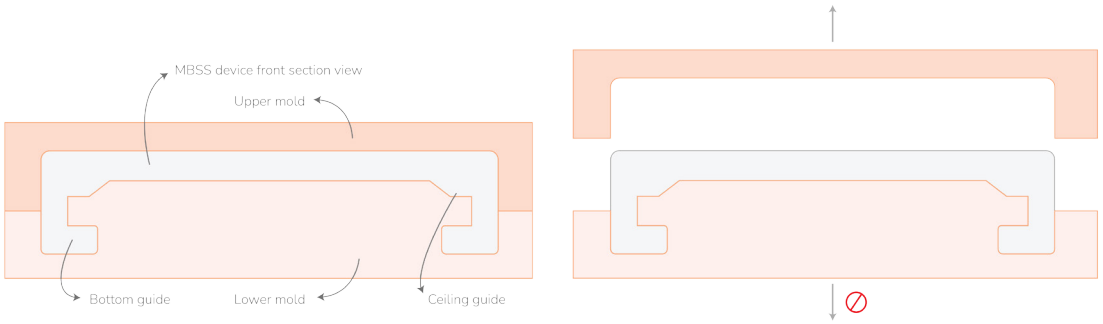


Figure 122: The current design is not able to be injection molded, as the lower mold cannot open due to the C-profile.

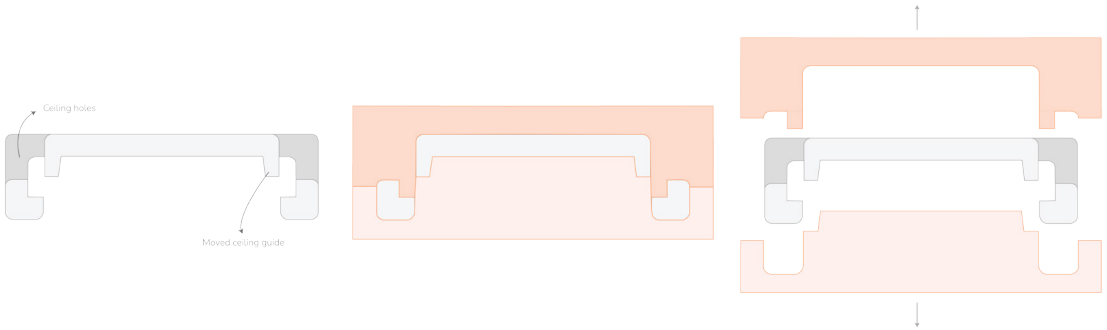


Figure 123: Gaps in the ceiling of the device enable both mold halfs to open and close.

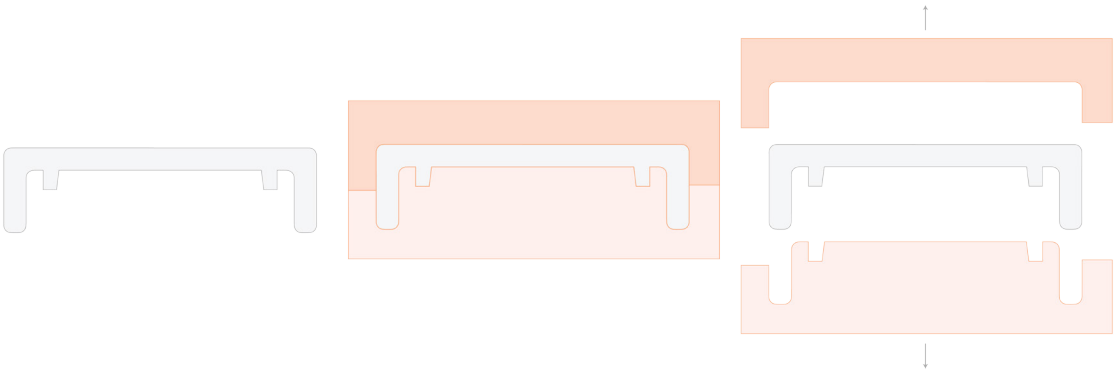


Figure 124: Gaps in the bottom guides enable enclosure of the ceiling.

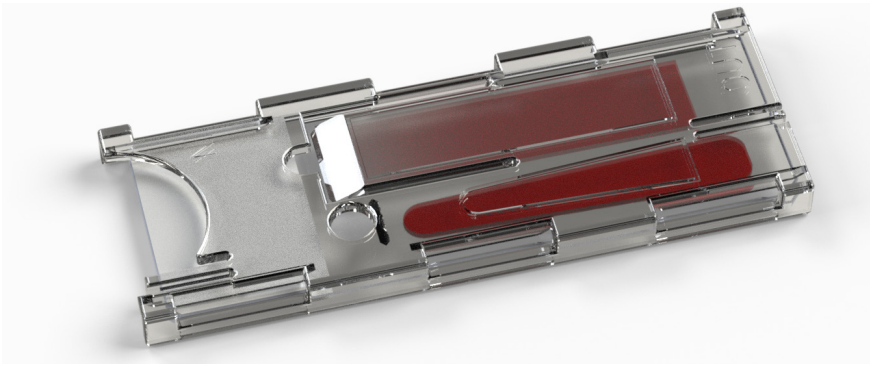


Figure 125: A render of the design thus far.

7.6 FINAL TESTING

Now that all the use case criteria and the parameters have been implemented in the design and the design is optimized for production, a prototype is built to validate the functionality of the MBSS at this stage of the design process. During this stage the device will develop from PL 4 to PL 5.

7.6.1 PROTOTYPE

Since FDM 3D printing doesn't meet the quality requirements for high quality smear preparation, a new prototyping method is found. SLA printers, such as the Formlabs printer, are accurate printers with a high quality surface finish. The Formlabs printer is used to prototype a model for the final couple of tests before the final iteration round. The prototype is almost a direct representation of the concept presented in chapter 7.5 (*Figure 126*), except from the larger open area to place the blood drops and the non-transparent material.

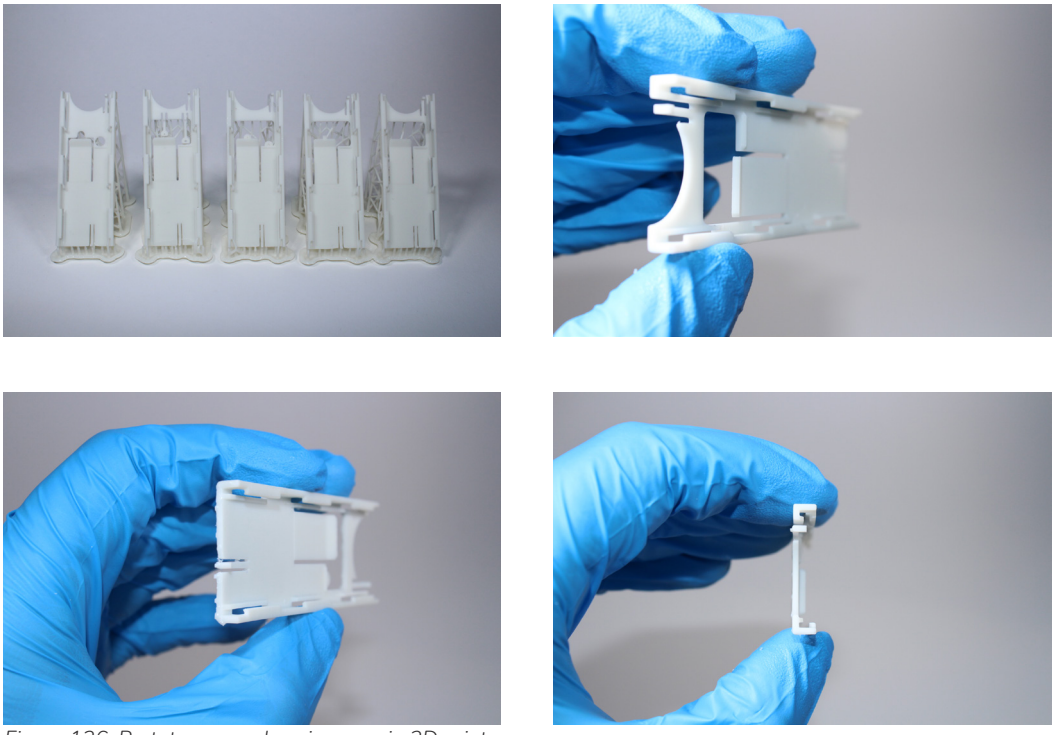


Figure 126: Prototypes made using a resin 3D printer.

7.6.2 METHOD

In total, ten tests have been performed, preparing both the thick and thin smear in one motion. To test the performance of the device, the smears have been assessed using the assessment template. The use of the prototype is as following (*Figure 127*):

1. The slide is pulled out of the device up to the point that it is still clamped by the thin smear spreader.
2. Blood drops are placed in front of the spreader in the open area of the device.
3. The slide is pushed inwards until the drop of blood touches the thin smear spreader and the blood is spread along its edge.
4. The slide is pushed inwards until a 'click' sound is heard, preparing both smears.
5. The device and is put away to air dry the slide.

