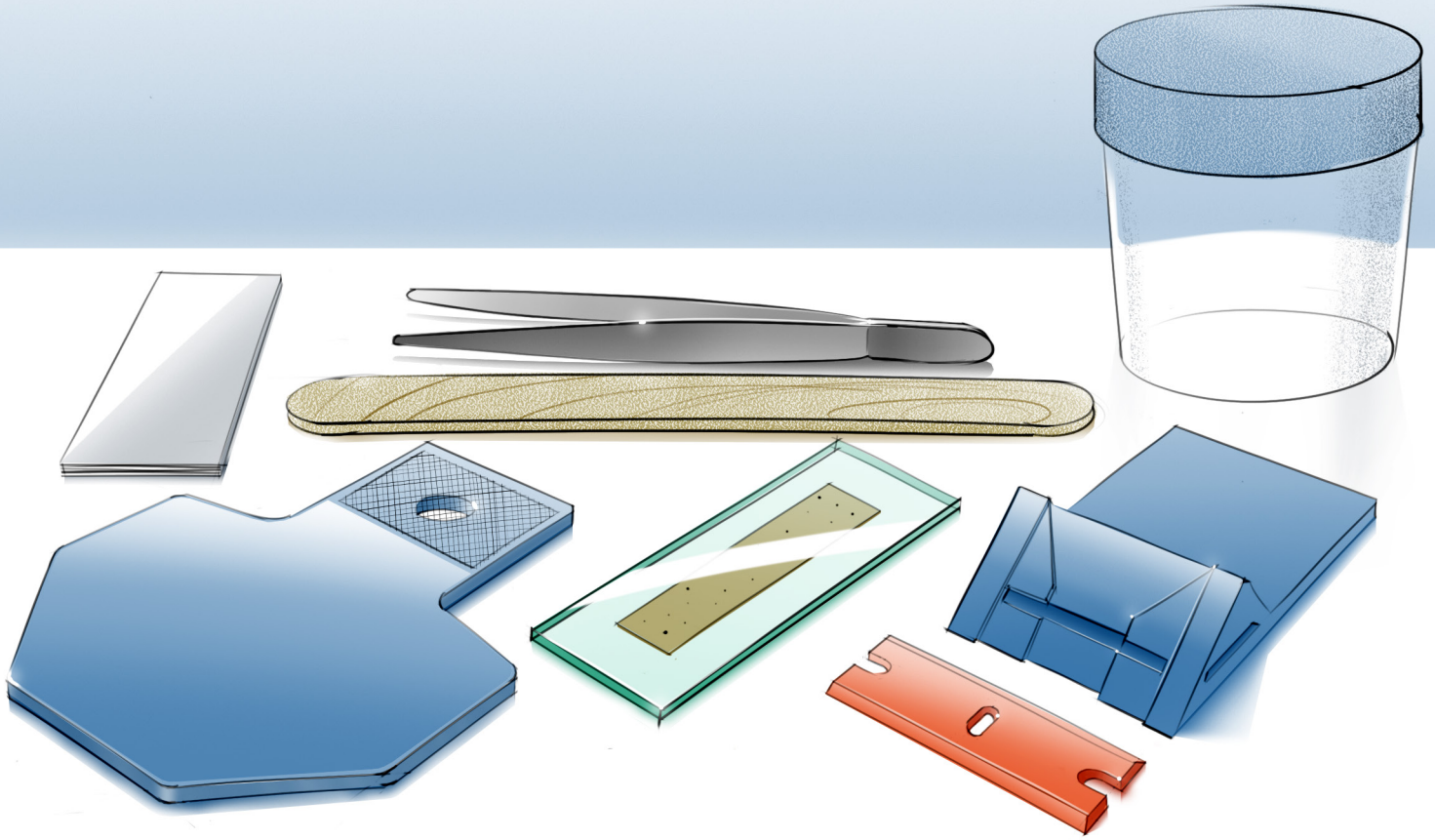

An Upgrade for the Kato Katz Method

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To God, Mom, Dad, & my little Sister

ACKNOWLEDGEMENTS

I would like to thank the following people, without whom I would not have finished this project:

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- My family, for their unending love, trust and support
- My fellow design students, whose ideas and enthusiasm are a constant source of inspiration
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EXECUTIVE SUMMARY

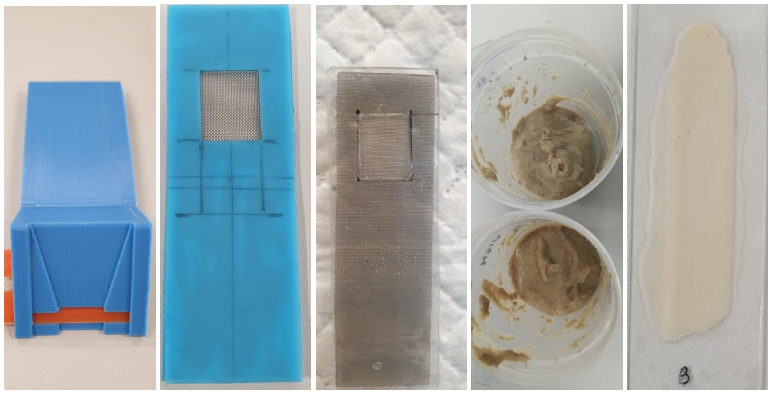
The kato katz method was, and still is, an invaluable tool to diagnose schistosomiasis mansoni en masse in high and medium intensity infection areas that are economically backward. Due to the current sensitivity capabilities of 24 EPG, the standard kato katz method is unable to diagnose very low intensity infections. And even though alternatives are sought, none are as affordable, cost-effective and easy to use as kato katz in the context of LMICs.

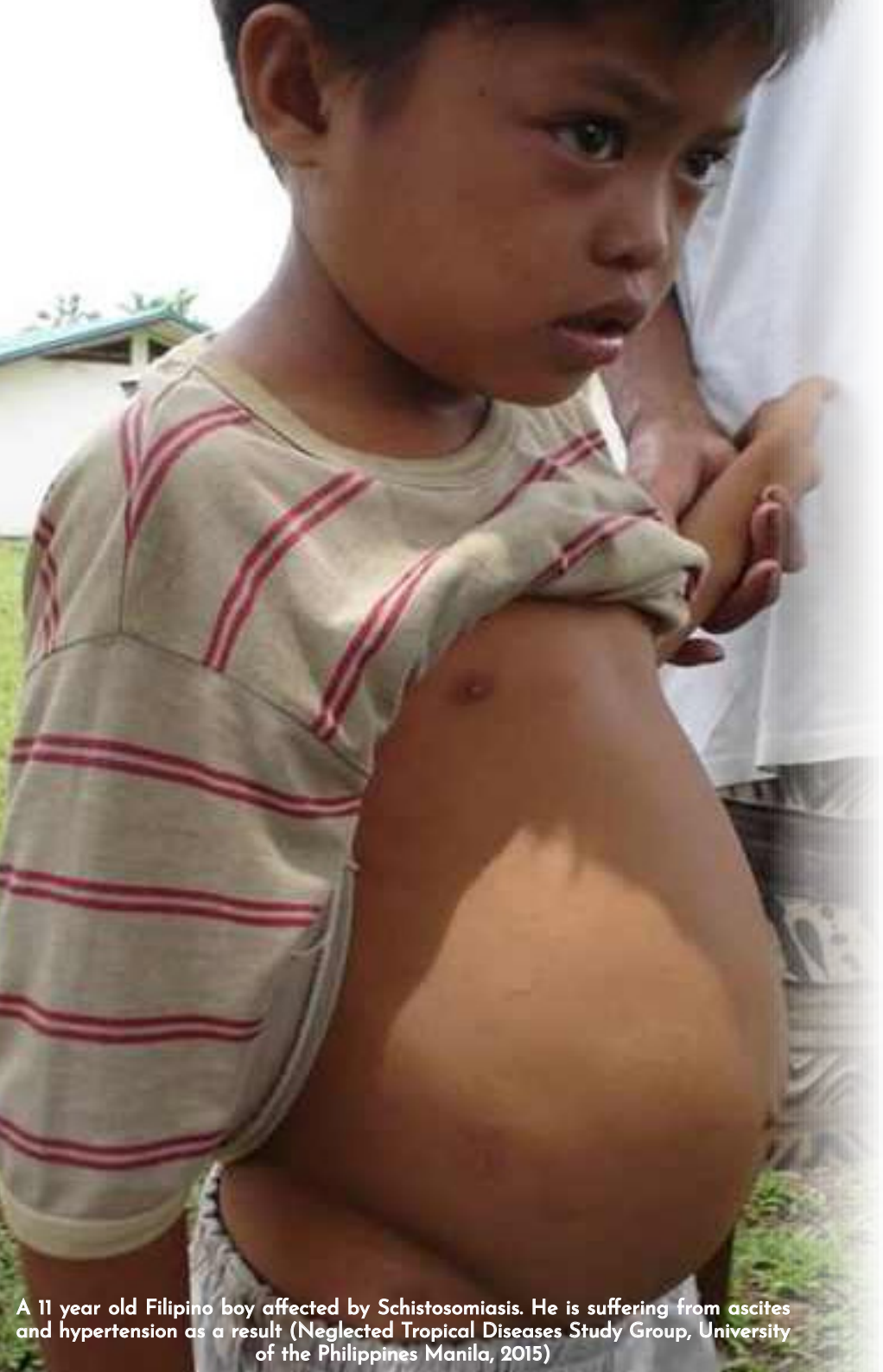
As the goals of the world health organization shift from disease mapping and treatment, to post treatment surveillance and eradication of S. Mansoni, more sensitive means of egg detection are necessary. The lack of a cost effective diagnostic tool will mean a rebound in the prevalence and intensity of infection, as medication cannot be given without a diagnosis. An accurate, cost effective, and easy to use method such as the currently used kato katz thus becomes a necessity. In this light, it was thought to be prudent to explore the idea of improving the kato katz process itself, in terms of sensitivity, ease of use and sustainability.

The context of this project was strictly limited to the sample preparation process only, and the seven steps recomended by WHO were classified into filtration and smearing phases. Three concepts were generated for each phase, and evaluated relatively using the harris profile. The template filter and the draw down smearing method were chosen to be the most desirable, and were finalized for prototyping and evaluation.

Material selection was based on their ability to withstand multiple cycles of cleaning using water and regular soap. Silicone strips with a stainless steel mesh insert and PLA for draw down smearer were chosen for their resistance to soap as well as economical value. Preliminary testing with synthetic feces showed that silicone strips were too flexible during the filtration process and did not allow for consistent deposit of fecal sample onto the glass slide. Hence the material was changed to a more rigid polycarbonate.

The components were taken to Nigeria to be tested on field by one of the stakeholders, Mr. Prosper Oyibo. He let two experienced lab technicians test the components using actual feces, and took notes of their feedback, based on a questionnaire prepared before hand. Overall, the lab technicians found the improved kato katz method to have potential for on field use, given that a certain amount of changes were made, such as reduction in the amount of fecal sample, and changing the template so that feces do not collect at the corners. It may be concluded that with certain steps taken in the future, the improved kato katz method will certainly be useful.





A 11 year old Filipino boy affected by Schistosomiasis. He is suffering from ascites and hypertension as a result (Neglected Tropical Diseases Study Group, University of the Philippines Manila, 2015)

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1. INTRODUCTION

1.1 Humans, Parasites & Feces - A History

The first written records of parasitic infections come from ancient cultures dating back to 3000 BC. The Chinese, Indians, Egyptians, Greeks and Romans and Arabs, all had physician's notes confirming this. Large worms, such as the roundworm and tapeworm, are clearly visible to the naked eye, and could certainly have been the first worms to be observed in an infected person's feces. It was also through the study of these worms by which scientists established the pathological process *(Cox, 2002)*. Several physicians sought to explain the presence of worms in humans via spotaneous generation theory, or metamorphosis of undigestible food in to worms. The late middle ages and renaissance periods did not have much progress in terms of parasitology. The discovery of the microscope in the early 16th century empowered parasitologists greatly. They observed eggs in parasites, but could not identify them as such yet *(Stewart, 1951)*.

In recent history, the first to observe parasite eggs in human feces was a German doctor working in Cairo, Theodor Bilharz (after whom Schistosomiasis, or Bilharzia, is named) in 1851, which is evidenced from a letter he had written to his colleague in 1852. In the same year, William Henry Ransom, an english physician, had also used this method to detect parasites in the feces of cats and dogs. He was the first to have the idea that this could be a diagnostic method to detect Ascaris lumbricoides in humans. He was able to confirm this hypothesis in July 1854, when he found Taenia and Trichuris eggs in a 9 year old girl's feces, who complained of chronic abdominal pain since six weeks. It must also be noted that microscopes were not yet common in the mid 1800s, and it took a long time before a universally accepted method to detect eggs was established. Towards the late 1800s and the early 1900s, it was getting clearer that more and more species of parasites could be detected by examining stools under a microscope *(Grove, 1986)*. By the beginning of 20 th century, 28 species of parasitic worms that affect humans had been identified. Parasitologists now faced the question - how did parasite eggs move on to a new host?

