ENHANCING ROBUSTNESS IN MICROSCOPIC SCREENING OF DENGUE VIRUS WITH A GLASS STRIP BLOOD SMEAR DESIGN

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Abstract

The development of staining device for lab microscope plays a crucial role in enhancing biological sample visualization and analysis. By improving contrast and visibility, this device aids in obtaining high-quality and clear images of the sample's structures and components. Traditionally, the determination of platelet count involves a manual process that includes collecting a blood sample, staining it, and observing it under a lab microscope with the assistance of a skilled medical lab technician. To address the limitations of this manual approach, we propose a glass strip blood smear design as an alternative method, focusing specifically on dengue virus detection. In this method, the blood sample is obtained through a simple pricking procedure and collected into a specially designed glass bio strip, similar to a glucose test strip. Within the strip, automated staining occurs, preparing the sample for observation. Unlike plastic strips used in sugar tests, glass bio-strip provides suitable surface for microscopic examination, whether using a lab microscope or a mobile phone microscope. The significant advantage of this alternative method is that it enables individuals without a medical background to perform platelet count analysis. By pricking the patient and inserting the blood sample into the glass strip, non-medical personnel can observe the stained sample using a mobile phone equipped with a microscope feature. This method facilitates the identification of platelet count deficiencies and holds potential for counting other components such as red and white blood cells, aiding in the diagnosis of various disorders. The improved visualization and clarity of the stained blood samples enable more accurate identification of platelet count deficiencies, which are often associated with dengue virus infection. This method allows for quicker and more convenient screening, as non-medical personnel can easily perform the analysis using a mobile phone equipped with a microscope feature.

Keywords: dengue virus, microscopic screening, glass bio-strip, blood smears, mobile phone microscope

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1. Introduction

Blood smears, also known as blood films, are microscopic slides prepared for microscopic examination by staining a slide with a thin layer of blood [1]. Blood smears are used to examine and visualize various blood components, such as platelets, red blood cells, and white blood cells. The writer examining the appearance, size, shape, and distribution of these blood cells, healthcare professionals can gather important information about a patient's overall health, diagnose various blood disorders or infections, and monitor the effectiveness of certain treatments [2]. Blood smears are commonly used in hematology laboratories and are an essential component of a complete blood count analysis [3]. It can be used as a diagnostic tool for screening and detecting the presence of the dengue virus in a patient's blood. In the case of dengue fever, blood smears are primarily used to visualize and identify specific changes in the morphology and count of blood cells that may indicate the presence of the virus [4][5]. During the acute phase of dengue infection, certain characteristic changes may be observed in the blood smear. These changes can include a decrease in platelet count, the presence of atypical lymphocytes, and the appearance of fragmented red blood cells [6]. Additionally, the observation of specific cellular changes, such as the presence of "dengue bodies" or "virological rosettes," which are clusters of red blood cells surrounding infected monocytes, can provide further evidence of dengue infection [7]. However, it's important to note that blood smear analysis alone is not sufficient for confirming a dengue infection. Laboratory tests such as enzyme-linked immunosorbent assay and polymerase chain reaction are typically used for definitive diagnosis [8][9]. Blood smears are often used as a complementary tool to support the overall diagnostic process and provide additional insights into the patient's condition. It is crucial to consult with a healthcare professional or a laboratory specialist for the proper interpretation and analysis of blood smears for dengue virus screening.

There are several types of blood smears that can be used for dengue virus screening. Thin blood smear [10] is the most common type of blood smear used for dengue virus screening. A thin blood smear involves spreading a small drop of blood thinly and evenly on a glass slide. It is then stained with a specific stain, such as Giemsa stain, to enhance the visibility of the blood cells and any abnormalities that may be present. Thick blood smear [11] may also be prepared for dengue virus screening. A thick blood smear involves applying a larger drop of blood onto a glass slide, which is then spread in a concentrated manner to create a thicker layer. This technique allows for a higher concentration of blood cells, making it easier to detect the presence of dengue virus particles or other abnormalities. The buffy coat smear [12] is a thin, whitish layer that forms between the plasma and red blood cells after centrifugation of a blood sample. It contains a higher concentration of white blood cells, which are the primary target for dengue virus detection. By preparing a smear from the buffy coat layer, the concentration of white blood cells is increased, improving the chances of detecting the virus [13][14]. Each type of blood smear has its own advantages and may be used in specific situations or in combination to increase the sensitivity and accuracy of dengue virus screening. The choice of smear type depends on the laboratory protocols, availability of resources, and the specific requirements of the diagnostic facility or healthcare provider conducting the screening [15].

When examining blood smears for various purposes, including dengue virus screening, it is crucial to obtain clear and well-defined images of the blood cells [16]. However, several factors can contribute to visualization and clarity problems in blood smears. One factor that can affect visualization is the technique used during smear preparation. If the blood is not spread evenly and smoothly on the slide at the appropriate angle and speed, it can result in uneven distribution of cells and poor visualization [17]. The thickness and uniformity of the smear also play a role in achieving clear images. Another factor is the staining method employed. Inadequate staining or improper timing during the staining process can lead to suboptimal visualization. Insufficient staining may result in faint or poorly visualized cells, while over-staining can cause cells to appear too dark and mask important details. The drying of the blood smear is also critical. Inadequate drying time can cause cells to stick together or become distorted, hindering clear visualization [18]. On the other hand, over-drying can lead to shrinkage of cells and the appearance of artifacts, affecting the accuracy of interpretation. The quality of the microscope slide used for preparing the smear is another consideration. Dust particles or contaminants on the slide surface can interfere with clear observation of the cells, compromising visualization. Additionally, microscope settings need to be optimized for clear visualization [19]. Proper lighting, focus, and magnification are

essential for obtaining detailed and accurate images of the blood cells. Adjustments to these parameters may be necessary to achieve the best possible visualization. To address these visualization and clarity problems, it is important to follow standardized protocols for blood smear preparation [20]. This includes ensuring consistent and controlled spreading of the blood, using appropriate staining techniques, allowing sufficient drying time, and using clean and high-quality microscope slides. Regular maintenance and calibration of microscopes are also essential for optimal visualization. By addressing these factors and implementing quality control measures, healthcare professionals and laboratory technicians can improve the clarity and accuracy of blood smear images, facilitating the detection and diagnosis of various conditions, including dengue virus infection.