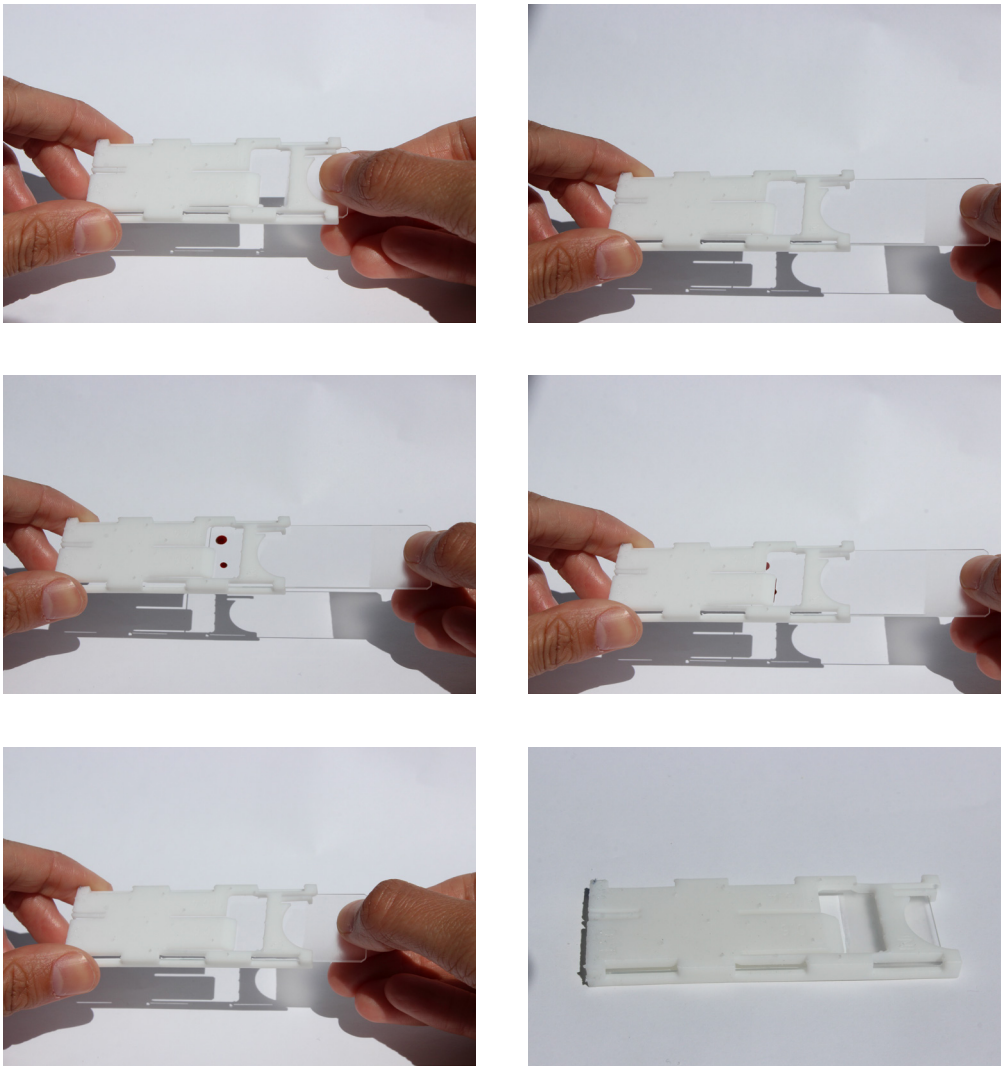


Figure 127: Smear preparation procedure using the protype.

7.6.3 RESULTS

Figure 128 shows the results of the prepared slides. *Figure 129* shows that the monolayer is visible in the thin smear.

Using the template and assessment procedure, *Figure 130* shows the categorisation of the thick and thin smears prepared on the same slide.

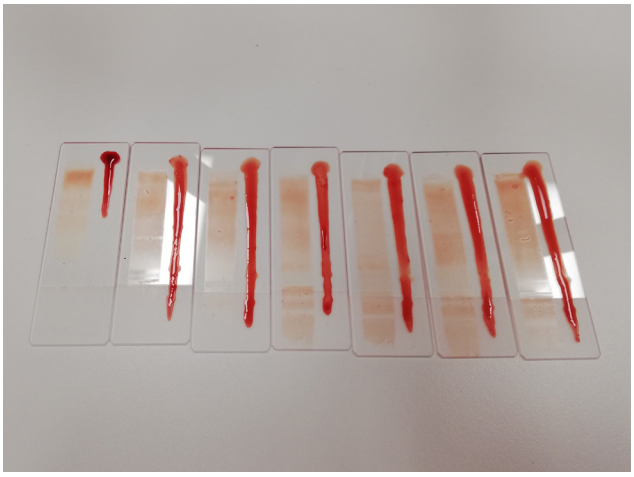


Figure 128: Results of smears prepared using the prototype.

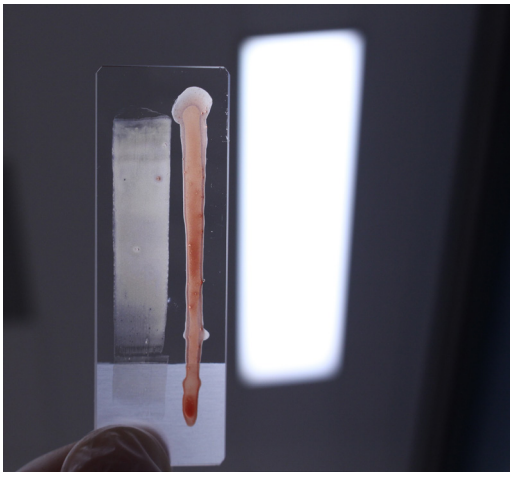


Figure 129: The 'rainbow' indicates the monolayer.

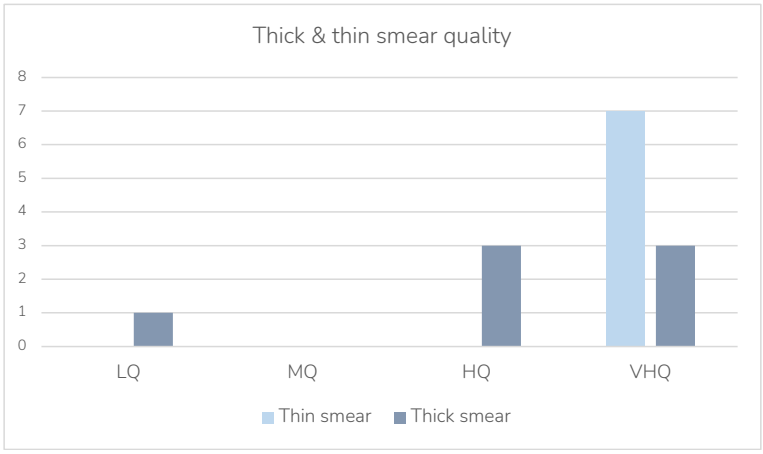


Figure 130: Quality categorisation of the smears.

7.6.4 OBSERVATIONS

- It was observed that:
- The quality of the smears in general is good. The thin smears are stretched over a long surface and the monolayer area is large. Most thick smears appear with an even thickness and a good surface area.
- Applying the blood at the front of the device is not optimal in terms of use.
- There are some streaks present in some of the thin smears. This could be due to inconsistent movement speed, or an unequal distributed pressure. However, when assessed under the

microscope, these streaks appear to have minimum impact on the smear quality. *Figure 131* indicates the 'streak line' when viewed at 1000x magnification, the cells are still well distributed, just a slight bit more apart from each other.

- When pushing out the slides while the smears are not fully dried, the thin smear leaves a track on the frosted end of the slide. This is unwanted, since this is the part of the slide that is held and it might affect the labelling.
- There was quite some leeway between the slide and the device's guides. This led to the fact that the downward pressure by the thin smear spreader was not as high as intended. Because of this leeway, the distance between the thick smear spreader and the slide was larger than it should be. This resulted in smears that appear too short and thick, this can be seen in the first prepared smear in *Figure 128*. After applying manual downward pressure, pushing the spreader closer to the slide, the smears were of better quality.

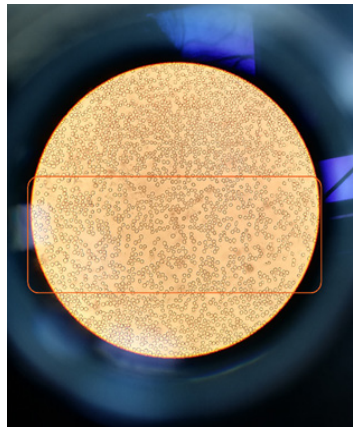


Figure 131: The 'streak lines' under the microscope.

7.6.5 DISCUSSION & CONCLUSION

It can be concluded that the MBSS produces high quality thick and thin smear pretty consistently. However, due to the leeway in the design, the consistency is not optimal yet. This is due to the fact that the thin smear spreader is not applying the required pressure and the thick smear spreader is too much apart from the slide's surface. This indicates that the final prototype must have less leeway and smaller tolerances. It also indicate that it might be a good option to also make the thick smear spreader springy. This will ensure that no matter the slight differences in dimensions of the product or slide, the distance between the thick smear spreader and slide is always the same. The fact that slight streaks in the smear's surface have minimum impact on the smear's quality is favourable. This means that even if the smear's surface quality is not optimal, it won't have much impact the quality of the smear.

The fact that the thin smear spreader can leave a blood mark on the frosted end when pulling out the slide, is unwanted. However, this is only the case when the slide is pulled out before the smear is dry. In the usual and expected case that the smear fully dried inside the device, the spreader won't leave a trace on the frosted end. This shows that it is of importance to properly dry the slide for the required time. On the other hand, it can also serve as an indication whether the smears have properly dread yet.

It was observed that the placement of the blood in front of the spreaders is not optimal in terms of use. This is due to the fact that first the device must be picked up, the slide must be pulled out and laid away, then the blood must be applied and finally the device must be picked up again and the slide must be pushed inside. For optimal use, it would be more convenient if the pull and push movement can be done subsequently, without steps in between. This suggests that the holes, used to apply the blood to the slide, must be located elsewhere. If these holes were located at the end of the device, the blood can be placed first, then the slide is pulled out until the drops reach the spreaders, after which the slide can be pushed back in straight away. It is believed this would enhance the use of the device.

7.7 CHAPTER CONCLUSION

The MBSS design at this stage of the design process, has incorporated the defined parameters from chapter 6.t This eliminates the angle and pressure variable from the preparation process and ensures consistent and accurate application of the best variable parameters. This is presumed to decrease the required experience for thin smear preparation and increase the consistency in smear quality.