Transmission of infection by ingestion of eggs was first demonstrated in 1862 by French scientist Casimir J. Daviaine. Later, Giovanni B. Grassi, an Italian scientist, infected himself with A. lumbricoides eggs, and found them in his feces. In 1902, the father of tropical medicine, Patrick Manson, mentioned in his writings that some zoologists may have found what may be an intermediate host for schistosoma. In 1915, Robert T. Leiper, the father of modern helminthology, established the complete life cycle of schistosoma *(Di Bella et al., 2018)*. In 1922, the migration pattern of roundworm larvae after entering the human body was established by Japanese pediatrician Shimesu Koino, who infected himself and later found large numbers of larvae in his sputum *(Cox, 2002)*.

Following this, several efforts were made to develop a stanardized method that can identify parasite eggs in a reproducible manner. Norman R. Stoll created the 'Stoll dilution egg counting technique' in 1923 *(Cort, 1931)*, through which he also established a standard-

ized fecal egg count of 'eggs per gram' of feces. The method was widely used for hookworm epidemiological studies *(Storey, 2015)*. Clayton Lane invented the direct centrifugal flotation method in 1923-24 *(Chandler, 1925)*, sedimentation by gravity was introduced by Faust and Meleney in 1924, and many iterations for improving the acid-ether centrifugation technique were undertaken by various researchers. For schistosomiasis, rectal biopsy was considered the most reliable option *(Maldonado & Acosta-Matienzo, 1953)*. In 1949, Paul C. Beaver invented the uniform density fecal smear method, which was considered to be very useful for large scale surveys because of its simplicity *(Maldonado, 1956)*. In 1954, Kan Kato and Momoshige Miura created the cellofane thick smear method that used a larger amount of feces (appx. 60 - 70mg), making it more sensitive than the currently popular direct smear method (appx. 2 mg). The method was modified and made easier for use on-field by Naftale Katz et. al *(Katz et al., 1971)*. Later, the WHO has acknowledged its wide use and adopted it as the gold standard for fecal analyses *(World Health Organization, 1991), (WHO Expert Committee on the Control of Schistosomiasis, 1993)*.

Parallel to the development of diagnostic tools, studies to identify endemic populations and regions were also on the rise, and some of the first estimates came to light. 72 million cases of various tapeworm species infections, and 34 million cases of various fluke infections were recorded across the world, as reported in 1924. By the 1940s, 46 million people are said to have been suffering from Schistosomiasis Japonicum across China, Japan and Phillipines, while a few thousand cases have been found in the Dutch East Indies. Schistosomiasis Mansoni was found to be the most widely distributed of all the schistosome species, with 29 million infections spread across Africa, West Indies, Venezuela and northeastern Brazil. 457 million cases of hookworm infections, 644 million roundworm infections, 355 million whipworm infections and 35 million cases of threadworm infections were also reported in the same time period. It was observed that there were approximately as many helminth infections as there were people in the world (Stoll, 1947).

In another parallel set of events, European colonization of countries around the world, and the subsequent establishment of trade routes via sea, ensured that human parasites were transported and distributed across the world, and port cities took the brunt of this burden. The Liverpool school of Tropical Medicine was established in 1899 in order to tackle this problem. The city was an important port for trade between England, Asia, Africa and the Caribbean. In the same year, another school, London School of Tropical Medicine (now known as the London School of Hygiene & Tropical Medicine) was founded by Patrick Manson, the father of tropical medicine. The goal of this institute was to train medical officers for employment across the british empire. Such was the need, that several institutes were established in Europe during the early 20th century to study tropical diseases. Although some of these institutes were established for colonial trade betterment, they later became pioneers in developing and testing methods to diagnose and treat tropical diseaes in poor countries in a large scale and cost effective way (Hotez, 2010).

In 1977, Dr. Kenneth Warren joined the Rockefeller Foundation as the director of health sciences, and started the 'Great Neglected

Diseases (GND)' of mankind program *(Ravo, 1996)*. He successfully appealed to the scientific & medical community that even though some diseases like schistosomiasis affected about 100 million people worldwide, less than $5 million was spent across the world on its study, and thus would qualify as being 'neglected'. He started a series of research institutes for the study of parasitic diseases, and many joined his cause. The World Health Organization (WHO), established in 1948, initiated the Special Program for Research and Training in Tropical Diseases (TDR), with the help of World Bank and the United Nations Development Program. The concept of mass drug administration came up in the 1950s, pioneered by Frank Hawkins for treating lymphatic filariasis. Entire populations endemic with a disease would be given an anthelmintic drug at the same time (and repeated if necessary), regardless of individual positive or negative diagnosis. The emergence of multinational pharmaceutical companies enabled further success of mass treatment programs, as they were frequent donors of large quantities of essential drugs. Economists like Jeffrey Sachs reported on how diseases like malaria trap populations in low and middle income countries in a cycle of poverty, leading to the formation of Millenium Development Global (MDG6), whose goal was to combat 'AIDS, Malaria, Tuberculosis & other diseases'. Unfortunately, the big three received much more attention and funds compared to the 'other' diseases. Some expressed concern over this neglect and pointed out how these 'other' diseases could also cause the same poverty cycles. The Bill and Melinda Gates Foundation (BMGF), established at the same time as MGD6, supported this cause by funding research on parasitology, as well as operational research that would solve practical problems such as identifying populations at risk, methods to mobilize and convince populations for diagnosis and treatment, and meeting large scale targets on time.

In 2005-06, 13 diseases were identified as 'neglected', and their extreme prevalence in many low income populations, and subsequent socio-economic decline was published. They also mentioned that these diseases can interfere with, or complicate the treatment of malaria and AIDS. The WHO responded by creating a new department for Neglected Tropical Diseases (NTDs) *(Molyneux et al., 2021)*, whose mission, as found on their website, is:

"The Department of Control of Neglected Tropical Diseases coordinates and supports policies and strategies to enhance global access to interventions for the prevention, control, elimination and eradication of neglected tropical diseases, including some zoonotic diseases." *(About Us, n.d.)*

Now that the history, significance and need for large scale treatment for NTDs has been established, the rest of the report will continue in the context of Schistosomiasis, and later will specifically focus on Schistosomiasis Mansoni, as this project is an attempt to improve the Kato-Katz method, which is a sample preparation process used to diagnose S. Mansoni.

1.2 Schistosomiasis - An Overview

Schistosomiasis is an NTD that is found in 78 countries, disproportionaltely affecting those belonging to socially and economically backward regions of the world. In terms of number of infections,

it is second only to malaria *(Barakat, 2013)*. In 2019, 236.6 million people worldwide needed treatment for the disease, of which more than a 100 million have received it. It was estimated that 600 million are at risk of infection worldwide *(Chitsulo et al., 2000)*, which has increased to 800 million by 2021 in Sub-Saharan Africa alone, which houses only about 13% of the world's population, but includes about 90% of schistosomiasis cases worldwide, with an annual human death rate of 280,000 *(Aula et al., 2021)*. The affected global population has lost a combined total of 4.5 milllion years of their lives, from the statistic known as disability adjusted life years (DALYs), as calculated by the World Health Organization (WHO) *(WHO Expert Committee on the Control of Schistosomiasis (2001 : Geneva & Organization, 2002)*.

Although highly preventable, schistosomiasis had to be classified as one of the neglected tropical diseases (NTDs), as it occurs predominantly in low and middle income countries (LMICs). Those who have it usually cannot afford treatment, and no access to proper sanitation and drinking water ensures proliferation of the disease *(winkler et al., 2018)*. Figure 2 shows the distribution and density of the disease across the globe. It can be obseved that the most affected population is in the Sub - Saharan Africa region. The two variants of the disease, intestinal and urogenital schistosomiasis, can cause multiple heath issues that affect quality of lives of adults, and cause growth and learning issues in children. Severe cases may also lead to death *(Schistosomiasis, n.d.)*.

The disease is caused by a family of parasitic worms called bood flukes (belonging to the class trematoda), which are transmitted to

Figure 1: Lifecycle of Schistosomiasis Mansoni. Humans wade into infested waters, schistosome larvae penetrate the skin, migrate to the intestines and start laying eggs, which are passed in stools. Eggs hatch, find snail hosts, reporoduce and produce more larvae.

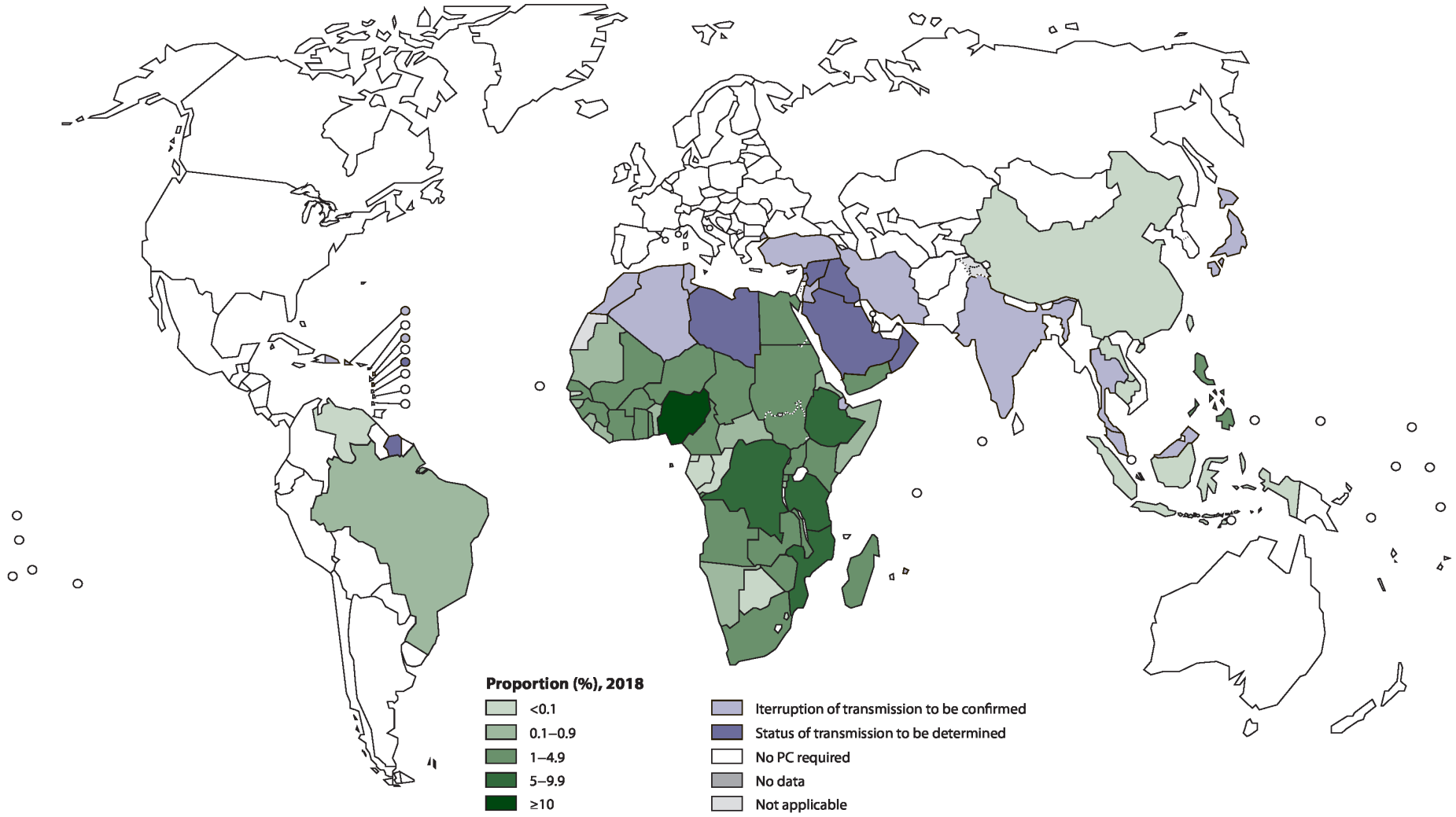


Figure 2: Map showing the percentage of people across the world that need treatment for schistosomiasis, according to the World Health Organization. It can be observed how schistosomiasis disproportionately affects those in low and middle income countries. Image taken from https://cdn.who.int/media/docs/default-source/ntds/schistosomiasis-%28bilharzia%29/proportion-global-population-requiring-pc-for-sch-2018.pdf?sfvrsn=852fb11b_6

humans by contact with infested waters. Schistosome larvae (called circariae) in infested waters penetrate the skin when humans wade into them (children playing, adults washing clothes, utensils, fishing, etc). They then migrate to the intestinal or urogenital regions, latch on to the inner walls of blood vessels, where they copulate, and the females release eggs periodically, which are usually passed via urine or stool, depending upon the species of infecting schistosomes. When a person infected with schistosomes relieves themselves in a water body such as a lake, some eggs are released into the waters. These eggs hatch and release miracidia, which search for an intermediate host (snails, in the case of schistosomiasis), where they multiply, mature into cercariae, and are released from the snail. The cercariae then swim the waters until they come in contact with humans (or other animals), thus completing the infection cycle *(CDC - DPDx - Schistosomiasis Infection, 2019)*. The general life cycle of one species, S. Mansoni, is illustrated in figure 1.

The report will now narrow its focus further to Schistosomiasis Mansoni, one of the five main subspecies of schistosomes, which affects more people than any of the other schistosome species *(Barakat, 2013)*. It is an intestinal schistosomiasis, where adult schistosomes inhabit the inferior mesenteric vein that drains the colon. Infected individuals show symptoms ranging from asymptomatic, to bloody stools, developing inflammatory colonic polyps, liver fibrosis, and ultimately, death *(Elliott, 1996)*. S. Mansoni affects about 54 million people worldwide, and puts around 400 million at risk of infection. It causes about 0.2 million deaths a year, and around 29% of those diagnosed late (with bleeding varices) die, even with access to the best healthcare available *(Gunda et al., 2020)*.

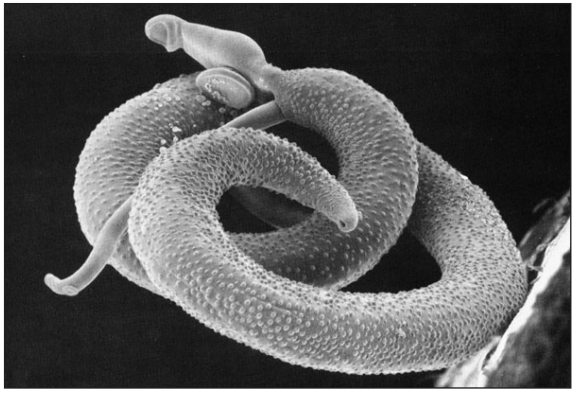


Figure 3: Schistosoma copulating. Adult males are long, wide and flat, while females are long, thin and cylindrical. The male wraps itself around the female *(Wikipedia, 2006)*

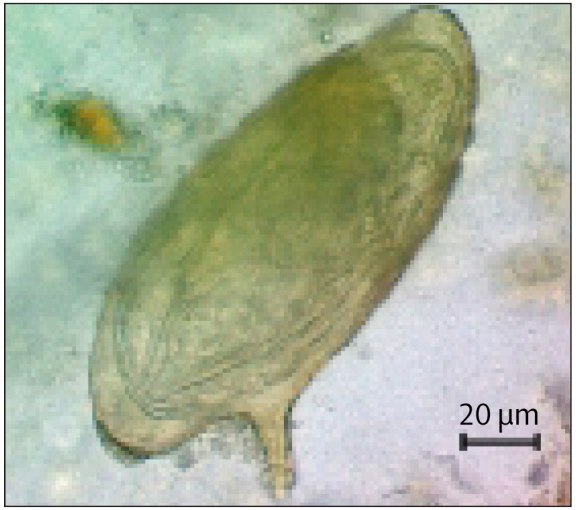


Figure 4: A schistosomiasis Mansoni egg. Notice the characteristic lateral spine on the bottom right. They typically measure about 140 microns *(Gryseels et al., 2006)*

Microscope examination of a standard quantity of feces is the preferred method of diagnosis for S. Mansoni. A small sample of fecal matter is deposited onto a standard microscope glass slide and prepared for reading. A laboratory technician then places the glass slide under the microscope and counts the number number of eggs in a methodical way. This process of preparing fecal samples for microscopy is called the Kato - Katz method, and is the 'gold standard' method for diagnosing several parasitic infections worldwide. The steps to prepare a fecal sample with this method, as recommended by the WHO, are illustratted in figure 5 *(World Health Organization, 2019)*.

An adult S. Mansoni pair can produce a maximum of about 300 eggs per day *(Nelwan, 2020)*, each measuring 60 x 140 µm approximately, and can be identified by their characteristic lateral spine, as shown in figure 4. The schistosomes cannot multiply inside their human hosts, and so the intensity of infection is entirely dependent on how many miricidia penetrated the skin of the infected individual. Some of the released eggs get stuck in tissue, triggering immune responses, while others are excreted when a person relieves themselves, so they can be found in stool samples collected from infected individuals. Infection intensities, defined as the number of eggs per gram (EPG) of feces, are classified as low (0 - 99 EPG), medium (100 - 399 EPG) and high (≥ 400 EPG) *(Coulibaly et al., 2011)*.

The current treatment method of choice is preventive chemotherapy (PC). The drug recommended by WHO is Praziquantel, as it is cost effective, safe, and works for all human infecting schistosoma species. The recommended dosage is 40 mg/kg bodyweight of the

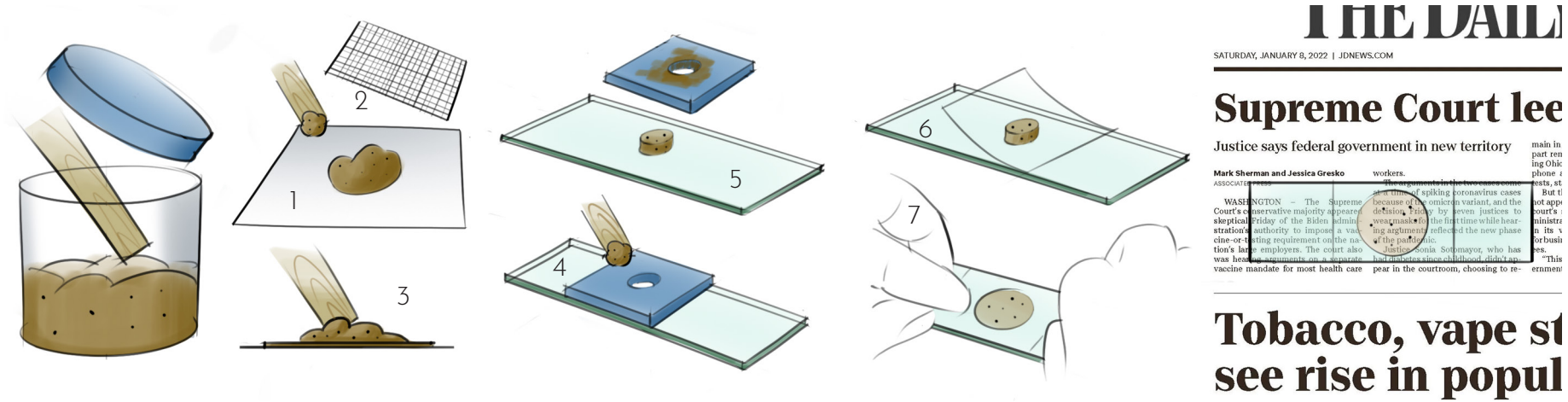


FIgure 5: The kato katz sample preparation method as recommended by the WHO. At the end of a successful 7-step sample preparation process, a thin transparent film containing 41.7 mg of feces is created. To test if a quality sample has been created, it is placed on a newspaper. If the print can be read through the stain, the sample is considered good for microscopy.

patient in one dose. Side effects include nausea, vomiting, abdominal pain and malaise, all of which are usually mild. Severe side effects were only noticed in patients with heavy infections, and include acute colic pain with bloody diarrhoea. The medication works by paralyzing the schistosoma and damaging their outer layer (called the tegument), which serves as the interface between the parasite and host by performing essential functions such as absorbtion, secretion, and protection. It must be noted that praziquantel has no effect on newly acquired infections after treatment, or eggs and immature worms present during treatment, so the patient will still release healthy eggs for several weeks after being treated. Patients will be re-examined after 4 - 6 weeks. It was found that after treatment, 70 - 100% of patients have ceased releasing eggs, and those that still do, have a reduction rate of about 95% *(Gryseels et al., 2006)*. Oxamniquine, which is only effective against S. Mansoni, is recommended as a second choice (*WHO Expert Committee on the Control of Schistosomiasis (2001 : Geneva & Organization, 2002)*.

1.3 Control & Elimination Strategies

As individuals cannot afford healthcare in LMICs, the burden of diagnosis, treatment, and monitoring falls to various global health institutions such as the WHO and local governments. Expert committees and researchers have generated, improved and validated various strategies for mapping, control, treatment and elimination of schistosomiasis. The first WHO expert committee meeting was held in 1952 in San Juan, Puerto Rico. In their report, they discuss bilharziasis, its epidemiology, and finally, procedures for surveying and controlling the disease *(WHO Expert Committee on Bilharziasis & Organization, 1953)*. In a later report, they discuss the variables that affect the objective of a control program - prevalence, intensity and incedence of disease, as well as availability of trained personnel and funds. Formulation of a program objective, a study of its feasibility, and a continuous evaluation process are the steps recommended in order to measure progress towards achievement of goals, funding and acceptance of program by locals *(Epidemiology and Control of Schistosomiasis, n.d.)*.

Subsequently, during the 1950s to 1980s, one of the more popular strategies to control schistosomiasis was to curtail the intermediate host population - eliminating the snail species responsible for schistosome proliferation. The goal was to use molluscicide in infested waters, either as a focused or as a blanket solution, to eliminate snails in proximity to human dwellings. This strategy provided mixed results. While snail populations decreased drastically, some places even reaching zero, there was always a rebound of the population within a few weeks/ months as even one snail can repopulate entire water bodies. This meant that water treatment had to be extremely frequent and focused, and the programs must be under constant expert supervision, all of which claw into already limited resources. Other modes of control were also explored, such as chemotherapy with drugs such as Ambilhar, which showed some serious side effects. Educating endemic population by public health services also showed some promise in the control of the disease (*Epidemiology and Control of Schistosomiasis, n.d.)*. In the late 1970s, oral treatment for schistosomiasis began to emerge, and this, along with other factors such as toxicity to soil, handlers and fish, pushed snail control programs out of preference, though not totally abandoned. They

are still used as a supplement to PC *(King & Bertsch, 2015)*.

Modern strategies for control and elimination of schistosomiasis were first outlined in a report by a WHO expert committee published in 2002. The first objective should always be morbidity control - a plan to reduce the intensity of the disease to a level where it is no longer a public health burden. This can be done by ensuring access to PC drugs at all health care facilities, and by frequent treatment of at-risk population. In high- prevalence areas, it was the practice that treatment was done without prior diagnosis, as this would be more cost effective, but due to the risk of developing a tolerance for the drug, it is now recommended that a well planned targeted approch that is integrated with the current local healthcare system would have all the benefits of being effective, efficient and economic. For a more permanent control, improved sanitation, clean drinking water, environmental measures (snail control) and health education is a necessity *(WHO Expert Committee on the Control of Schistosomiasis (2001 : Geneva & Organization, 2002)*. Meanwhile, other studies focusing on the relation between diease prevalence and patient age showed that the most affected age group for schistosomiasis is 6 -15 years, which is predominantly school going children *(Coulibaly et al., 2013)*. Based on this data, the strategy of mass drug administration (MDA) in schools was developed. In this program, all school going children in endemic areas would be administered praziquantel annually or biannually, depending on the intensity of infection, at school. Treating school children for control of schistosomiasis was a cost effective option, as they represented a large percentage of the infected population, and schools were great platforms for mass treatment as it was easy to gather and treat them. But although these programs proved to effectively reduce infection intensities, other studies, disease transmission and economic models suggested that by expanding these programs to sustained, community-wide treatments would reduce rapid reinfections, and would also be cost effective in the long run *(Lo et al., 2016)*. Other studies where adults at high risk areas were also treated along with school children showed evidence that annual integrated treatments can significantly reduce the prevalence and intensity of schistosomiasis, as well as other NTDs *(Zhang et al., 2007)*.

The latest guideline for control and elimination strategies provided by WHO is as follows: To interrupt transmission of schistosomiasis, large scale preventive chemotherapy must be combined with providing potable water, proper sanitation, hygiene-education, snail control and environmental modification. The five phases of schistosomiasis control and elimination are 1) morbidity control, 2) elimination as a public health problem, 3) interruption of transmission, 4) post-transmission surveillance and 5) verification of elimination. Treatment strategy recommended by WHO are as follows: For high risk areas, school children and adults are treated annually. For moderate risk areas, school children and adults are treated once every two years, and for low risk areas, only school children may be treated twice across the duration of their pre-school years. The WHO emphasizes that there is no 'one size fits all' strategy, and recommends a tailored program for each endemic area*(World Health Organization, 2022)*.

1.4 S. Mansoni, A Case Study - Mapping, Treatment & Post Treatment Surveillance

It was known that schistosomiasis was endemic in Sierra Leone, but comprehensive epidemiological data was unavailable, especially in the northern and eastern regions. Hence, in 2008, a national survey project was undertaken by the National Neglected Tropical Disease Control Program (NTDCP) to map schistosomiasis and soil transmitted helminths across the country, in order to provide data for a mass drug admistration program. The survey was done based on the newly released guidelines for preventive chemotherapy by the WHO in 2006 *(World Health Organization, 2006)*. This section will only highlight the relevant processes and results in the context of S. Mansoni and this project.