The features from the user scenario that make the use of the product easier and more effective have been incorporated in the design. The features encourage that the product is used in the intended way and prevent unintended use.

A preliminary material analysis is performed, stating that PET is a very suitable material for this device, as the material is low cost and has good optical and mechanical properties. The product will be produced through injection molding. The product has been optimized to enable this production process, eventually resulting in the concept visualised in *Figure 132*.

Eventually the design was prototyped and tested, showing consistent preparation of high quality smears (apart from one thick smear). The thin smears show a good stretched body with a large monolayer surface area. The cell distribution turns out to be good for diagnosis. The thick smear shows a good stretched and thick body, with few irregularities in its surface. This is promising. From the testing some optimisation aspects came forward, to further improve the perfomance of the device.

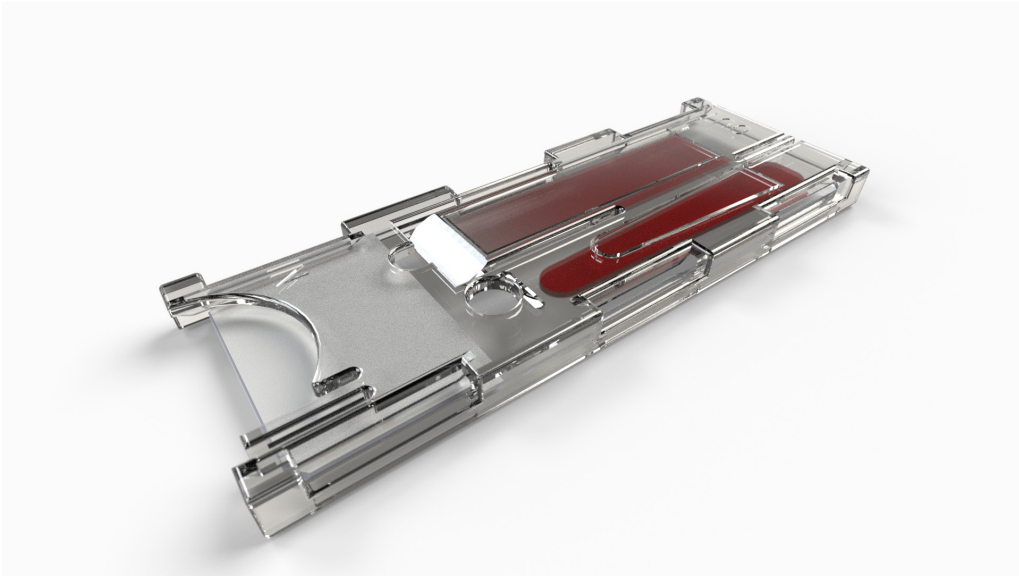


Figure 132: Render of the envisioned concept.

7.8 CRITERIA & CHALLENGES

24. Production

24.1 (cr) The mold must be able to open and close (7.5).
24.2 (cr) The product must have draft angles (7.5).
24.3 cr) The product must be uniform in wall thickness (7.5).

25. Product

25.1 (cr) The product must be made from PET (7.4).
25.2 (ch) The blood application holes in the device must be located elsewhere, to enhance the use of the device (7.6).
25.3 (ch) The thick smear spreader needs a 'distancer' that ensures the spreader is a fixed distance apart from the slide (7.6).


MBSS
Malaria Blood Sampling System
Instructions
FRAGILE

CHAPTER 8

FINAL DESIGN

The MBSS is a low-cost, compact, field compatible and simple solution to the problem of the frequent poorly prepared blood smears. The tool enables inexperienced users to prepare a high quality thick and thin blood smear in a few simple steps. This 'human empowering' tool creates benefits for both the medical staff and patient. Enabling efficient and effective preparation of a thick and thin smear on one slide in a single movement and protectively air drying the smears inside the device's body, makes this tool a valuable new asset in the fight against malaria. The MBSS consists of a foil packaging, containing a MBSS preparation device with an integrated slide and a plastic capillary tube.

8.1 SPECIFICATIONS & COSTS

8.1.1 PLUG & PLAY

One of the main problems leading to poor quality smears are the speed, angle and pressure parameters that precisely need to be applied. The MBSS eliminates the angle and pressure variable, by having the optimal parameter values integrated in the design. The fixed angle and spring system in the thin smear spreader ensure consistent preparation of high quality thin smears. Similarly, the thick smear spreader is distanced (criteria 25.3) from the slide with a fixed distance through a small notch and ensure no more manual pressure is required as indicated in chapter 7,6. This ensures that the thickness of the thick smear is consistent over different slides.

Testing shows that there is no need to fixate the speed parameter, since the speed range in which high quality smears are prepared is wide. This brings the accompanying advantage of keeping the process human, ensuring trust with participants and a feeling of pride with the operator. The fact that the MBSS eliminates two of three skill based variables and the impact of the fourth variable (blood volume) is minimised through the capillary tube that is added to the package, takes away the need for experience and skills in the smear preparation process.

8.1.2 SIMULTANEOUS PREPARATION

Where normally thick and thin smear preparation requires two separate actions, the MBSS facilitates simultaneous preparation of both smears in one action, on one slide. Not only does this increase the workflow efficiency, it also is a more controlled way of smear preparation. The smears are always prepared at the same spot on the slide, with the same dimensions and without overlap. This eases the diagnostics process, since the microscopist always receives similar quality slides.

8.1.3 PROTECTION FROM ENVIRONMENTAL CONTAMINATION

The smears are prepared during the insertion motion of the slide in the MBSS. Once prepared, the slide is stored inside the body of the device. This enables protective air drying of the smears, without risking environmental contamination, which is a frequently observed problem in the field. The fact that dust, flies etc. cannot settle on the smears, increases the ease and accuracy of diagnosis.

8.1.4 EASY APPLICATION OF THE REQUIRED BLOOD VOLUME

The included capillary tube in the MBSS package enables accurate measurement and appliance of blood volume. The indicator line on the tube show how much blood is required for preparation of the smears. The blood is easily applied on the slide, by touching the slide with the tube through the opening in the MBSS. The capillary tube increases consistency of the volume that is applie and therefore increases the consistency in blood smear quality.


THIN
THIN
MBSS
Malaria Blood Sampling System
Instructions

8.1.5 INCLUDED SLIDE

The MBSS has a slide included in its body. Including the slide ensures having control over the quality of the slide, thus the smear, as it avoids (re)usage of unclean and low quality slides. High quality clean slides are of big importance for the success of the smear. By including a high quality slide, the quality of the smear can be assured. An additional advantage is that the slide is protected during transport, because it's inside the body of the device. This reduces the risk of hazardous situation when slides break.

8.1.6 FOOL PROOF

It is of importance the MBSS is used as it is intended. Therefore several features are integrated, that avoid unintended use. The front snap connection disables pulling out the slide from the MBSS, once the smears are prepared, which would result in ruining the smears. The snap connection at the end ensures the slide is not pushed inwards too far and enables pushing the slide out of the body once the smears have dried. The circular opening at the front enables pulling out the slide easily from the device and is an indicator the slide is supposed to be held here. It ensures that the slide is held only at the frosted end, eliminating the risk of touching the slide at the intended smear space.

8.1.7 DIRECT QUALITY FEEDBACK

The transparency of the MBSS enables the operator to see the smear results through the device. The indication lines at the top of the product indicate the desired shape of the smears. It can be visually assessed whether the length of the smear meets the length of the indicator lines, providing direct feedback on the quality of the smears. If, for some reason, the smear doesn't meet the length or width of the indication lines, it provides direct feedback that the smears should be re-prepared.

8.1.8 COSTS

The cost price is estimated based on the cost price sheet by Erik Thomassen, see appendix 11.16 for the full calculation. One of the main influencers of the cost price is the price of the mold. It is assumed that the mold price will cost somewhere between €50.000-100.000, therefore a price range is given depending on the costs of the mold. With only 5 grams in weight, the MBSS' material price (PET) is only €0,0054 per cassette. Adding the production costs, the device's cost price is between €0,09 - €0,15, depending on the mold price. The included capillary tube and slide are stock components, the slide must be assembled inside the device and the capillary tube must be added to the package. Taking into account the purchase price of these components the assembly and the packaging, the total cost price of the package is estimated to be between €0,21 - €0,27.


THICK
THIN
IN
OUT

8.2 FINAL PROTOTYPE

A final prototype has been developed, using the SLA printing technique with transparent resin. The prototype is meant as a close representation of the final design that is envisioned. *Figure 133* present the final prototype.

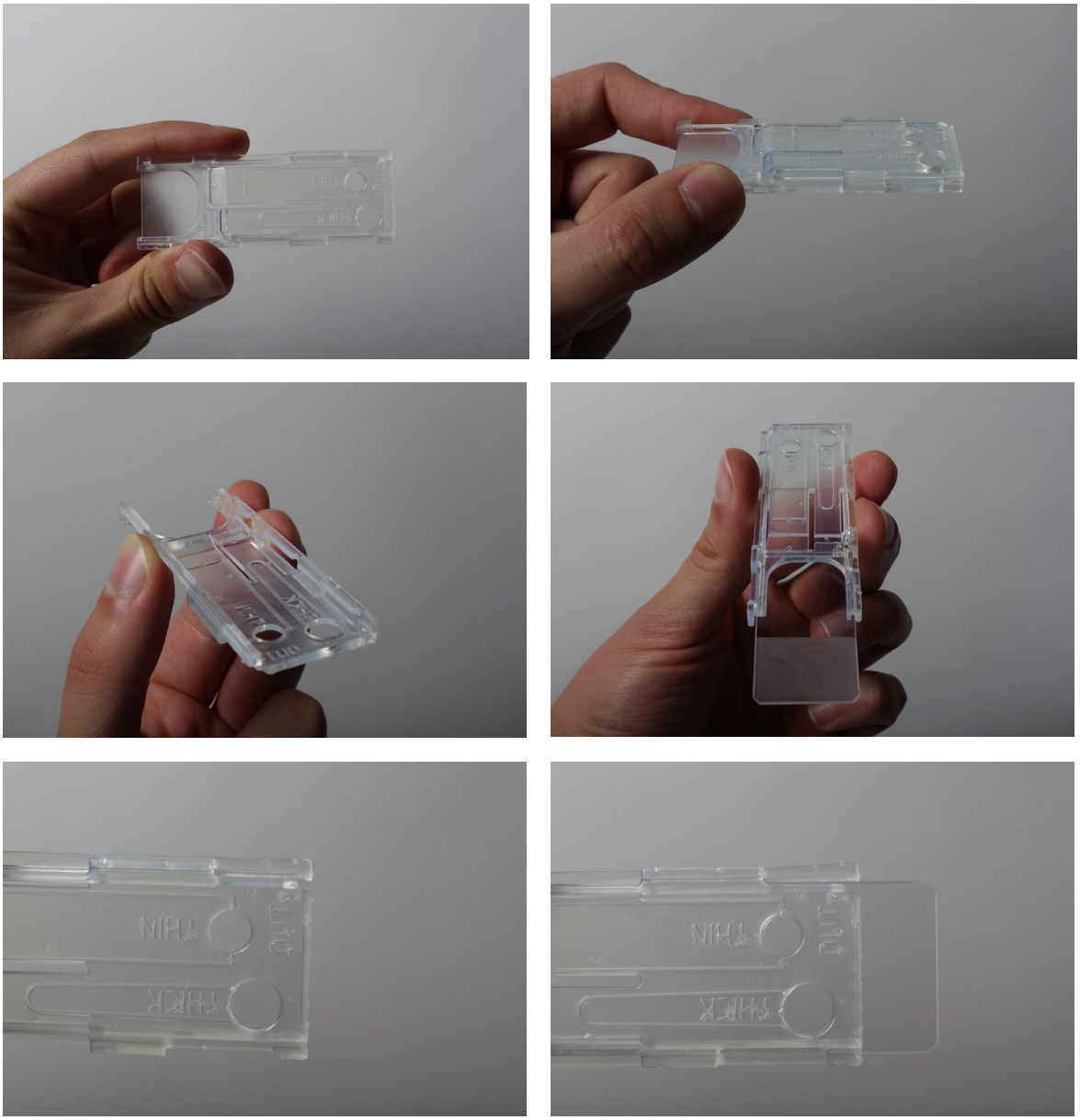


Figure 133: The final prototype, made with the Formlabs SLA printer.

8.2.1 USE

Thick and thin smear preparation using the final MBSS prototype is done in fotur simple steps:

1. Apply the blood for the thin smear in the dedicated hole.
2. Apply the blood for the thick smear in the dedicated hole.
3. Pull out the slide up to the point where the blood drops touch the spreader and spread along their edges.
4. Push the slide back in, in one smooth consistent movement.
5. Lay away the device and let the smears protectively air dry.
6. Once the smears have dried, push the slide out of the end of the device.

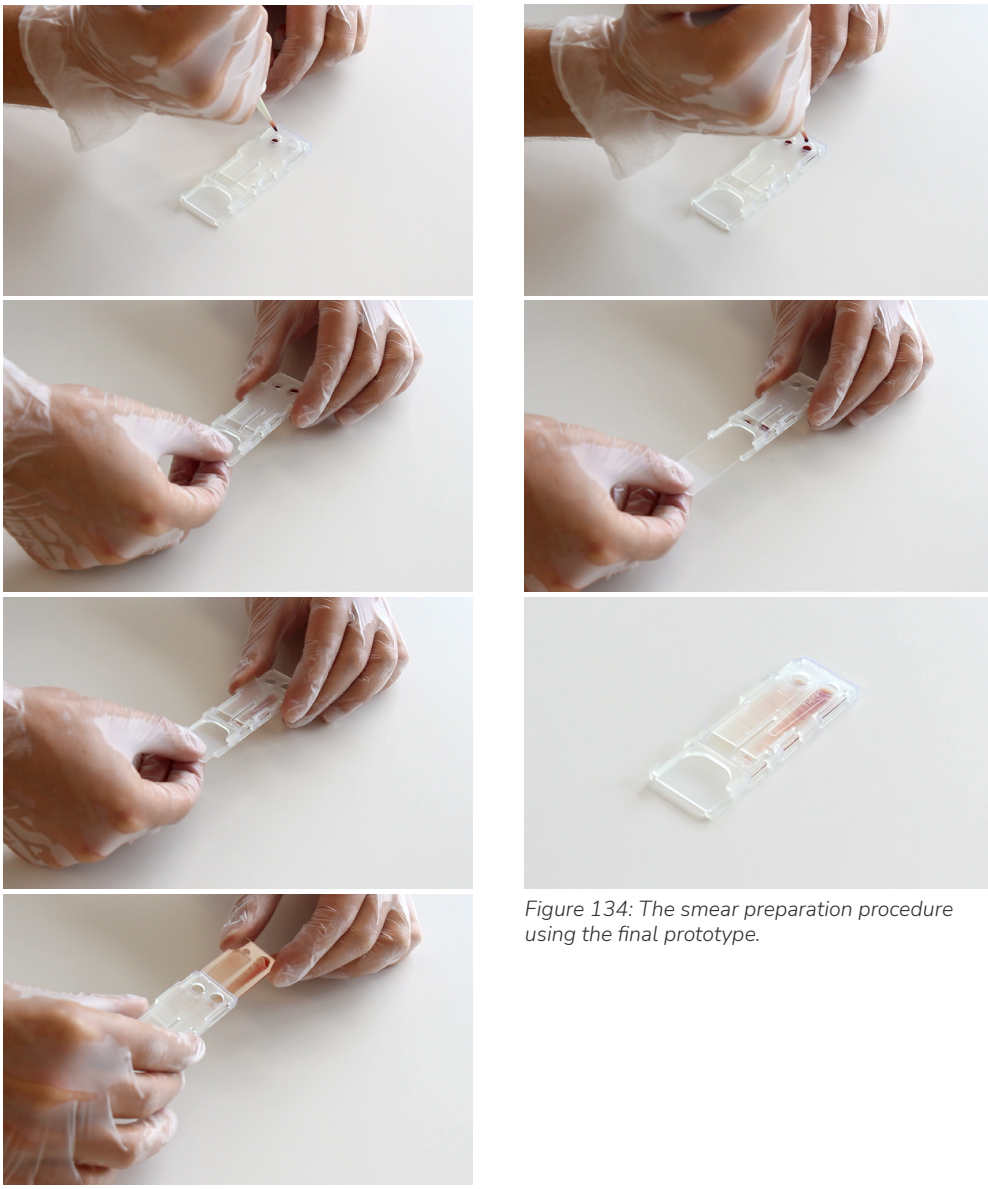


Figure 134: The smear preparation procedure using the final prototype.

CHAPTER 9

VALIDATION

With the final prototype, final validation tests are performed with non-experienced and experts in smear preparation, in order to assess the cassette on its performance and usability. This test compares the conventional, manual smear preparation method with smear preparation using the MBSS, on the following aspects: smear quality, user friendliness, required experience, consistency and time. The results are used to make claims on the efficiency and effectiveness of the product and for recommendations for further development. In this final stage, the product will develop from PL 5 to PL 6.

9.1 METHOD

The participants have been asked to prepare four thick and thin smears in total; two through the conventional manual method and two using the MBSS device. For each of the methods an instruction video is recorded, which is shown to the participants before smear preparation. This ensures that the participants are all provided with the same information about the smear preparation methods, enabling data comparison of the participants. After preparing two slides using each method, the participants are asked to fill out a questionnaire with a 7 point Likert scale (for the questionnaire see appendix 11.17. The questionnaire asks the participants to rate their experiences on the following aspects:

- Ease of smear preparation (1: very difficult – 7: very easy)
- Required experience (1: much – 7: none)
- Presumed smear quality (1: very low – 7: very high)
- Likeliness to consistently prepare high quality smears (1: very unlikely – 7: very likely)
- Required preparation time (1: very much – 7: very little)

In the data analysis and visualisation of the data, three distinguishable data categories are made: the data from all participants, the data from the experienced participants (experts) and the data from the non-experienced participants. Separating the data enables making statements on the impact of the device on the mentioned aspects, with regards to the experience and expertise levels of the user. Unexpectedly, there was the ability to compare data on another aspect; the impact of movement smoothness. As during some tests the insertion movement of the slide was not smooth, due to tight dimensioning of the prototype, a re-test was done with these participants with a smoother running prototype. This provided the opportunity to compare the data of both tests and make a statement about the impact of a smoothly running device on the effectiveness and efficiency of smear preparation.

In total four non-experienced and seven experts in smear preparation participated in the test.

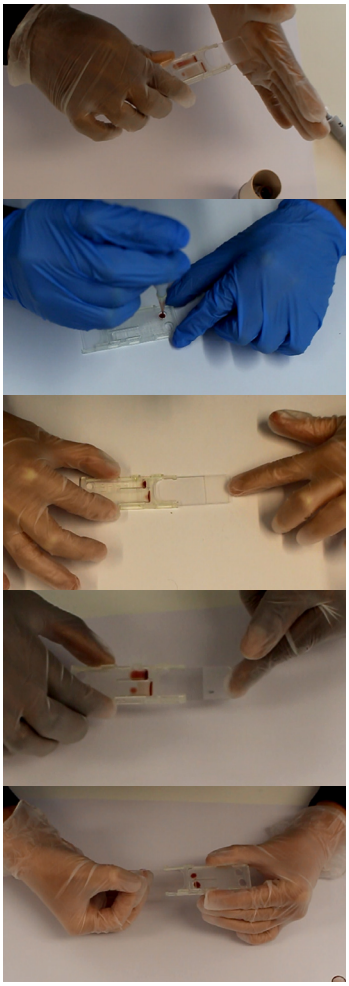


Figure 136: A couple of snapshots of the performed tests.