Community sensitization was the first step (spreading awareness of NTDs and explaining plans for treatment). Then, 53 schools were selected at random, distributed throught the country at an average of 4 schools per district. About 100 students between the ages of 6 - 15 years were chosen from each school for stool sample collection, while maintaining a balance between sexes and ages. Each student was given a bottle for a stool sample, and was assigned an identification number. As soon as the samples were collected, one slide per sample was prepared using the kato katz method. The GPS co-ordinates of each location of sample collection, infections and its intensities, differences between ages and sexes etc, were all noted. All data was stored in microsoft excel.

Each district was categorized as low, moderate and high risk areas, as defined by the WHO PC guidelines. All rural populations in districts where schistosomiasis was detected were considered at risk, as people were involved in professions dealing with water - fishermen, farmers, women doing chores etc. So all school children in endemic communities, along with adults in high risk areas were the target population for annual PC program. S. Mansoni was found to be heavily prevalent in the north eastern part of the country, and was found to be directly proportional to multiple environmental factors. Elevations above 250 m, and higher population densities seem to have higher prevalences and intensities. A total of 5691 school children were tested for the survey, out of which 5069 data entries were valid. Overall, the point prevalence across the country was 18.4% with a range of 0 - 82.1%. Five districts were classified as high-risk and two were classified as moderate-risk *(Koroma et al., 2010)*.

As only one stool sample was prepared and examined per person, and that only four schools per district were used for testing, it was noted that prevalences and intensities may have been underestimated. Hence, a complementary survey would be conducted in 2009, where school children in the seven districts classified as moderate and high risk would be retested. 59 schools that were not chosen in the national survey, but existed within the moderate and high risk areas, were selected for the complementary survey, from each of which 30 children of ages 9 - 14 years were tested. A total of 1,760 fecal samples were tested, and it was found that the overeall prevalence of S. Mansoni was 40.2%, and intensity 95.4 EPG, which means over 20% of children have moderate or heavy infections *(Hodges et al., 2011)*.

In parallel, a sentinel survey took place to measure disease prevlence and intensity before, and six months after treatment. Fifteen schools that showed a high prevalence rate of schistosomiasis (≥50%) in the 2008 and 2009 surveys were chosen for re-evaluation of disease prevalence and intensities. As before, 30 students from each school (ages 9-14) participated, and one slide was prepared per person for microscopy. A total of 448 children were tested. Prevalence and overall intensity before treatment were found to be 69% and 170.8 EPG respectively. 35.6% children had moderate to heavy infections.

After the survey, MDA was started by the NTDCP in a phased manner. The school children from moderate and high risk districts identified in the national and complementary surveys were treated with Praziquantel, which were a total of 562,980. 94% coverage was achieved in this first round of treatment. Six months later, the 448 students from the sentinel survey were tested again. This time, the prevalence and intensity of S. Mansoni were 38.2% and 47.3 EPG respectively, reducing the proportion of children with moderate to heavy infections to 9.9%. Before MDA, 13 of the 15 sentinel schools showed high prevalence, which reduced to just 4 after, indicating a significant improvement in overall health of the children *(Hodges et al., 2012)*, and validate the reliability of the guidelines laid down by the WHO.

1.5 Kato Katz - The Gold Standard?

Since its modification for field use in 1972, the kato-katz method has been the "gold standard" for preparing stool samples for microscopy, as recommended by the WHO *(Krauth et.al, 2012)*. It gives standardized results, and can be easily taught to those who collect and prepare samples *(Speich et al., 2010)*. It is the most cost effective technique currently available for fecal diagnostic tests, and is widely used not only as a tool for screening and mapping endemic areas for neglected tropical diseases (NTDs) *(Developing Capacity to Monitor Parasitic NTDs through Kato-Katz in Cambodia, 2020)*, but also for monitoring infections and post treatment surveys to measure changes in intensities, making it an essential health care aid for diagnosing multiple parasitic infections in LMICs and elsewhere.

Though widely used because of its economic benefits and reasonably accurate diagnosis, Kato Katz method has quite a few setbacks. It has poor sensitivity when it comes to low intensity infections. Theoretically it cannot detect an infection intensity of less than 24EPG, as one slide holds only 41.7 mg of feces. It can also show a high variation in number of eggs per sample, even though all were prepared from the same stool sample, which may be due to eggs clumping together or skill levels of the technicians *(Kongs et al., 2001)*. Some samples, such as the ones being tested for hookworm infections, must be examined within 30 minutes of preparation. It is difficult to do this, for example, in a setting where there are a large number of samples to process in a short time. Also, recent studies show that the cost of implementing this method at a large scale

may be more expensive and logistically more cumbersome than what is currently assumed *(Turner et.al, 2017)*. Due to these factors, the drive for searching alternative diagnostic methods is becoming greater.

Despite the shortcomings, abandoning this method for newer diagnostic tools will cause significant reduction in delivering healthcare to a wider section of affected population. Novel techniques such as point of care - circulating cathodic antigen test (POC-CCA), and up-converting phosphor - lateral flow circulating anodic antigen test (UCP-LF CAA), are extremely sensitive, but are not suitable for surveying post treatment endemicity, as antigens are unaffected by treatment. So they can only be used in areas where no treatment has been never given, and for newborns in areas where transmission has been completely stopped *(Sousa et al., 2019)*. Also borrowing from the field of diagnostics for sexually transmitted infections (STIs) in LMICs, two of three product development strategies WHO recommends for coming up with new diagnostics is improving existing tools, and adapting existing platforms. Although they recommend that all three strategies applied together gives the best results, the estimates demonstrate that improving/ adapting takes significantly less time and resources compared to coming up with new diagnostics, as shown in table 1 *(Kettler et al., 2004)*.

Strategy	Process	Resources	Timeline
Improving Existing Tools	Develop Specifications; Fund, Manage and Co-ordinate Private Sector or Universities	USD 2-3 M	2-3 Yrs
Adapt Available Platforms	Develop Specifications; Fund, Manage and Co-ordinate Private Sector	USD 5 M	3-5 Yrs
Develop New Platforms	Search for Bright Ideas	USD 10-12 M	≥10 Yrs

Table 1: A reproduction of the product development strategies for improving current diagnostics for STIs table, and an estimation of time and resources each strategy takes, according to the WHO *(Kettler et al., 2004)*

Further evidence to support the improvement of kato katz method is presented in table 2, where other diagnostic methods used in LMIC settings are compared with kato katz in terms of accuracy, cost effectiveness, the three main factors in selecting a diagnostic test in an LMIC setting *(Ajibola et al., 2018)*. As seen in the table, none of the other tests satisfy all criteria. This makes kato katz an ideal diagnostic tool, especially in high and medium infection intensity areas, and for disease mapping. If it can be improved so as to accurately and reliably diagnose low infection intensities, it would greatly contribute to the continuation of treatment and monitoring programs at low costs, atleast until a more accurate, cost effective and reliable tool emerges.

Category	Method	Sensitivity	Cost of One Test (Equipment + personnel)	LMIC Compatibility (Accurate, Cost effective, Easy to use)
Microscopy	Kato Katz (gold standard)	98.6% (Duplicate KK. at HII*) *(Lamberton et al., 2014)* 77.4% (Triplicate at MII*) (Glinz et al., 2010)	US$ 1.73 *(Speich et al., 2010)*	A, C, E
	Conventional Formalin Ether Concentration (C-FEC)	85% (Single at MII*) *(Glinz et al., 2010)*	US$ 0.3 *(Zeeshan et al., 2011)*	A, C
	Mini - Parasep	93% (Single at MII*) *(Adugna et al., 2017)*	US$ 0.9 *(Zeeshan et al., 2011)*	A, C
	FLOTAC	91.4% (dual at MII*) *(Glinz et al., 2010)*	US$ 2.83 *(Speich et al., 2010)*	A
Antigen Detection	Point of Care- Circulating Cathodic Antigen Test	91.7% (Single urine test at HII*) *(Lamberton et al., 2014)*	US$ 7.26 *(Worrell et al., 2015)*	A, E
DNA Detection	PCR Test	100% (Single urine test at LII*) *(Enk et al., 2012)*	< US$ 4 *(Enk et al., 2012)*	A

Table 2: Current 'gold standard', Kato Katz, compared to other diagnostic tools used in LMIC settings to detect Schistosomiasis Mansoni. Sensitivities, affordability and ease of use are the basis of selection. Kato katz, as the gold standard, satisfies all three conditions.

* These represent the mean infection intensity in an endemic area. HII : High Intensity Infection, MII: Medium Intensity Infection, LII: Low Intensity Infection

2. PROJECT BRIEF

2.1 Resaerch Question, Goals & Scope

In the previous chapter, it has been shown that the kato katz is a very cost effective and fairly accurate method, and is widely used for diagnosing and monitoring S. Mansoni. It has very low sensitivity when it comes to low intensity infections (0-99 EPG). Hence, the overarching objective of this project is to improve the kato katz method so it may help healthcare professionals accurately and reliably identify low intensity infections, while being just as cost effective as the current method. The research question for this project, therefore, is:

"*Can the Kato-Katz method be improved interms of sensitivity, ease of use, and sustainability, while maintaining current reliability and cost effectiveness?*"

There are three major goals of this project:

1. Increase sensitivity to to detect very low intensity infections

2. Reduce human skill depenency to increase reliability

3. Replacing single use materials with sustainable ones

The scope of this project is limited to the WHO recommended kato katz method, which means sample collection, transportation, microscopy, treatment etc will not be considered. Only sample preparation, which is the step after transportation, and before microscopy is the focus of this project. The seven steps will be analyzed, and depending on the need, will be modified, combined or discarded.

2.2 Stakeholder Analysis

A stakeholder(s) is an individual or a group that can either affect, or is affected by, the decisions made as a part of implementing a project. They are classified into different classes based on how many of the three main attributes they possess, which are as follows: 1. *Power to influence*, which is a stakeholder's ability to make the project manager implement or change an aspect of the project that they would not have normally done, or resisted doing. Power weilded may be coercive, utilitarian, or normative 2. *Legitimacy of stakeholder-project relationship*, which shows if this relationship is viewed as 'good' or 'bad' in a social value construct. The constructs may be individual, organizational or societal. 3. *Urgency of claim*, which is the stakeholder's capability to attract immediate attention and call to action. Urgency may be due to time sensitivity, or criticality of the stakeholder's need. This classification helps the project manager list and prioritize stakeholders and their interests. It must be noted that these attributes are dynamic, and are subject to change across the duration of the project. They are also social constructs, and not objective truths, and hence there will be a considerable influence of the 'sub-conscious belief system' on decisions taken.

Another point of view that helps with this prioritization is 'Stakeholder Salience'. Among the stakeholders that are in the same class, the project manager may 'find' that some stakeholders are

more important than others. This may be a conscious or sub-conscious decision due personal, organizational or societal beliefs, desire to complete project objectives and achieve intended outcomes, influence stakeholders etc. Salience is not a recommeded step in stakeholder analysis, but rather an observation that it is an inevitable phenomenon. It is the 'means' through which a project manager achieves an 'end'. Salience, like stakeholder attributes, is also dynamic, and varies throughout the project. The different classes of stakeholders based on their attributes and overlaps is illustrated in figure 7 *(Mitchell et al., 1997)*.

Based on the theory presented above, multiple stakeholders were identified and classified into various groups:

- *The Community Level:* Patients, parents of minors, community leaders, medical vendors, traditional healers
- *The Formal Healthcare Level:* Community Health Extension Workers (CHEWs), doctors, laboratory technicians
- *The Organizational Level:* Medical officers of health(MOHs), disease surveillance and notification officers (DSNOs), school teachers
- *The Economic and Policy Level:* Financial donors, academia, non governmental organizations (NGOs), and the regional and national governments

Each stakeholder's presumed level of interest and influence is shown in figure 8. At the community level, patients and parents have relatively low interest and influence, as they are wary of giving samples, and if forced to participate due to suspected infection, having to forego work, and thus losing the day's wages. Community leaders have medium influence and interest, as they would like to see their community healthy, and are capable of mobilizing people to treatment programs. Traditional healers and medicine vendors have low interest and influence as well. At the formal healthcare level, CHEWs have high interest but medium power, as they are the ones who collect samples and distribute medication. Doctors have medium-high power and influence, as they can instruct both CHEWs and patients. Lab technicians have high interest, as they are responsible for sample preparation, microscopy, data storage and organization, but only low-medium influence, as they can only influence changes in the sample preparation process. At the organizational level, DSNOs and MOHs have high influence and interest, as community health is their responsibility, and owing to their positions, they are respected and listened to. Teachers play a crucial role in organizing sample collection and MDA events in schools. They have low-medium power and low interest. Lastly, at the economic and policy level, financial donors, regional and national governments have high influence and high interest, as they approve and govern the programs themselves. Academia has high interest but medium-high influence, as their contributions are crucial but mainly non-financial, while NGOs have medium interest and influence due to their allround nature of work. As the main objective of this project is to improve the kato katz method, and in-line with the concept of 'salience', the focus for this project will be on 'lab technicians', as they will be the end users of this method.

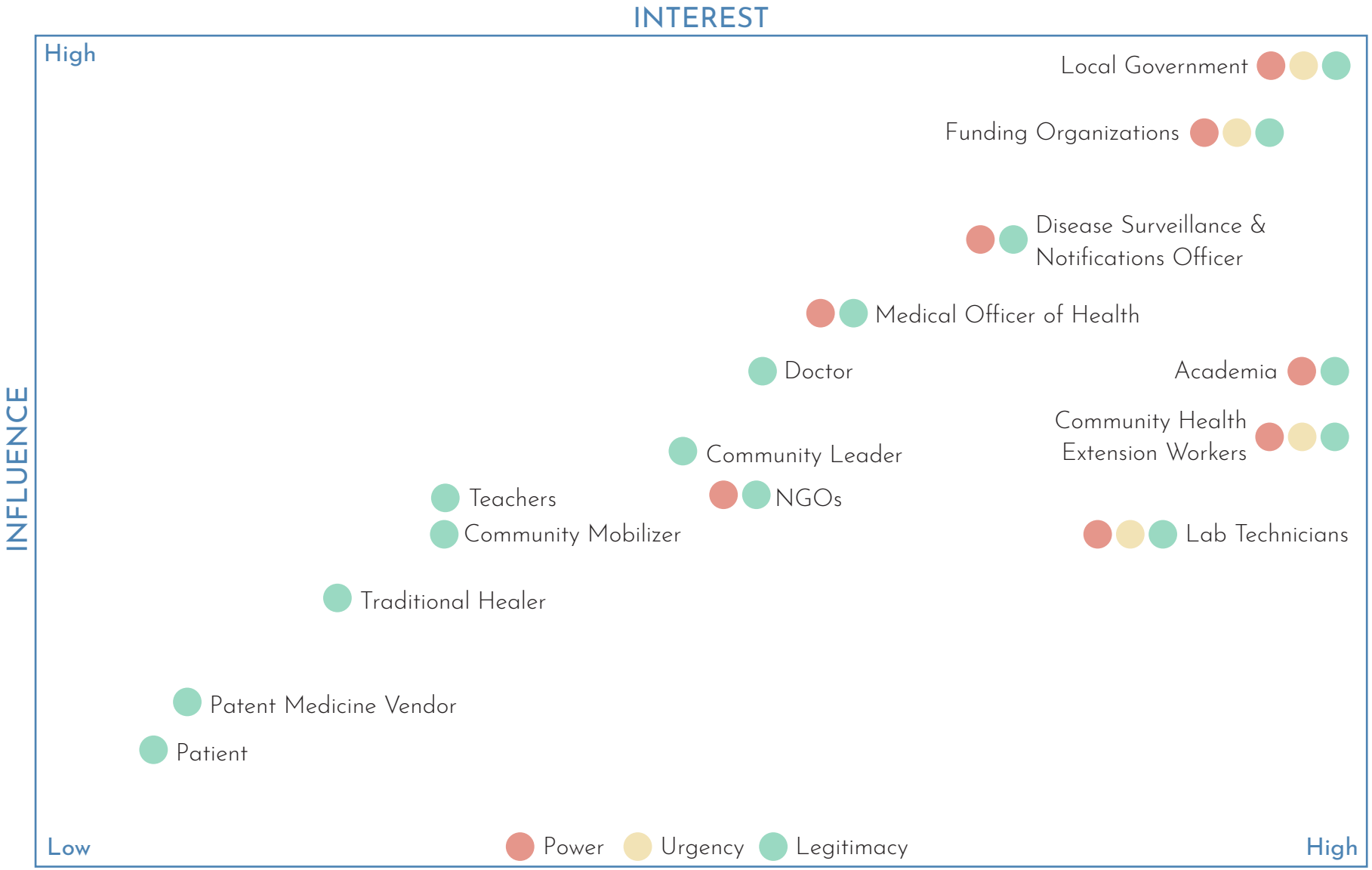


Figure 8: Various stakeholders related to this project shown in an influence vs interest chart. Each stakeholder's salience is also shown, depending on how many classes each falls into. Stakeholders with all three classes are definitive stakeholders, who are given top priority. Stakeholders with two or one class(es) are moderately salient and latent stakeholders, whose needs are prioritized next and last respectively *(Mitchell et al., 1997)*

2.3 Lab Technicians - Needs and Wishes

Two people with on-field experience of using the kato katz method were chosen to fill in the role of 'lab technician'. Online interviews were conducted separately, and information was collected regarding their observation of sample preparation journey, their specific roles and duties, pain and gain points, and finally needs and wishes. The interview points are presented in appendix B. Following is a brief introduction of the stakeholders and summaries of their interviews:

Mr. Prosper Oyibo is a PhD student at the faculty of Systems and Control Engineering, TUDelft, had developed an AI based automatic schistosome egg detection machine for reading fecal samples prepared using the kato katz method, named 'The Schistoscope'. He has tested the device several times in Nigeria, where studies were being conducted for MDA. As a part of his learning, he has trained under professional lab technicians, and has prepared several fecal samples under their supervision. He has an in-depth understanding of the sample preparation and microscopy processes. The end result of this project will be designed in such a way that it can be examined using manual microscopy, as well as Mr. Oyibo's Schistoscope. His experiences and opinions about sample preparation are:

- Was present for sample collection. Participated in sample preparation and microscopy
- Daily routine of lab technicians: travel to field -> sample collection -> travel to lab -> prepare sample -> analyze sample -> data collection and storage -> clean up
- On average, 20 - 30 samples are collected and analyzed per day.
- Various samples are collected for various tests. It is rarely only one type of sample collection/ testing, so isolating time for just stool sample collection is difficult
- Personnel are allotted depending on study size and availability
- Usually there is a sample prepper and a microscopist. Who does what is very fluid, and depends on who is free at the time

Mr. Brice Meulah is a PhD student at Lieden University Medical Center (LUMC), as well as a scientific researcher at Centre de recherche médicale de Lambarene, Gabon. He has extensive knowledge and experience with the kato katz method, and has worked everyday with it everyday for 1.5 years. Following are a list of his experiences and opinions:

- Number of participants enrolled to give samples on a particular day makes a lot of difference in work load
- With 2 microscopists, 30 sample analysis a day was good. 40 was too much
- A microscopist needs atleast 5 min to read a slide. Ten if the egg count is very high

- Quality of the sample prepared is more important than speed of preparation
- Usually microscopy is slower than sample preparation
- Things to improve: quality and consistency, timing, reduce contact with stool on gloves

Based on the information provided by the stakeholders, and literature review a journey map has been created showing the pain and gain points of each step, illustrated in figure 9. Adding to this the limitations of kato katz method observed from literature, and given the goals and scope of this project, the following needs and wishes have been established, which are later converted to design specifications that can be validated via qualitative and quantitative methods.

1. Increase the sensitivity of the kato katz method to detect very light infections
2. Increase reliability of the method. Every sample should be of the same quality irrespective of the skill level of the technician
3. Make the sample preparation process quicker and easier while maintaining the costs
4. Reduce contact with stool on gloves
5. Use reusable and sterilizable materials wherever possible
6. Prepared sample must be fit for analysis on a conventional microscope, as well as the Schistoscope

2.3 Design Specifications

The needs and wishes previously mentioned are now translated into design specifications. Each need/ wish is assigned a target value that the final prototype must reach or exceed in order to satisfy the requirements of end users. Below is the list of design specifications:

1. Must be able to detect eggs for very low intensity infections, i.e. ≤ 10EPG *(Bärenbold et al., 2021)*
2. Preparation time for one sample must be below 2.58 minutes *(Speich et al., 2010)*
3. The cost of a kit that lasts for 500 samples must cost less than $136.15 *(Kato Katz Kits - Diagnostic Test Kits | Sterlitech, n.d.)*
4. Sample preparation must be done in 7 steps or less *(World Health Organization, 2019)*
5. Technician skill must be eliminated from sample preparation. Every smear must measure to be the same thickness
6. Materials used must be washable with common dish soap (non reactive according to manufacturer specifications)

3. DESIGN & EVALUATION

Now that the design specifications have been established, ideas to improve the kato katz method can be generated. The first step was studying the technique. It can be divided into three important steps: Homogenization, Filtration, and Smearing. Homogenization refers to distributing the eggs evenly in a stool sample, so that any unit weight of stool taken at random would have the same number of eggs. Homogenization is currently done by the lab technician using a wooden spatula. The stool sample is mixed for a number of times. No alternatives that are more efficient, cheaper, and easier to clean were found compared to the gloved human hands. Hence, idea generation was limited to the phases of filtration and smearing.

3.1a Brainstorming - Filtration Methods

Filtration is the step that follows homogenization. In the traditional kato katz method, a small amount of a homogenized stool sample, about the size of an almond is scooped up and placed on a scrap piece of paper. A fine mesh (60 - 105, made of nylon or steel wire) of size 5 x 5 cm is used to press against the stool sample and filter it. Larger debris remains behind, while the filtered stool is scraped with a spatula to continue with the rest of the process. As stated in the sample preparation journey map, in order to reduce or eliminate the issues, three concepts were developed using the brainstorming method. Each of the ideas are explained in the paragraphs, below, and illustrated in figures 10 A, B, and C.

The **template filter** concept combines the filtration and the first step of the smearing phase. The mesh is sandwiched between two templates, so that the holes align, with the mesh showing through. The user scoops a sample of the homogenized feces and puts it directly on to the template filter, and scrapes it through the mesh until the cavity is filled and lifts the template up. A measured amount of fecal sample is now ready for smearing. The advantages of this method is that it reduces one step in the kato katz method, and eliminates the need for scrap paper and reduces waste as the template filter will be designed to be reused for many cycles.

The **piping filter** works in a similar fashion to regular piping bags used to decorate cakes. In this version, a thin, flexible, but durable sheet (e.g. silicone, 10 x 10 cm) is taken, and a hole is made in the center. A reusable mesh is adhered to the sheet on top of the hole. The user scoops a small amount of homogenized feces, and places it on top of the mesh, aligned with the hole. The four corners of the sheet are lifted and held together to form a bundle, with the hole pointing downward. The bundle is now squeezed to filter the feces out of the mesh, and onto a standard template placed on a glass slide. Once the template is filled, the excess is scraped off, and the template is lifted, leaving a standard amount on the glass slide. The advantages of this method is its portability and ease of use.

The **centrifuge filter**, as the name suggests, uses centrifugal forces to filter the feces. In this method, the sample container given to the patients has a removable and washable mesh layer at a third of the total height from the bottom. After sample collection and transportation, the user places a batch of the containers in a mechanical

centrifuge, and spins it by hand. Precise speeds are not necessary, and visual inspection is sufficient to check if most of the feces has been filtered. It is important, however, to ensure that most the fecal matter has been filtered in all of the containers, and only fibers and other large particles remain. This is to make sure that all eggs have passed through, as different stool samples have different consistencies, and one sample is only half filtered, there is a chance that eggs remain in the unfiltered feces as they tend to clump together sometimes. Once filtration is complete, the user pulls out the mesh layer, homogenizes the filtered stool, and proceeds to the template. This method is very advantageous to the user, as they do not have to filter the feces by hand. Even with gloves on, handling fecal matter is the most uncomfortable step for a lab technician. Also, as multiple containers can be filtered with a centrifuge in every cycle, a lot of time is saved. There is also no need for scrap paper, single use meshes, and spatulas for filtering.