Figure 135: An expert assessing the smears under the microscope.

9.1.1 ALL PARTICIPANTS

Figure 137 shows the plotted data of all participants. The results show the potential of the MBSS device; the device scores better on all aspects compared to the manual preparation method.

The ease of preparation for both the thick and thin smear is similar when using the MBSS device, which is logical since the two smears are prepared in the same motion. However, the difference in ease of preparation is particularly significant in thin smear preparation. The data shows that the required experience is much lower using the device. Especially with thin smear preparation the difference is more significant than with thick smear preparation. In general, the presumed quality of both smears is higher when using the device. Again, the difference in presumed quality regarding the two preparation methods, is more significant with thin smear preparation. The likeliness to consistently prepare high quality thick and thin smears is higher using the MBSS device. Finally, the data shows that using the device, also has a positive impact on the preparation efficiency.

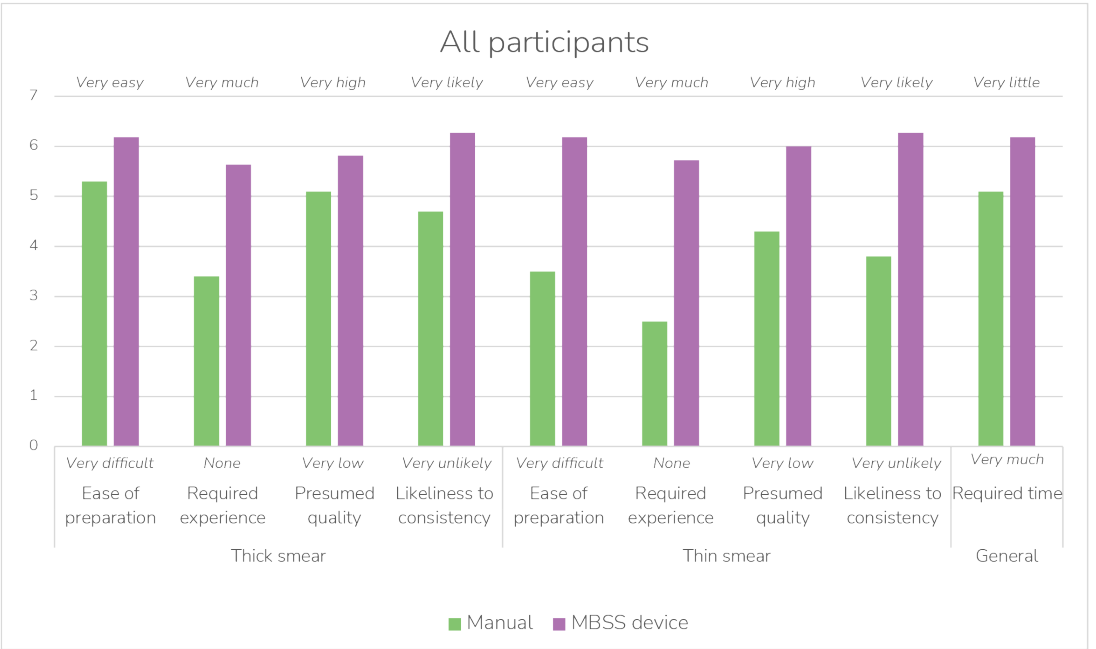


Figure 137: Data visualisation of how all participants score the use and quality of the MBSS device compared to the conventional smear preparation method.

9.2.1 EXPERIENCED PARTICIPANTS

The data of the experienced participants shows a plot (*Figure 138)* where the bars lie more closely together compared to the plot of all participants, this is especially the case with thick smear preparation. The ease of preparation, presumed quality and likeliness to constantly prepare high quality thick smears is similar for both methods. The only deviating bar regards the required experience in thick smear preparation, where the required experience using the MBSS device is significantly lower. Interesting in this bar is the fact that the standard deviation is large, indicating a relatively large difference in answers by the participants. When looking at thin smear preparation, the differences between the two methods are more significant, especially on the ease of preparation, required experience aspects and likeliness to consistency aspects. On the presumed quality, both methods score similarly.

It is noted that the standard deviations are larger with the manual smear preparation method, compared to using the device. This means the scores with manual preparation are more scattered.

Figure 138: Data visualisation of how experienced participants score the use and quality of the MBSS device compared to the conventional smear preparation method.

9.2.2 NON-EXPERIENCED PARTICIPANTS

The results of the non-experienced participants show the most significant differences between the two preparation methods (*Figure 139)*. On every aspect, preparation using the MBSS device scores significantly higher, especially with thin smear preparation; on every thin smear preparation aspect the MBSS scores at least twice as good compared to the manual preparation method. With the thick smear the differences are not as significant, but certainly present. Similarly to the expert chart, the standard deviations with the conventional method is larger compared to using the device.

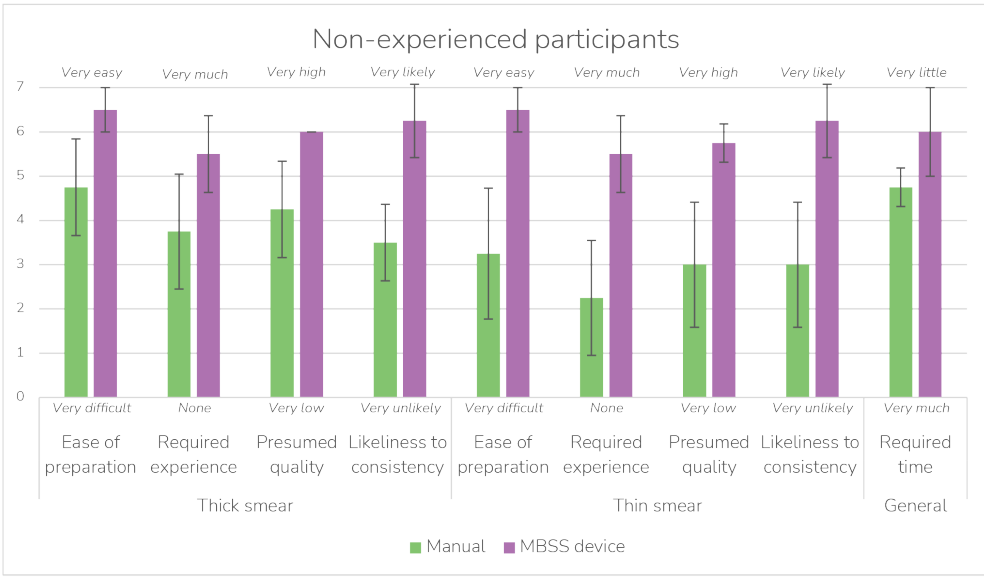


Figure 139: Data visualisation of how non-experienced participants score the use and quality of the MBSS device compared to the conventional smear preparation method.

9.2.3 IMPACT OF MOVEMENT SMOOTHNESS

A re-test was done with three participants (1 non-expert, 2 experts), as their initial test was with a non-smooth running prototype. The plot of *Figure 140* shows that the impact of a non-smooth running device is significant. Especially the required experience shows a significant better result with the smoothly running prototype.

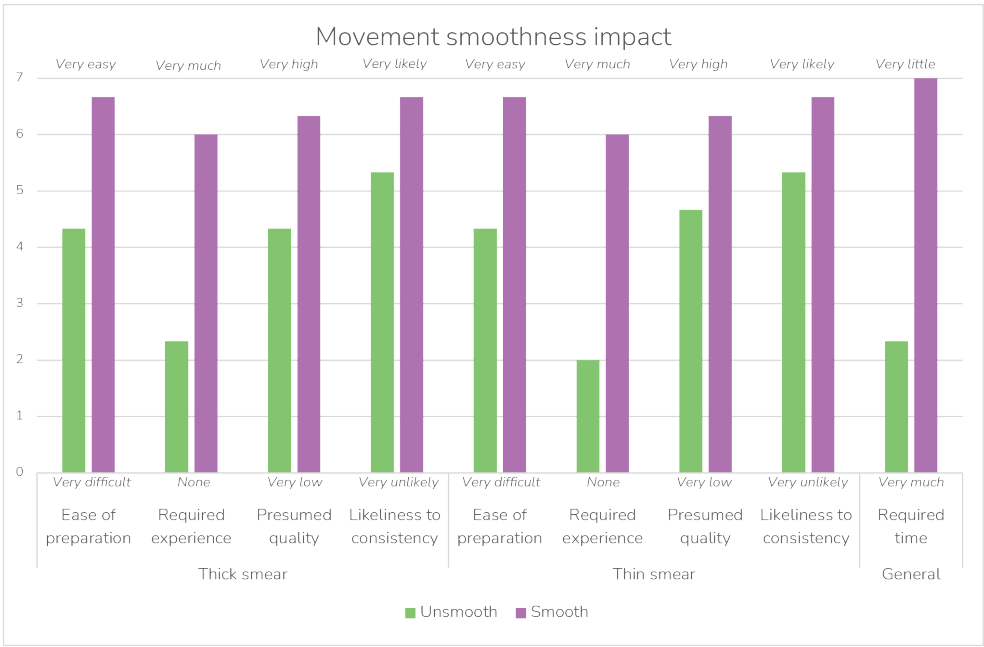


Figure 140: Impact of insertion smoothness of the slide.