3.1b Brainstorming - Smearing Methods

The step after filtration is smearing. Smearing is the process of dispersing the measured amount of stool deposited onto the glass slide into an even, semi-transparent film. When placed on newsprint, the lab technician must be able to read the text through it. This is the recommended test for the verification of a successful smear. In the traditional kato katz method, 41.7 mg of feces is deposited through the template and on to the glass slide. The template is removed, and a hydrophilic cellophane tape that has been soaked in a glycerol-malachite green solution for 24 hours is placed on top of the fecal sample. Now the slide is turned up side down, with the

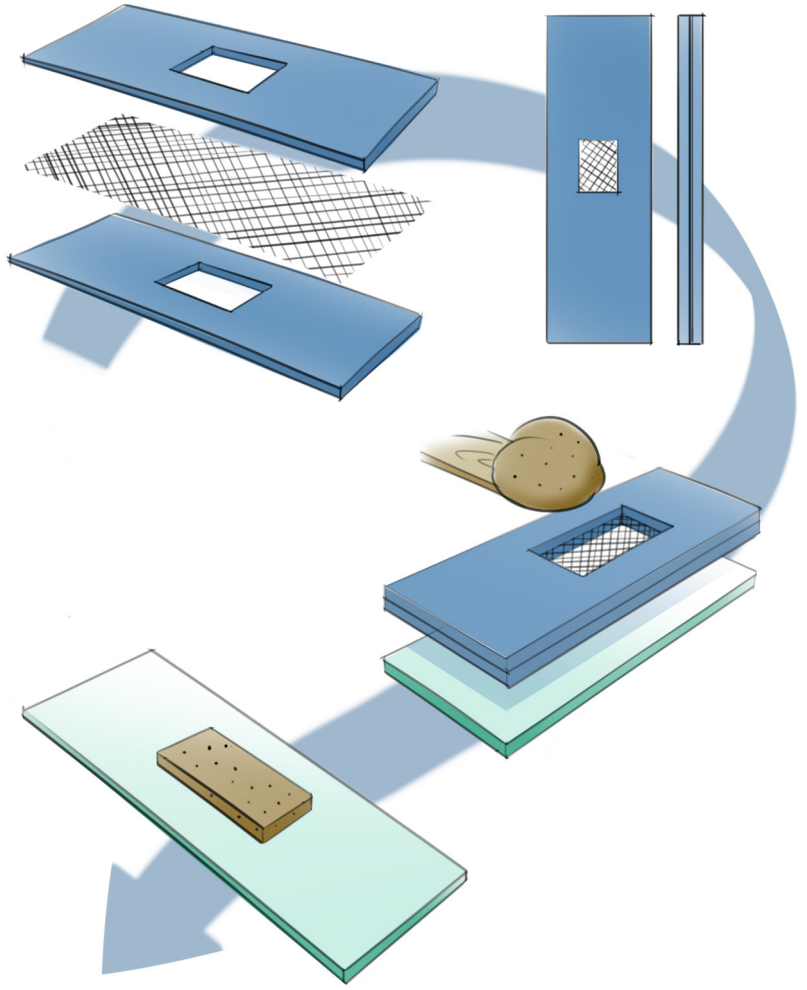


Figure 9: The template filtration method. Two strips of silicone are fixed with a stainless steel mesh inbetwen. The feces is pushed through the mesh, and leaves a measured quantity of sample on the glass slide

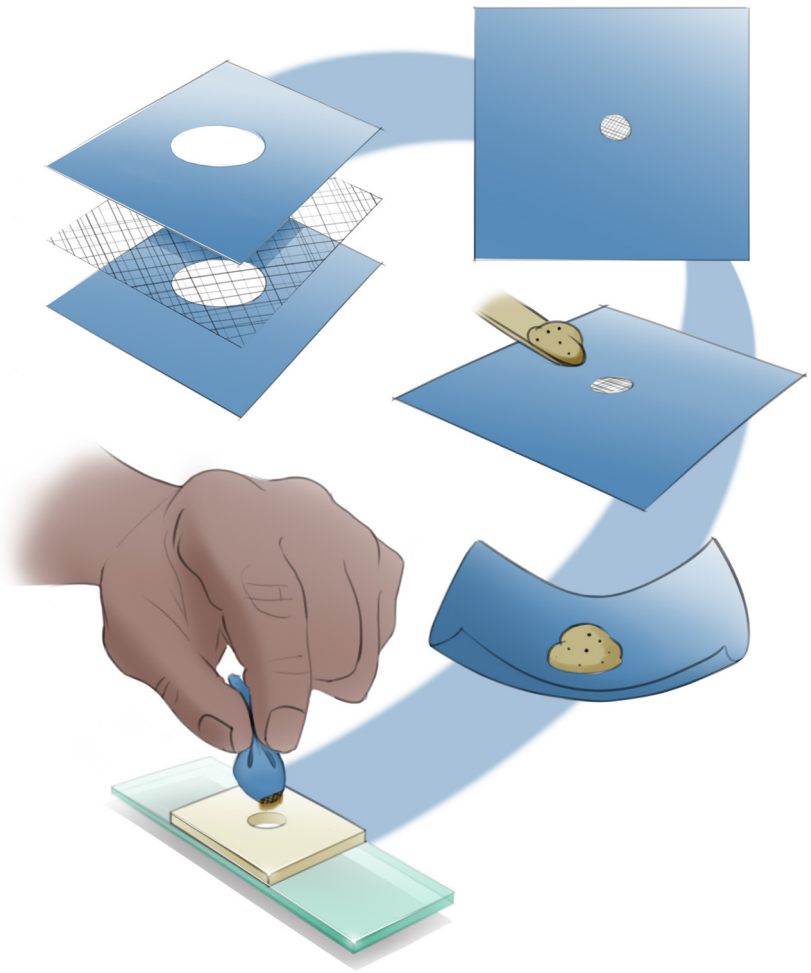


Figure 10: The piping filtration. Similar to the template filter in assembly, the piping filter uses a nylon mesh that is sandwiched between silicone thin sheets. The sample is deposited, and the sheet is made into a bundle and squeezed to filter the feces

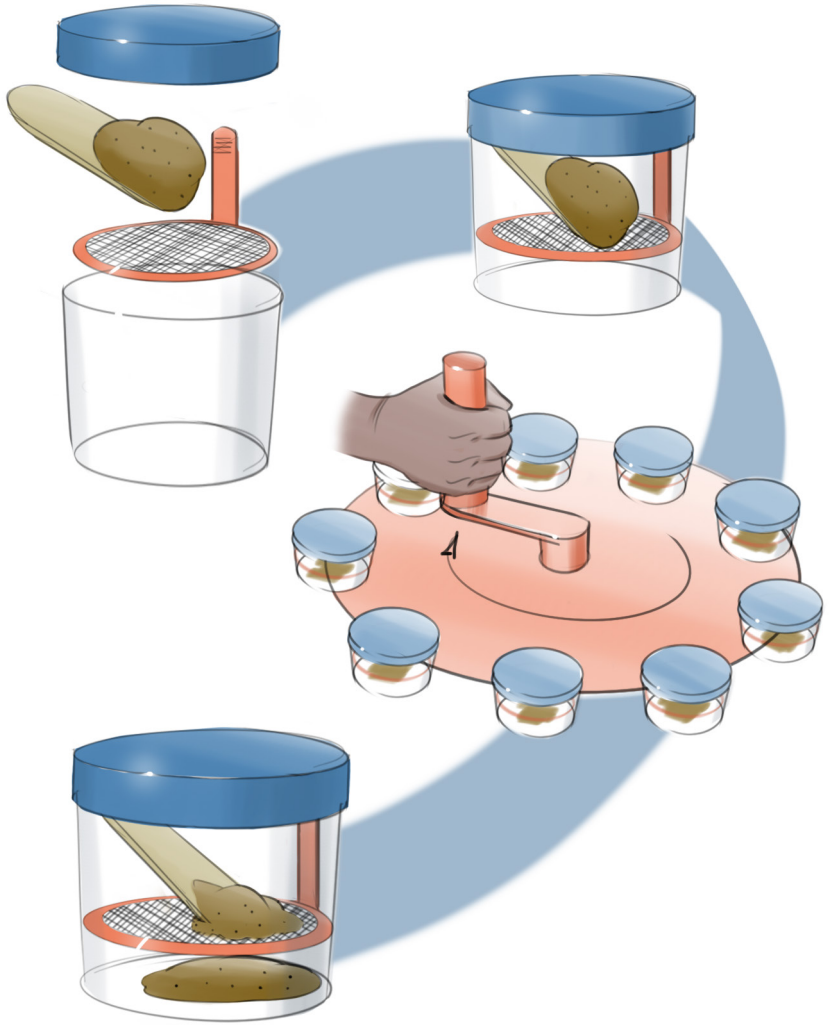


Figure 11: The centrifuge filter uses an inbuilt mesh with every sample container given to patients. It is then put in a hand centrifuge and spun, during which process the feces is filtered through centrifugal forces

cellophane facing the workbench or table, and the glass slide is pressed gently but firmly with both thumbs, until a circular smear is formed. The three smearing ideas are explained in the paragraphs below, followed by their illustrations 10 D, E, F.

The **stamp smearing** method utilizes a spring loaded mechanism, which functions similarly to the mechanism of an old fashioned mechanical date stamp. When placed on the glass slide and depressed, a plate is lowered, and presses the fecal sample in to a smear. This is similar to the traditional kato katz smearing method, but inverted. It has the advantages of speed and ease of use.

The **roller smearing** method uses a roller to flatten the measured fecal sample. The sample is first covered with the soaked cellophane tape, and then the roller is applied from one end of the slide to the other, ensuring even pressure throughout its length. Advantages of this method are speed, portability and commercial availability. Any low-cost print roller available locally will produce good results. A perceived disadvantage of this method is variation in pressure exerted as the roller is moved along the slide, creating tapering smears.

The **draw-down smearing** method uses a straight, rigid scraping edge fixed at a height equal to the desired thickness of the smear, from the top of the glass slide. After placing the standardized volume of fecal sample on the glass slide and covering it with presoaked cellophane tape, the scraping edge is placed on one end of the glass slide, and pulled towards the other end, causing the fecal sample to drag and form a smear along the length of the slide. The

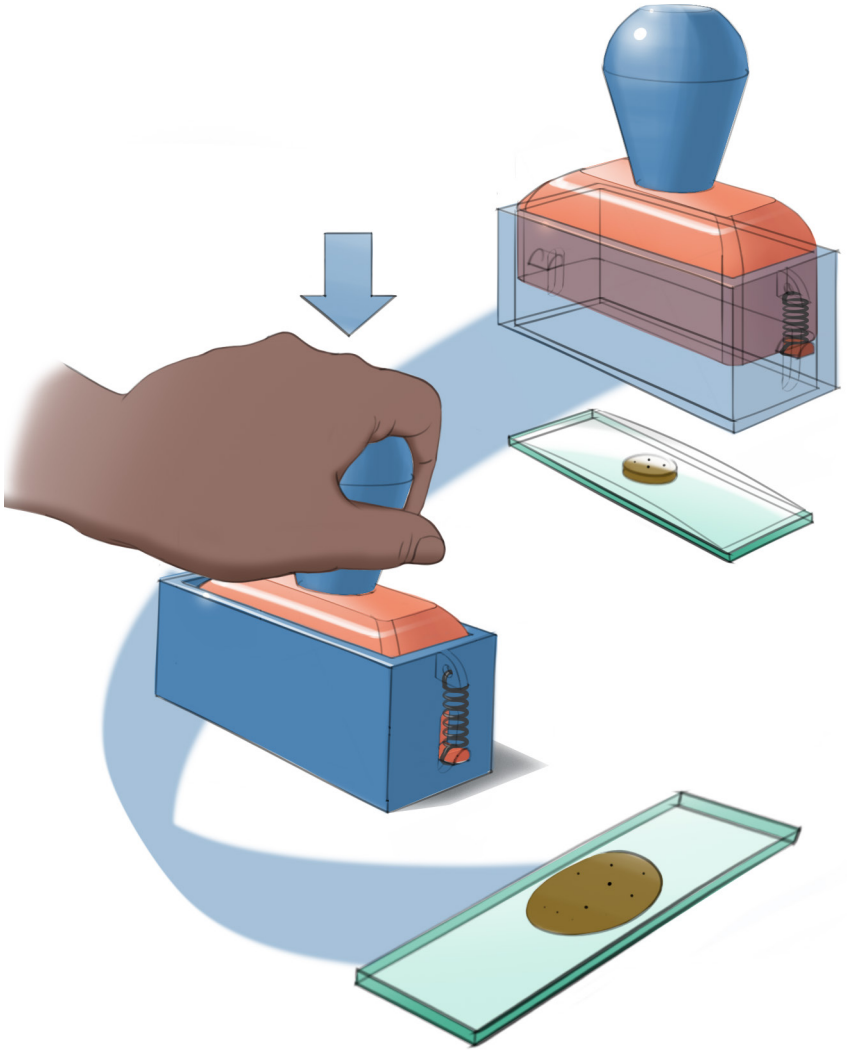


Figure 12: The stamp smear works like an old fashioned ink pad based stamp. After depositing the fecal sample and placing the cellophane on top, the user uses the stamp to exert uniform force on the feces to form a thin smear