9.2.4 COMMENTS & OBSERVATIONS

- Fixation with this smear layout might be challenging, as it might be difficult to only fixate the thin smear. It is unwanted to fixate the thick smear. Where normally the thin smear is fixated by vertically dipping the slide in methanol, now the slide must be dipped horizontally. A specially developed fixation 'cup' can eliminate the risk of accidentally fixating the thick smear.
- The layout of the smears is something to get used to. Some expert participants prefer a circular drop as a thick smear. At the same time they do admit that this thick smear preparation method might provide more diagnosable FOVs.
- There are application possibilities for clinical and laboratory purposes in an advanced and regulated context, with slight adjustments to the product. For example, in this context it is preferred to have the smears on separate slides, since there is enough money and equipment available. Therefore it was asked whether it is possible to develop a device that facilitates preparation of two thick and two thin smears on separate slides. 'If so, I would like to use it'.
- It was stated that even though the intention is not to re-use the product, it will happen. It might be a challenge to fight re-use, in order to assure the smear quality. In addition, when the product might be deployed in more advanced and controlled clinical and research environments, the consideration to allow re-use of the products in this environments can be made. Since proper cleaning and sterilizing of the product can be more assured in such an environment.

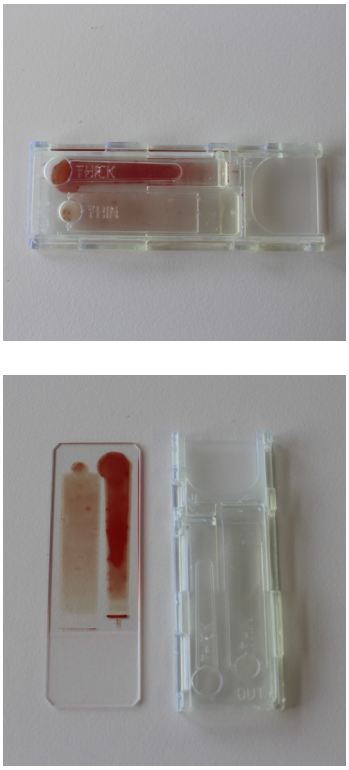


Figure 141: A result from one of the tests.

9.2.5 DISCUSSION

In general, the MBSS device scores higher on all aspects compared to the manual smear preparation method. Especially regarding thin smear preparation the differences are significant. This can be explained by the fact that conventional thin smear preparation is considered more difficult and error sensitive compared to thick smear preparation. This results in lower scores for conventional thin smear preparation compared to thick smear preparation. Since the MBSS scores on thick and thin smear preparation are similar to each other, because both smears are prepared in the same motion, it is logical that the differences between the two methods in thin smear preparation are more significant in thin smear preparation compared to thick smear preparation.

In general, the standard deviation lines are larger with the manual smear preparation method compared to using the MBSS device. This is especially the case in the 'expert' category. The wider range in their answers in manual preparation is thought to be due to a difference in experience level between the experts. The highly experienced participants most likely rated e.g. the ease of thin smear preparation higher than the less experienced ones. Interestingly, the standard

deviation with the MBSS device is in general smaller, also with the experienced participants. This suggests that the device decreases the differences in experience level and their influence on the rated aspects.

Through using the 7 point Likert scale, the data enables comparison of the two preparation methods on a more 'abstract' level. It cannot be concluded with specific and quantitative data that the MBSS actually performs better on all aspects. This would require a more scientific approach to this test, by for example comparing the amount of readable field of views of both methods. However, this test does give insights in the perceived use and performance of both methods by the participants, which is also likely to be reflected when applying a more scientific approach to this research.

For high quality thin smears prepared using the MBSS, the smoothness and consistency in speed is of major importance. It was sometimes observed that the slide was not pushed inside the device with a consistent speed, affecting the smear quality. This was due to the fact that the slide was too tightly dimensioned inside the device, resulting in friction at some points. This was especially the case with the first couple of expert participants, because at that point the prototype was more untouched because of little usage. This might have skewed the results and possible give a misrepresentation of the performance of the MBSS device. Therefore, the participants where the device didn't run smooth were asked to redo their test with a smoother running prototype. Eventually the results of this test were used for the final data. Without predefined purpose, this data was eventually used to compare the initial and secondary test, to assess the impact of a unsmooth running device. It appears that the impact is significant and it shows the importance of a smooth running product. Therefore, extra attention to perfect dimensioning, in order to enable a smooth insertion movement from first use, should be taken in optimizing the product.

9.2.6 CONCLUSION

It can be concluded that smear preparation using the MBSS device scores better on all aspects compared to the convention manual preparation method. Especially regarding thin smear preparation, the data shows that the ease of thin smear preparation is significantly higher using the device. Especially for inexperienced users the device adds a lot of value, it provides better quality smears, is easier to use and the likeliness to consistently prepare high quality smears is much higher. In addition, the presumed quality of the smears using the device is higher. The fact that this counts for both experienced and non-experienced users, shows the potential of the product. It means that this product is not only beneficial for inexperienced people, but also for experienced ones, which is a nice side-effect. The fact that both the participants used to the field setting and advanced clinical and laboratory setting see the potential of the product and envision application in both contexts, shows the potential to widely deploy the MBSS in multiple contexts.

9.3 QUALITY VALIDATION

Besides validation with experts and non-experts, also validation on the smear quality and quality consistency of the device has been done. This is done by subsequently preparing 10 thick and thin smears, using the prototype. Their quality is assessed using the quality assessment template, after which they are designated to their corresponding quality categories. *Figure 142* shows the prepared slides.

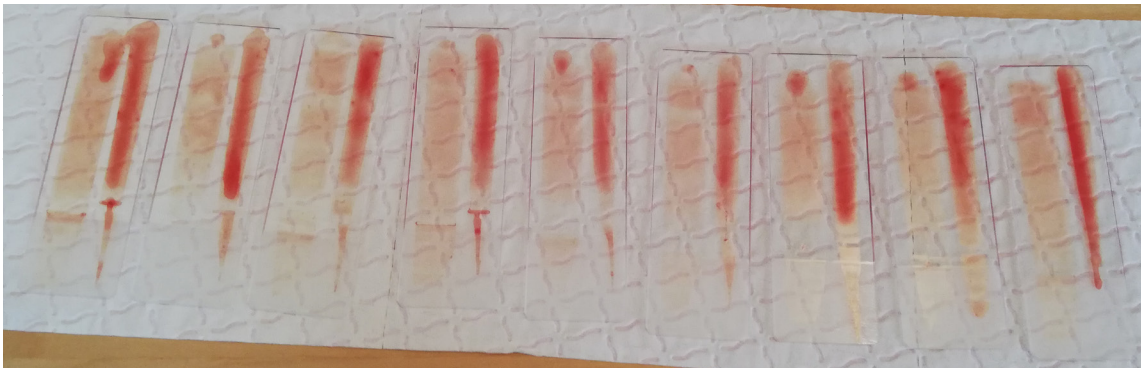


Figure 142: The prepared slides during the quality validation.

Using the quality assessment template, the smears have been assigned to their designated quality category (*Figure 143*).

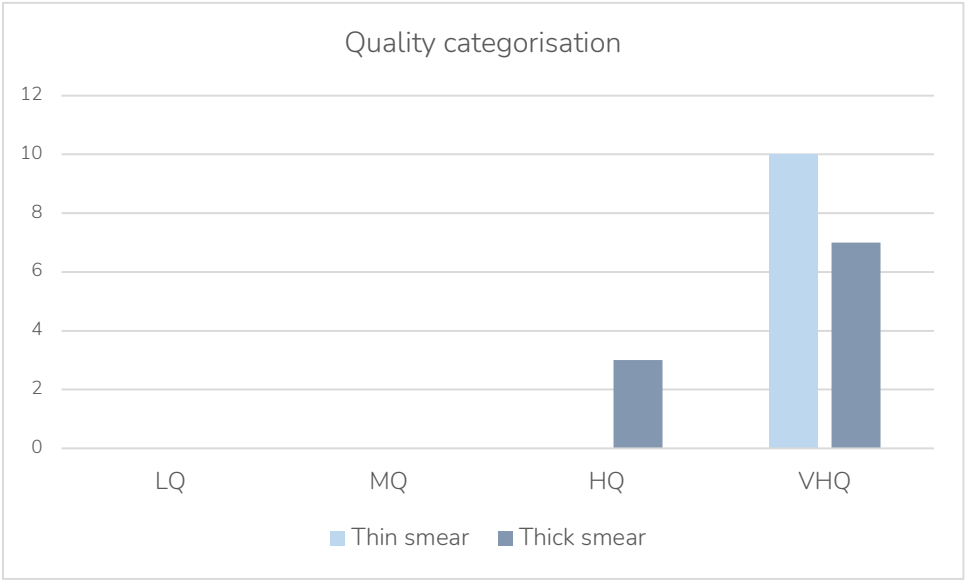


Figure 143: Quality categorisation of the prepared smears.

Some of the smears have been fixed and stained by the LUMC staff, the results of the thick smear are shown in *Figure 144* and of the thin smear in *Figure 145*.

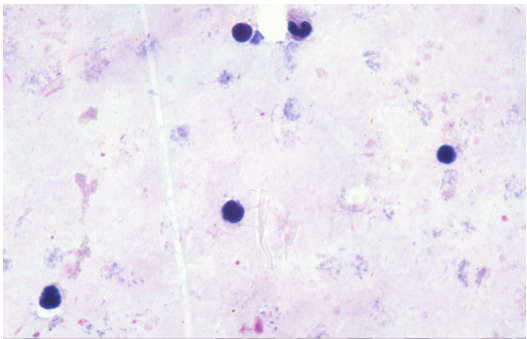


Figure 144: Microscopic view after staining the thick blood smear prepared with the MBSS.

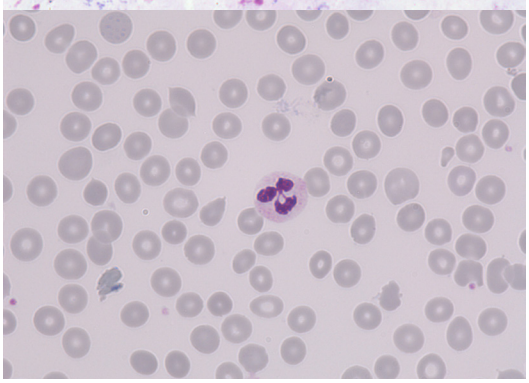


Figure 145: Microscopic view after staining the thin blood smear prepared with the MBSS.

9.3.1 DISCUSSION & CONCLUSION

The results show that every thick and thin smear is categorised in the 'high-quality' category or higher. This indicates that the quality of the thick and thin smears prepared by the MBSS device is high. The fact that this was the case for 10 subsequent tests also shows that the quality consistency of the smears is high. The thin stained thin smears show that the cells are lying next to each other, thus are well distributed. Also, the surface area of the monolayer was large and more than sufficient for proper diagnosis. Accidental fixation of the thick smear was thought to become a problem, however, the LUMC staff indicated that this was not the case, which is positive. The thick smear cells are nicely coloured and the 'background' looks good, the thick smears are considered good for diagnosis. However, the thick smear does show some inconsistencies in thickness. There are places where the required 10-15 leukocytes per FOV are present, but also places with 1-5 leukocytes per FOV. This indicates that further optimisation is required, to ensure a consistent thickness of the thick smear and a good distribution of the leukocytes. In fact, increasing the overall thickness is a relatively simple adjustment, as it is dependent on the distance between the spreader and slide and the amount of blood used. This indicates that further tweaking of these parameters is required to obtain the best result and consistency in thickness. Overall it can be concluded that the performance of the device is good. 'The smears show high quality bodies that are good for diagnosis' (expert 1).

CHAPTER 10

CONCLUSION

10.1 CONCLUSION

In this thesis, a new method towards enhancing the quality of field prepared malaria blood smears is developed through the use of a newly designed, innovative tool. The Malaria Blood Sampling System (MBSS) was developed to increase the quality and the quality consistency of blood smears by reducing the impact on the quality caused by human and environmental factors, as well as the limited resources available. The MBSS is not just a tool, it is a new system, a new way of working and a new approach to blood smear preparation. It provides a more consistent, effective and efficient way of preparing blood smears in the field.

This is achieved by reducing the human impact; experience and skill are some of the main influencers on the smear quality. The fact that there is a lack of experienced field staff preparing smears, results in inexperienced staff preparing low-quality smears. The MBSS has proven that compared to manual smear preparation, it requires significantly less experience to prepare a thick and thin smear. This is due to the fact that the MBSS eliminates two of the four skill based variables that influence the quality of the prepared smears: angle and pressure, by integrating the best performing variable parameters in the design. Additionally, the MBSS system minimizes the impact of the blood volume variable through the capillary tube that comes with the package, which eases blood volume measurement and application. The only variable that is left is the applied speed, which is manually applied by the operator. The deliberate choice is made to let this variable remain human-controlled, as the speed range in which high quality smears can be prepared turns out to be wide. Another reason is that this prevents the product from becoming complex and requiring high-end resources, which are unavailable in the field. Besides, keeping the human touch to the process is of importance for the trust of the participants and the employment of the staff.

Additionally, the ease of preparation using the MBSS scores higher; just by one straightforward movement, both smears are prepared. The data shows this also has positive effects on the efficiency of the smear preparation process, reducing the time to prepare both smears.

The environmental impact is reduced by eliminating the possibility of dust, ants, flies and other debris to settle on the smears. The ability to air dry the slide inside the device enables protective air drying. This makes diagnosis easier and more efficient, since unwanted particles will colour through the stain, leading to much ‘noise’ in the smear and possible interpretation errors.

One of the critical aspects of being capable of producing high-quality smears, is making use of high-quality supplies. Since the MBSS package includes a high-quality glass slide and a capillary tube, the influence of the material used is controlled, and the quality of the smear can be guaranteed. Including these items in the package eliminates the risk of using non-sterile, unclean or contaminated components, supplies or resources. Besides, where usually the blood volume is inaccurately applied straight from the finger on the slide, or through the use of inaccurate equipment, the capillary tube offers high

accuracy and precision in blood volume measurement and appliance, which is a critical factor for the smear quality.

The MBSS has proven to deliver high quality thick and thin smears. Both experts and non-trained staff in smear preparation were impressed by the quality output. The cells are nicely distributed with the thin smear, with the Red Blood Cells lying next to each other. The fixed parameters ensure that the thin smear is spread over the entire length of the slide, resulting in more than sufficient monolayer surface area to diagnose. The thick smear shows consistent high-quality results as well. With the fixed distance of the thick smear spreader corresponding to the slide, the thickness and surface area of the smear is consistent and show few irregularities.

It can be concluded that the MBSS enables the preparation of high quality thick and thin smears, with a higher quality and quality consistency compared to the conventional manual preparation method. The required experience is significantly lower and the ease of preparation significantly higher, making the MBSS a valuable product to deploy in both low- and high-expertise areas. The MBSS is a game-changer that will assure consistent, high-quality smears in low-resource and expertise settings, such as the field. Deploying the MBSS in the malaria diagnosis 'infrastructure and system' will establish and ensure higher efficiency and accuracy in malaria diagnosis, leading to an increase in the number of right treatments and fatality reduction.

10.1.1 CONTRIBUTION TO THE FIGHT AGAINST MALARIA

During this graduation project not only a new, innovative product has been developed, also the current situation and way of working in the field has been mapped in depth. Through interviews, the current way of working and how it differs from the theory has been defined. Next to that, the current problems and their impact leading to poor quality smears have been closely defined. This not only provided grip to the development of the MBSS during this thesis, but it can also provide grip and insights for future projects and interventions related to the topic of blood smear preparation (in the field context).

10.2 LIMITATIONS

This project worked towards increasing the quality of malaria blood smears. The goal was to develop a product or method that guarantees consistent preparation of high quality thick and thin blood smears. By increasing the quality of the blood smears, more accurate diagnosis can be performed and better patient treatment can be realised, reducing malaria fatalities. The design project was led by three main phases, each with their own research questions. The limitations during every phase will be discussed.

Analysis phase

One of the main limitations during the analysis phase was that there was no possibility to visit the given context by AiDx Medical, due to COVID-19. The context research was performed through desk research and interviews, but no personal experience and observations were done in this research phase. It is believed that if the possibility would have been there, more accurate data could have been collected about the current situation and the way of working in the field context and the current problems that are being faced. It is believed the designer would have gotten a better feeling and understanding of the context if he could have experienced it personally, besides it would have been easier to extract the most important data. He would probably have been able to identify key issues based on visual observations and, as a result, the interviews with experts would potentially have been more sharp, efficient and productive. The fact that this was not possible made that the results were reliant on the available information on the internet and the input accuracy and completeness and cooperation of the interviewees. The first was challenging, as there is little data to be found about blood smear preparation in the field setting. Therefore online interviews were conducted with experts from the field, whom were in general cooperative. The fact the interviews had to be conducted online brought limitations and challenges in terms of making thoughts clear to one another, reading bodily language and unstable internet connection, this could have led to misinterpreting information. During the interviews it was experienced that because of personal opinions, experiences and operation location of the interviewees, the data was relatively scattered, it was challenging to find perfectly matching data. For example, interviews were conducted with persons from Nigeria and Gabon; their procedure and definition of a field trip is not completely the same. Also, the main defined problems were reliant on the interviewee's own experience and skills in blood smear preparation; an experienced staff member might face different problems compared to an inexperienced staff member. The slight differences made it difficult to find a common ground, made drawing conclusions harder as well as making generic statements on the current field situation. Possibly the 'field context' was a too broad context, which should have been narrowed down towards a more specific context like; 'Nigerian field context'. This would have made it easier to draw more specific conclusions, but on the other hand would have created a design foundation that is based on specific data, resulting in a context specific design that might not be deployable in a wider context range.

Despite the challenges and limitation faced during this phase, it is believed that accurate and reliable results have been generated and decisions have been made, that have laid a solid foundation for the upcoming design phase. Next to that, it is believed that the current root problems that have most impact on the blood smear quality have never been identified as concrete as done in this thesis.

Design & prototyping phase

During the design and prototyping phase the main limitation was the fact that the final product was designed in The Netherlands at a technical university, while it will be deployed and used in a totally different context. The limitation of making assumptions based on western Google desk research, interviews with e.g. Dutch tropical doctors and western education and designer mentality, creates 'western' biases, which without a doubt have influenced the results, conclusions and product design. This is not a bad thing, however, it does pinpoint the necessity to eventually test and evaluate the design, thus the underlying conclusions and choices, within the actual operating context and with the envisioned users. This in order to eventually eliminate the western biases from the design, making the product fully fit the operating context.

A challenge during this phase was the amount of problems that were identified in the analysis phase, which required an overarching and integrated solution. The challenge to combine and connect individual problem so-lutions, so that one fitting solution for all problems was developed, was a difficult one. In a certain way the amount of problems made the design 'space' so wide that it was considered as a limitation to the design pro-cess, as the wide design space made it harder to have a focussed design process. Perhaps more concrete de-sign boundaries should have been set, in order to more specifically find a solution to a limited number of prob-lems.

A limitation regarding prototyping was the access to accurate prototyping resources. The first couple of proto-types were developed using MDF 3D printers, which gave good tangible insights. However, the prototypes did not suffice for accurate tests on performance, limiting the depth of the research on these prototypes. More accu-rate prototypes needed to be developed, for example through SLA printing. However, the accessibility to these printer is much lower and more costly compared to MDF printing, decreasing the efficiency of the prototyping and testing process. Suddenly slight changes in the design required much more time to apply in practice. Even though I'm thankful there were SLA printers at my disposal, the effort and time it took to develop accurate pro-totypes was a limitation to the project.

Even though the final product is designed by a western designer, in a western context with western biases, it is believed the final product does meet the demands of the operating context. The found data, on which the design relies, is believed to provide enough grip and depth for the design, to suffice and work in the field context, with possible necessary adaptions to the design. Although the design space was wide, it is believed that the final product does solve many

of the problems within the design space. The mentioned suggestion of limiting the design space, resulting in a more specified and further developed design, can be rejected by the obtained result. The final product shows that more specific boundaries probably wouldn't have led to such a 'wide' problem-solving product.