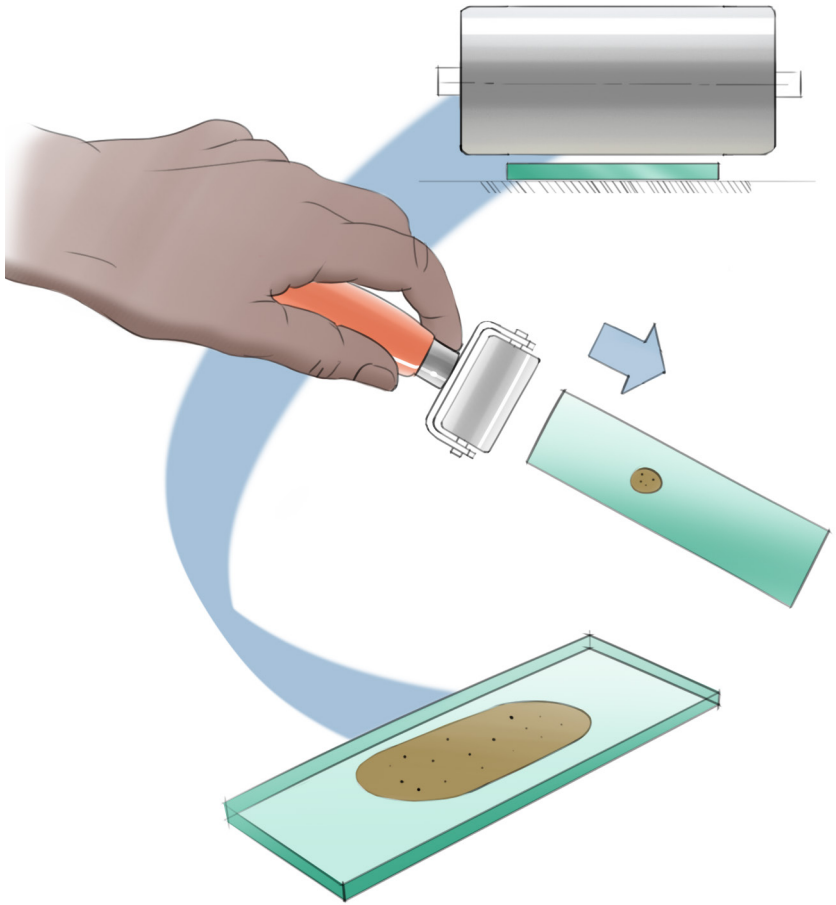


Figure 13: The roller smearer uses a roller to distributee the fecal sample in a uniform smear across the glass slide

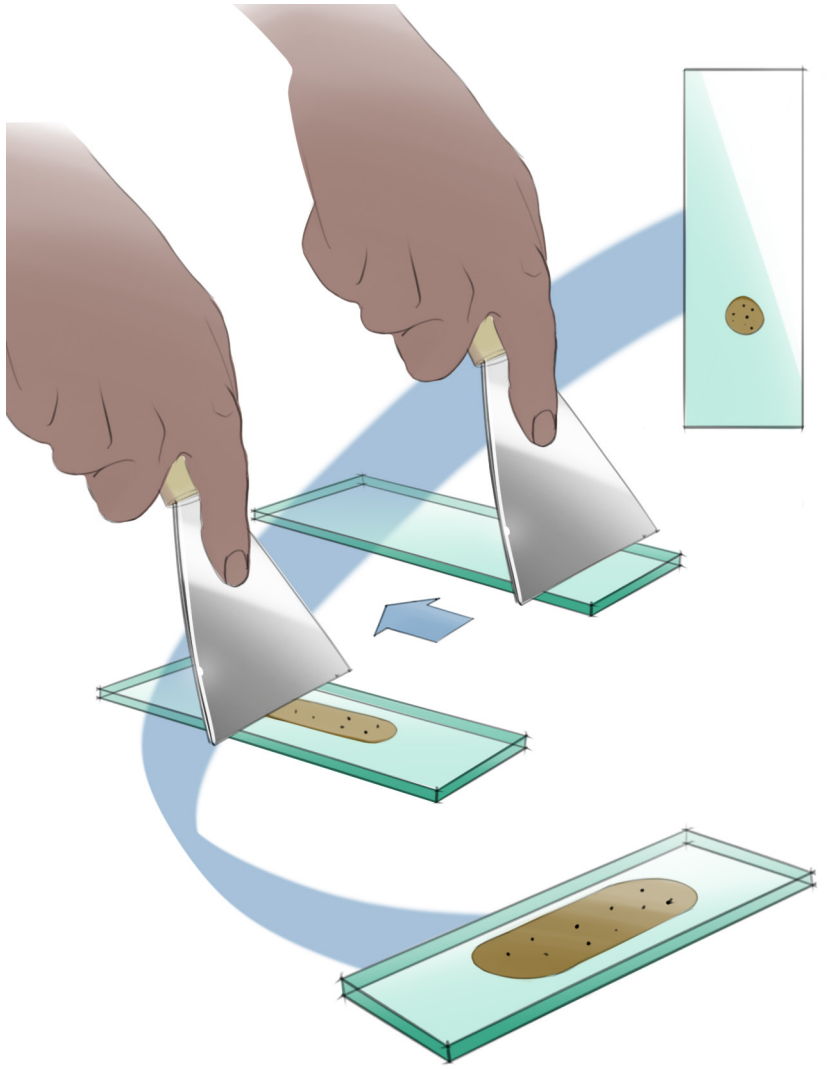


Figure 14: The draw down smearer uses a flat scraper to scrape the fecal sample across the glass slide

perceived advantages of this method are speed, ease of use, very low cost (commercially available paint scraper can be modified), and consistency of smears.

3.2 Preliminary Concept Evaluation

Each of the three concepts generated for filtration and smearing processes were compared with each other, and their perceived performance against each criteria chosen was measured. The Harris profile method was chosen for this purpose, as the method is specifically useful after multiple ideas have been generated, but specific details have not been laid out yet. The designer can 'estimate' if and by how much the idea generated may positively or negatively satisfy the design specifications (van Boeijen et al., 2014). The following were the criteria chosen for evaluating filtration concept ideas, based on design specifications generated:

1. Filtration time - one sample
2. Filtration time - ten samples
3. No. of steps to filtered feces
4. Ease of cleaning and drying
5. Fecal contact with gloves
6. Perceived unit cost
7. Perceived unit use cycles

Similarly, following criteria were chosen for evaluating smearing concept ideas:

1. Smearing time - one sample
2. Smearing time - ten samples
3. No. of steps to smeared sample
4. Ease of cleaning and drying
5. Fecal contact with gloves
6. Perceived unit cost
7. Perceived unit use cycles
8. Perceived smear consistency

Tables 3A and 3B show the filtration and smearing concept ideas' perceived performances respectively. It is clear from them that for the purposes of filtration and smearing, the template filter and fecal draw down concepts have the least drawbacks, and thus, are good candidates for further refinement, prototyping and testing.

Criteria	Template Filter				Piping Filter				Centrifuge Filter			
	-2	-1	+1	+2	-2	-1	+1	+2	-2	-1	+1	+2
Filtration Time - One Sample		■					■		■	■		
Filtration Time - Ten Samples		■					■				■	■
No. of Steps to Filtered Sample			■	■		■			■	■		
Ease of Cleaning & Drying			■	■			■		■	■		
Fecal Contact with Gloves			■		■	■					■	■
Perceived Unit Cost			■				■	■	■	■		
Perceived Unit Use Cycles			■			■					■	■

Table 3A: Relative comparison between the filtration methods

Criteria	Stamp Smear				Roller Smear				Draw-Down Smear			
	-2	-1	+1	+2	-2	-1	+1	+2	-2	-1	+1	+2
Smearing Time - One Sample			■	■			■	■			■	
Smearing Time - Ten Samples			■	■			■				■	
No. of Steps to Smeared Slide			■				■				■	
Ease of Cleaning & Drying			■			■				■		
Fecal Contact with Gloves			■	■			■	■			■	■
Perceived Unit Cost		■				■					■	
Perceived Unit Use Cycles			■	■			■				■	
Perceived Smear Consistency		■				■					■	■

Table 3B: Relative comparison between the smearing methods

3.3 Simulated Feces for Testing

Before proceeding further with concept refinement, prototyping and testing, it was deemed necessary that a substitute be employed in the place of actual feces (AF), as it makes testing, handling and cleaning of components a lot more convenient and hygienic. Ideally, the simulated feces (SF) must be able to mimic the physical (rheological) properties of actual feces as accurately as possible. Chemical properties maybe ignored, as long as none of the components needed to perform the kato katz technique reacted with the SF.

A literature search was done to see if there were any standardized formulations that would serve project needs. NASA developed five variants of SF using the same list of ingredients for studying waste management processes in space for short term and long term missions. The SF variants formulated were physically and chemically similar to actual feces, as they had to test transportation, compacting (water-removal) and incineration of feces to avoid bio-hazard issues *(Wignarajah et al., 2006)*.

Variant 4 was chosen for this project, solely for aesthetic purposes, as it was mentioned that it retained the dark color of actual feces. Of the different ingredients used, E. Coli, the bacteria was substituted with baker's yeast, as it readily available, does not pose a bio-hazard risk, and has been used in developing this variant, and the switch to E. Coli was only made because yeast would create pockets of air over time, reducing the density of SF and making storge difficult. The solution was to make smaller batches of SF only when necessary, and if stored for more than an hour, a good stir would remove all the air bubbles, restoring the original density. Another change made was inorganic components. It is 5% by weight and mainly consists of Calcium, Potassium and Phosphorus. As a substitute, daily multivitamin tablets were ground into a powder and mixed in with the rest of the ingredients. Table 3 below shows

Ingredient	Weight % (dry)	Weight % (wet)
Baker's Yeast	30	
Polyethyene Glycol (PEG)	10	
Psyllium Husk	5	
Peanut Oil	20	
Miso Paste	30	
Inorganics (powdered multivitamins)	5	
Dried, coarse ground vegetable matter (ground coriander seeds)	50 mg	
Tap Water		50

Table 4: Ingredient list for synthetic feces #4, as developed by NASA for their experiments with waste management for space missions (Wignarajah et al)

Figure 15: A picture of the list of ingredients of the preparation of synthetic feces. Inset: A sample of prepared synthetic feces

the ingredients, and their respective percentage dry weights. Water content in human feces varies between 50 - 90% of dry weight *(Nishimuta et al., 2006)*. For the purposes of this project, it was assumed to be 50%. All ingredients were measured and put into a standard urine and stool sample container (100 ml volume) and thoroughly mixed, except for yeast. This is because PEG and psyllium husk need time in order to soak up moisture and change into a gel like consistency. Once the mixture is rested for an hour, yeast is mixed in, rested again for ten minutes, and thoroughly mixed. SF is now ready for use.

The SF is used with a traditional kato katz kit to measure if the weight deposited on to glass slides is similar to 41.7 mg, the weight of actual feces deposited when the standard template is used. As

Figure 16: The weights of filtered fecal deposits consistently measure 40mg, showing that the sythetic feces created is reliable for testing

seen from figure 12, the weight of two empty glass slides were first measured to be 4.88 gms and 4.80 gms (noted on the left bottom of the slides), and after depositing SF sample using the standard template with a hole of diameter 6mm and height 1.5mm, the weights were found to be 4.92 gms and 4.84 gms respectively. This shows that SF deposited has a consistent weight of 40 mg. The difference in weight between AF and SF is 1.7 mg, which is within the acceptable 6.4% mean coefficient of variation for AF *(Katz et al., 1971)*.

3.4 Material Selection for Prototyping

From the design specifications, it may be derived that materials used to manufacture the new kato katz components must be easily available, economical, durable, chemically inert with common dish soap, and sustainable. Steam sterilizable materials are a bonus. Based on these requirements, a list of materials was made that satisfied most or all of the conditions. Materials for both filtration and smearing components are listed together as both have the same requirements:

1. Aluminium Sheet
2. Standard Microscope Glass Slide - 75 x 25 mm
3. Stainless Steel Mesh
4. Nylon Mesh
5. Polycarbonate Sheet

6. Stainless Steel Sheet
7. ABS Filament for 3D printing
8. PLA Filament for 3D printing

The following table compares each material in terms of the requirements stated above. As can be seen, silicone sheet with stainless steel mesh for template filter, and 3D printed PLA for the scraper were the obvious choices.

Material	Availability	Durability	Economy	Washable	Sustainable	Autoclavable
Aluminium Sheet	■	■	■	■	■	■
Glass Slide	■	■	■	■	■	■
Polycarbonate Sheet	■	■	■	■	■	■
Silicone Sheet	■	■	■	■	■	■
Stainless Steel Sheet	■	■		■	■	■
ABS - 3D Printing	■	■	■	■	■	
PLA - 3D Printing	■	■	■	■	■	
Stainless Steel Mesh	■	■	■	■	■	■
Nylon Mesh	■	■	■	■	■	■

Table 5: Perceived performances of different materials that could be used for manufacturing components of the improved kato katz method. The criteria were chosen based on design specifications generated

3.5 Final Concept - Design & Prototyping

Now that the materials have been selected, they may be used for manufacturing the prototypes. The paragraphs below explain how the filtration and fecal draw down designs were finalized.

The **template filter** will consist of two 75 x 25 x 0.8 mm rectangular silicone strips, that have a rectangular hole in the center, specifically measured and cut so that 100 mg of SF will be deposited on the glass slide when the hole is filled through the mesh. Between the two silicone strips, a 304 stainless steel mesh of a size between 60 - 105 is fixed using a high temperature silicone adhesive. This unit, when cured for 24 hours, will create a template filter that can be used multiple times by cleaning with dish soap as is standard practice in LMICs, but can also be autoclaved if necessary. A batch of fifty silicone template filters will last for a heavy day's work per lab technician, and may be washed and reused for hundreds of cycles, as both silicone and stainless steel are very resilient materials.