Testing & validation phase

One of the main limitations of the testing and validation phase was the access to blood. Eventually animal blood was used for testing. However, the properties of animal blood might differ slightly from human blood, which could potentially have influenced the results of the tests. Even though the impact on the design of this is presumed to be low, e.g. the viscosity of the blood might differ slightly, possibly requiring slightly different parameter values for human blood. If this is the case, it is a matter of simple adjustments of the design, to adjust it to the human blood properties. Besides the possible differences between human and animal blood, there might also be differences between non-infected and infected human blood. Because of no access to malaria blood, the actual performance of the device on preparing infected smears, and thus assessment on the readability of the parasites in the prepared, could not be done. For example, the temperature in the field could have an unpredicted impact on the device, what does this do with its performance?

During the tests, the smear quality was mostly visually assessed through the use of the assessment template and procedure and the obtained experience of the designer. The assessment template does provide a good method for visually assessing smears on their main quality aspects such as; surface area, thickness and surface irregularities. However, the exact cell distribution can only be seen through the use of a microscope. Due to time constraints, the majority of the prepared smears could not be assessed under the microscope. This could have resulted in in wrongly assessing some of the smears on their quality and allocating them in the wrong quality category.

Another limitation during testing of the prototypes was the fact that the product is designed to be a single use disposable product. However, due to limitations on prototyping resources, the tests were performed multiple times with the same prototype. This led to deterioration of the prototype, affecting its performance. Interestingly, its performance was often affected in a good way. Since the prototype was dimensioned a bit too tight, the insertion movement was not always as smooth as intended. However, after using the prototype multiple times, the movement became smoother as material was 'scratched' away by the slide. Nevertheless, the fact that the prototype was used multiple times, where the envisioned product should only be used a single time, is considered as a limitation to the accuracy of the results.

10.3 RECOMMENDATIONS

Obviously, the MBSS is not fully developed yet. Therefore this chapter lists multiple recommendations for further development and research on the MBSS, in order to optimize its performance and make it market-ready.

In context testing

Due to COVID-19 and time limitations, the product has not been tested within the envisioned context yet. Since the product has reached a level op development that it works in a controlled environment, the next step would be to test the product in the field setting. It is believed that interesting insights will be obtained from such an experiment, that will lead to further adjustments for the good of the MBSS. It would be insightful to see how the people work with the device in such a challenging and unregulated environment. It would also be a make or break moment of the concept, will the people there accept it? Will they use it on the long term? and do they see the benefits? Those and other questions can only be answered after testing in the context.

Fixation

Fixation of the thin smear was one of the mentioned challenges by experts, as the smear layout has changed. It would require a new type of cup to dip the slide in horizontally instead of vertically, so that the thick smear is not accidentally fixed. The design of this cup, or possible other solutions need further research. Also, the ability to fixate the thin smear while the slide is in the device, would be beneficial as it will be longer protected against the environment.

Smoothness of movement

It turns out that the movement smoothness, with which the slide is inserted in the device, has a big impact on the output quality. Since in the first couple of test-runs the device didn't run smooth, it is undesired that this might also happen with the final produced product. Therefore, close attention must be paid to properly dimensioning the device, so that it runs smooth from the first (and only) use. This is a matter of tweaking.

Capillary system

The capillary system that was envisioned for the MBSS device, turned out not to work as expected. However, it is believed that despite the challenges that were faced with this system, it has potential to work. Integrating a capillary system, which facilitates accurate blood measurement and application straight from the finger, would help in more efficient and accurate blood application on the slide. Besides, it would make the additional capillary tube redundant, ensuring fewer components and potentially lowering the costs of the MBSS.

Disable unwanted 'pull-out'

One of the main weaknesses of the current design is that the slide can be pulled out of the device too far. The slide must be pulled out up to the point the drops of blood touch the spreaders. However, e.g. impatient users can quickly pull out the slide and accidentally pull it out too far. This can lead to difficulties in pushing the slide back in. Once this happens with blood on the slide, the slide can be considered as useless as the blood drops are ruined. Therefore, it would be interesting to find a way to disable the slide from being pulled out too far. The easiest way to do this, is through making adjustments in the slide or a develop a custom slide. It should be further researched how this slide should be developed and what the impact on the costs would be.

Blood viscosity

Literature states that in manual smear preparation the angle of the spreader slide should be adjusted to the viscosity of the blood. Thicker blood requires a smaller angle, whereas thicker blood requires a larger one. Since the angle is fixated in the MBSS device, the device does not facilitate adjustments of the angle based on blood viscosity. Therefore the quality of different blood viscosities, using a fixed angle with the MBSS, requires further research. Based on the outcome it can, for example, be decided to develop two devices, each with a different angle or keep it with one device if the quality turns out to be high with different viscosity levels.

Relaxation of the material

Relaxation of polymers is the steady decrease in force under constant applied deformation or strain. The thin smear spreader is applying a downward force to the slide, through deformation of the material. Since the slide is included in the package and inserted in the device, the spreader is deformed up to the moment of use. The production/ assembly time to use time can be months. This means that it is likely the downward force of the spreader will decrease over time, due to relaxation of the material. Since the best possible downward force is closely defined, impact of relaxation on the smear quality must be researched. It is expected the impact is low, because the required downward force is low.

Recycling

Since the MBSS regards a single use disposable product, a new waste stream will be created. The possibility to recycle the device, slide and capillary tube requires further research. It is common knowledge that recycling of medical waste is difficult, since it can be infectious and toxic. However, currently there is an increase on researches on this topic, that investigate if and how certain types of medical waste can be recycled. Recycling of the MBSS would result in a more environmental friendly impact and can be cost beneficial.

Dual slide preparationt

The MBSS facilitates preparation of a single slide with a thick and a thin smear. However, frequently two slides per patient are prepared, one for diagnosis and one for the archive and in case something happens with the first slide. Preparing two slides would require two MBSS devices. It would be interesting to further research the actual necessity of preparing two slides per person, because the MBSS now enables consistent preparation of high quality smears. If this necessity still exists, then it would be interesting to see whether one MBSS device can be adjusted such that it can prepare two slides (possibly at the same time). It is envisioned that this would be the same device, but wider or longer, so that two slides can fit in the device.

Instructions

The use of the MBSS requires instructions. It is recommended that before first time use, the operator has received instructions through either a personal human demonstration or a video demonstration. The best way of demonstrating and explaining the device and its use must be further defined and developed. Next to that, the cover of the packaging contains the instruction steps on how to use the product, in case the operator needs to freshen up his memory. These instructions, how they are written and visualised also need to be developed in an simple and understandable way – take the Ikea instructions as an example, with the simplicity of the instructions everyone can assemble a closet.

Optimizing for mass production

There is a link between the MBSS material volume and the costs of the product. The dimensioning of the product requires further development, in order to minimize the costs and maximize the usability. Even though the low price of PET, with a batch size of e.g. 100.000 MBSS devices every cent that can be saved in material will have a significant impact on the total cost price. Additionally, the dimensions of the product need to be optimized to the precision and tolerances of the production method (injection molding). This to ensure smooth movement of the slide in- and out of the device.

10.4 PERSONAL REFLECTION

To conclude this report I would like to reflect upon my personal experiences and ambitions during this project.

I remember the beginning of this project, where I was a complete rooky on the topic of malaria and blood smears. As a designer you need to be capable of doing a deep dive into a topic you're not familiar with. You need to get an understanding of the full picture and I have never experienced it before in this fashion. I am surprised and impressed by myself, by the amount of knowledge and expertise I have gained in the past couple of months. Nevertheless, the unfamiliarity with this topic pushed me out of my comfort zone more than I expected. It was a challenge to get the full picture of the current situation, as I was unable to go there myself. This truly was a deep dive, with a lot of unknowns at the beginning, but with a lot of confidence at the end.

One of my personal project goals was to enhance my 3D printing skills and learn how to prototype fast, to get quick tangible results. Looking back, I believe I have more than achieved this goal. I've made so many prototypes that I quickly tested, threw away and made a new one. The amount of plastic prototypes in my room is insane (sorry nature). I truly believe I increased my 3D modelling and prototyping skills. And wow, I'm really impressed by the final prototype. The quality of the SLA printing technique was beyond my expectations and I'm really happy to have such a beautiful tangible end result.

Another goal was to proper document on the run. I must say that this goal has not fully been achieved. I bought a sketchbook, which I used to write thoughts and decisions down, but sometimes I forgot to do this. Yes I know this is a weak excuse, but it often felt like hurdle to document, like a waste of time because other 'more important things' can be done. However, as experienced before, this gave me a difficult time at writing my report, requiring me to work on it fulltime for a couple of weeks in a row. Looking back, it would have been better to just take the last hour of the day and use it to write, so that the distribution of 'important stuff' and documenting would have been more uniform over time.

Somewhere at 70% of the project I found out that my project had more structure than I thought. It sometimes felt like I just was doing 'something', but not necessarily with a purpose. It felt like this, because I didn't have a clear predefined work structure from the beginning of the project, I did what felt right at the moment. It appeared that my intuition was actually pretty structured and I was happy and relieved to see this while writing my report. It means that my gut feeling is good, but it also means it would provide more grip in the future to think of a predefined structure beforehand.

Now at the end of my thesis I did fall in love with this topic. Not always is it that it truly feels good to design something new, but in this case it did. I feel like my work contributes to the fight in malaria and that it could possibly help a lot of people around the world in a better workflow and a better malaria treatment. There is still a lot to do to bring this product to the market, but I personally really believe in the potential of the system and device that has been developed. It would be a pity to leave it on the shelf, therefore I hope this project will be continued with!


MBSS
Malaria Blood Sampling System
Instructions
FRAGILE

10.5 REFERENCES

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