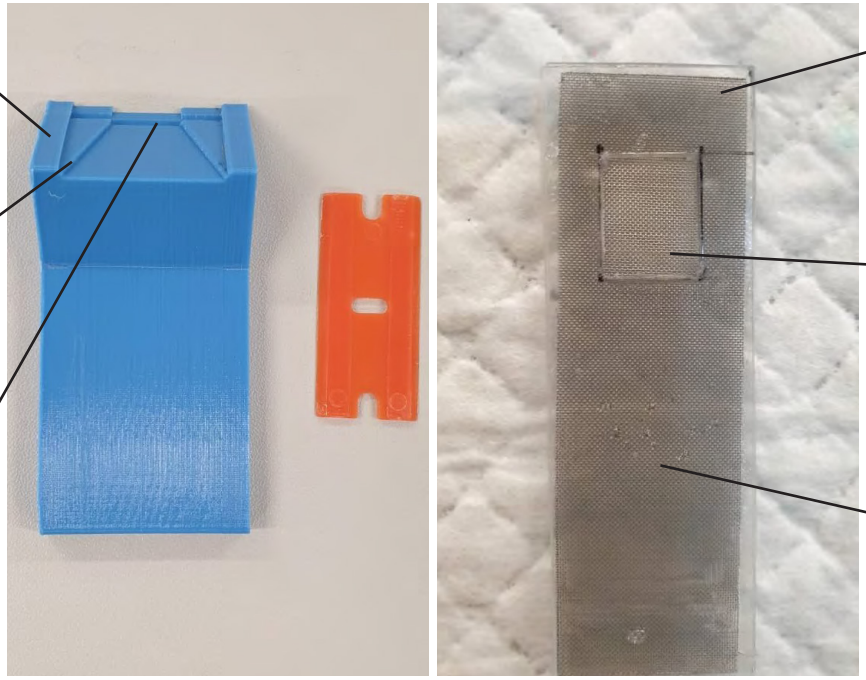
The **draw-down smearer** was more complicated to finalize in terms of manufacturing method selection. Initial idea was to use commercially available metal paint scrapers, and then mill their flat, scraping edges to have an inward rectangular notch, the dimensions of which would be the thickness and width of the desired fecal smear on the glass slide. 100 mg of SF would be deposited, a soaked cellophane would be placed on the sample, and would be drawn from one end to the other, using the milled paint scraper edge. Though this method would produce highly accurate fecal thin films, the cost, time and skill needed to produce multiple scrapers would be a disadvantage. Also, if the desired scraper is discontinued, the process of replacing it with a new one must be undertaken.

Keeping these difficulties in mind, it was decided that 3D printing a holder for a commercially available standard razor blade would be a feasible option, as the blades are mass produced, with good structural and dimensional stability. They can be bought commercially for very low prices, and a variety of material choices are also available. 3D printing with materials such as ABS or PLA will be very cheap and easy to manufacture at a local makerspace, and could be safely cleaned and reused for multiple cycles. PLA was chosen for this project as it was more sustainable compared to ABS. The holder would have a slot in to which the razor would be pushed in to form a tight fit. When aligned with the glass slide with the fecal sample and cellophane, the edge of the razor blade will be at a height equal to the desired thickness of the smear. The sample is then drawn down from one end of the slide to the other. The software 'SolidWorks' was used to model the holder, and was printed using a PRUSA i3 MK3 3D printer. ABS razor blades were bought online, which can cost a s little as 1¢ a piece retail. An experimental prototype was made with five slots, each of which holds the razor edge at a different height from the glass surface. The ideal thickness of the smear was calculated by measuring the thickness of a glass slide, preparing a kato katz sample with the traditional method using the same slide, measuring the total thickness of the glass slide + smear +cellophane, and finally subtracting the thickness of glass slide and cellophane from the total. Once the best thickness was found, a final model of the scraper was printed, this time with just one slot. The final concepts are illustrated in figure 13

Draw down scraper 3D printed using PLA material. It aligns with the glass slide at an angle of 45 degrees, and the guides on both sides prevent it from sliding toward the eges while drawing down the smear

A converging set of guides make sure that the smear does not exceed 13 mm in widht during draw down. This is not necessary for manual microscopy, but is important for testing with Mr. Oyibo's automated microscope

A slot that houses the commercially available ABS razor blade. It has tight toerances, and fits the blade snugly so it does not slide out. When assembled, the edge of th blade sits above the glass slide



Polycarbonate sheets of thickness 0.8 mm were used to create the template filter. Rectangular holes were cut, and a 80 mesh was sandwiched in between the layers. The template would deposit 100 mg of feces

Both polycarbonate and stainless steel are resistant to multiple cycles of washes using water and regular dish soap. The rigid nature of polycarbonate ensures that excess feces is not pushed through once it is full

The adhesive used for joining the layers is a high temperature silicone gel. If necessary, lab technicians may also sterilize the component using a steam autoclave. Polycarbonate may not last many cycles in this case

Figure 17: The final prototypes for the draw down smearer and template filter

3.6 On-Field Testing & Feedback

Mr. Prosper Oyibo, one of the stakeholders who was interviewed previously for the role of a lab technician, had a visit to Nigeria scheduled, where he was to test his Schistoscope. He generously offered to test the prototype on field, and proposed to hand them to two professional lab technicians who were working on a study, so they could test the prototypes and provide real-world feedback. Before his departure, the silicone template filter and the draw-down smearer were tested for the accuracy and reliability of filtered SF deposited on to the glass slide. Five smears were prepared, and their weights were checked after each stage- empty slides, after using template filter, and after draw down smearing. These are shown in figures 14. Two problems were immediately apparent:

1. SF was sticking to the underside of the silicone template filter when it was lifted after filtration, so for some samples the weight was too low, and for some it was too high because the user was trying to push through a larger quantity of SF to compensate for the sticking, and since the template is flexible, it curved upwards and too much SF was forced through the mesh

2. Some of the smears spilled out during the draw-down process, indicating that 100 mg may be too much for one slide. But because both samples that had spillage measured more than a 100 mg after filteration, it was assumed that the additional SF deposited was the culprit

Figure 18: Steps showing the filtration and smearing processes using the prototype. As can be seen, fecal deposits were incosistent due to flexibility of silicone

The template filters were redone using rigid polycarbonate strips with stainless steel mesh, keeping all the measurements the same. It was hoped that this would stop excess amount of sample to be forced through. The template filters, draw-down smearer and ABS razor blades were taken to Nigeria, and two professional lab technicians tested the new kato katz kit with actual fecal samples. They were each given a set of questions and asked for their type and level of emotions experienced after using these components. The questionnaire was designed based on the 'Premo' method of measuring emotions *(Laurans et al., 2017)*. Due to unforeseen troubles with printing out the questionnaire, Mr. Oyibo had to verbally ask the lab technicians the questions, while showing them the emotions on his phone, and noting down their answers. Hand written notes of the interviews, as well as the questionnaire can be found in appendix B. The feedback received from the lab technicians was as follows:

1. Filtering feces through the template was difficult as the area is too small, and it takes about 30 seconds of scraping the feces through the mesh. It was not a pleasant experience
2. Cleaning the template is difficult as particles of feces remain in the corners of the rectangular profile
3. The draw-down smearer is not intuitive. Could not figure out how it worked
4. 100 mg is too much for one slide. Spills over the slide and sticks to scraper
5. Scraping might break the eggs (opinion only)
6. Self aligning to slide is good
7. Does not create a transparent enough film, and is also not of consistent thickness
8. Pressing, instead of draw-down action maybe better as it spreads the sample evenly

Based on the feedback received, the following ideas were generated as possible solutions:

1. For the template filter, remove top layer of polycarbonate strip. This will automatically give the user more area to scrape the feces through the mesh, and the user is no longer restricted by the boundaries of the rectangular hole
2. Change the shape of the hole in the template from a rectangle to a circle. This way, there are no unreachable corners to clean
3. Decrease the capacity of the template hole to 80 mg instead of 100 mg. A duplicate kato katz sample will still be able to measure 6.5 EPG, thus satisfying the requirement of diagnosing very light infections
4. Decrease the distance between the razr edge and the glass slide to create more transparent films
5. Create a reliable sliding mechanism to maintain constant gap between razor and slide

4. DESIGN UPGRADES & FUTURE

It was decided that it would not make sense to try and find the right razor blade edge to cellophane distance (or fecal smear thickness, FST), as while SF did produce desirable film thickness during trials, actual feces does not seem to replicate the same transparency with the designed draw down smearer. Trials to find the optimal FST may only be made after finding the mean film thickness of prepared samples using the traditional kato katz method, along with actual feces.

4.1 Upgrading Filter-Template

Based on the feedback received from the lab technicians, a newer version of the template filter was created. As seen in figure 15, the new template has a recess along its length to hold a standard microscopy glass slide. Two flaps on either side enable the user to hold the slide down firmly in place with their thumb and middle fingers. On the other side of the template, a circular hole with a stainless steel mesh on top is provided. This gives the user ample space to move around the feces and push it through the mesh. Lack of corners in the hole make it easier to clean after use. Also, the amount of filtered feces that this template can hold has been reduced to 80 mg, as 100 mg samples have shown instances of spillage. In this case, each slide prepared is capable of a sensivity of 12.5 EPG, and a duplicate sample will double the sensitivity to 6.25 EPG, making it possible to achieve the target of measuring very low intensity infections (≤ 10 EPG).

Figure 19: Improved template filter manufactured based on feedback from lab technicians

Due to its relatively complicated geometry, 3D printing was deemed to be the best manufacturing method for the improved template filter. The same material chosen for manufacturing the draw down smearer, PLA, was used for filter template as well. This template ensures that filtered feces is deposited at the same location on every glass slide, making uniform smears, and avoids spillage.

4.2 Discussion & Conclusions

The traditional kato katz process has been divided into the filtration and smearing phases for easier study. Three concepts have been generated, and evaluated relatively based on design specifications. The template filter and the draw down smearer have been deemed to be the most suitable for making the kato katz process affordable, cost effective and eas to use. The first prototypes were manufactured. The template filter was made of silicone and stainless steel mesh, while the draw down smearer was 3D printed using PLA filament and a commercially available ABS razor blade. Preliminary tests showed that silicone template was too flexible to allow for consistent weight deposit of fecal sample, hence it was changed to a more rigid polycarbonate material.

On field testing in Nigeria showed that 100 mg was more than what a standard glass slide can handle. And the lab technicians found it harder to filter the feces using the template, as well as cleaning it. Hence a new template was created based on their feedback, that would allow for this. The suggestions, as well as the 'hopeful' reaction of the lab technicians show that there is potential in taking this project further. Greater speed and reliability, sustainable materials and easy to use methods will certainly help reduce overall sample preparation time, personnel and equipment costs, and make the experience of preparing samples less uncomfortable for the lab technicians.

There are, however, certain limitations to this method. Due to the limitations in size of the glass slides, further increasing sensitivity, even by preparing multiple slides from the same sample, becomes exponentially harder. For example, for the current sample weight of 80 mg, a sensitivity of 12.5 EPG can be obtained. A duplicate sample will give a sensitivity of 6.25 PG, and a triplicate sample, 4.11 EPG. In order to reach 1 EPG, 12 duplicate samples must be made. Therefore, diagnosing extremely low infections (≤5 EPG) will not be practical using this method. Also, due to the larger dimensions of the fecal smears created, manual microscopy will be relatively more tediuos, and the technician may be more prone to making mistakes due to fatigue. The process may be much more suitable for automated microscopy.

In conclusion, it may be said that the research question has been answered in a positive manner, meaning that sensitivity, reliability and sustainability of the kato katz method can be improved, with a few reservations. When the ideal film thickness of a prepared slide with actual feces is measured, further tests and trials will be possible, and a standard method that has been invaluable and unchanged for over 50 years may be used for early post treatment surveillance as well.

4.3 Future Scope

There are many aspects of the project that may be taken further to validate the use of this improved kato katz method on field. Following are the actions that may be undertaken in the future:

1. The mean thickness of a fecal smear formed using the traditional kato katz method, using actual feces must be found, as

SF does not seem to replicate the same thickness and transparency. Once this is achieved, the optimal FST can be found out through trial and error

2. Theoretical sensitivity of the improved kato katz method can be validated using a known number of S. Mansoni eggs in SF

3. The enhanced design of the filter template based on user feedback can be validated by on field tests

4. User testing may also be used to verify zero dependence on skill

5. No. of use cycles maybe estimated. Once achieved, cost comparison may be made with a standard commercial kato katz kit of 500 units

6. Further research on materials for 3D printing, as well as shape optimization for the draw down smearer, as well as the improved template filter may be done to reduce costs and extend durability

7. Further expansion of the kato katz method - interms of increasing sensitivity through use of larger quantities of fecal samples, larger glass slides, along with automated microscopy methods must be explored

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