Our contributions. In order to improve the visualization quality of blood smears and enhance the detection of dengue virus, we propose the design of a glass strip blood smear as an alternative method. Our focus is specifically on developing a rapid diagnostic test (RDT) that offers better visualization and clarity of stained blood samples, enabling more accurate detection of the dengue virus.

- The new design of the glass strip blood smear aims to provide a convenient and efficient screening process. It allows for quicker analysis, and even non-medical personnel can perform the test using mobile phone equipped with microscope feature. This innovative approach not only enhances visualization but also offers greater accessibility and ease of use in dengue virus detection.
- To validate the performance of the proposed glass strip blood smear, we conducted comprehensive evaluations using various quality measures. We compared the results obtained from our method with those from existing RDTs used for dengue virus detection.

By conducting comparative analysis, we aim to demonstrate the effectiveness and superiority of our glass strip blood smear in terms of visualization quality, accuracy of dengue virus detection, and overall performance when compared to existing RDTs. The validation process enables us to assess the potential of our proposed method for widespread implementation and use in the field of dengue virus screening. The rest of this paper is organized as follows. Section 2 provides a comprehensive review of the existing literature on blood smears for dengue virus detection. In Section 3, we present the background study of blood smear techniques. Section 4 outlines the methodology of our proposed model. We describe the step-by-step process and techniques employed in designing the glass strip blood smear for enhanced visualization and clarity in dengue virus screening. In Section 5, we present the validation process and the results obtained from comparing our proposed glass strip blood smear with existing RDTs used for dengue virus detection. Finally, in Section 6, we provide a concise conclusion summarizing the key findings, contributions, and implications of our study.

2. Recent works

In this section, we present a comprehensive review of recent works and studies conducted in the field of blood smears for dengue virus detection. The review focuses on the advancements, methodologies, and key findings of these studies, shedding light on the current state of research. By exploring the recent literature, we aim to provide a solid foundation for our proposed model and identify gaps that our study seeks to address.

Mello et al. [21] proposed the use Polyester-derived acetate slides for standardizing the preparation and staining of clear acetate slides containing both thin and thick blood smears. When compared to conventional slide preparation, acetate sheets provided a platform that was both more consistent and reliable for the preparation of blood smears. Smears made from both thick and thin acetate sheets were stained with the Giemsa method by the researchers. High-quality images of blood cells and parasites were found when prepared smears were examined microscopically. Stains were able to stand out clearly on the transparent acetate background, which reduced the amount of paint residue present. The researchers were able to identify significant morphological characteristics of Plasmodium, neutrophils, and platelets thanks to this clarity. Vu et al. [22] addressed the significance of an external quality assessment (EQA) scheme in evaluating laboratory performance, particularly in the context of blood smear preparation for morphological analysis. The blood smear preparation procedure must be robust and consistent, according to ISO 15189. There are no published guidelines for the preparation of a series of blood smears with high homogeneity and long-term stability, especially for EQA purposes, despite the fact that blood smear preparation is a common practice in

medical laboratories. In order to fill this void, researchers have examined a number of factors that influence the preparation of blood smears. The appropriate reagents, parameters for each step, and the amount of time the collected blood samples were stored prior to fixation were some of these factors of the preparation process, such as fixation, staining, and timing. Each experiment conducted by the researchers was assessed based on the characteristics of homogeneity and stability. The goal was to establish a standardized procedure that consistently produces blood smears with uniform morphological features and exhibits long-lasting stability.

Chibuta et al. [23] proposed an innovative and cost-effective approach in low-resource settings for the rapid detection of malaria and the reduction of the need for manual microscopy. They developed a real-time, low-cost video processing pipeline based on a modified YOLOv3 detection algorithm to accomplish this. The analysts assessed the exhibition of their answer utilizing two distinct informational indexes. The microscope camera was used to collect the first set of data, and the cell phone camera was used to collect the second set. Their model, in particular, had an impressive accuracy of 99.07% on the microscope camera dataset and 97.46% on the mobile phone camera dataset. Beckman et al. [24] conducted a study with the objective of identifying specific clinical scenarios where the review of blood smears could provide valuable insights beyond the results obtained from automated laboratory testing. The researchers examined the practices of blood smear evaluations at three distinct facilities and examined the indications and interpretations of physician-initiated smears. The percentage of smears that might have additional clinical value was their objective. During the study period, 515 consecutive physician-ordered smears were performed. About 23% of these smears produced interpretations that may have additional clinical value. This indicates that in a significant proportion of cases, the review of blood smears provided valuable insights beyond what could be obtained through automated laboratory testing alone.

Chen et al. [25] conducted a retrospective study aimed at reducing proportion of inappropriate Pap smears performed using two slides and saline smears. Patients at a medical center in eastern Taiwan were the focus of the study, which looked at 5,000-6,000 Pap smears performed annually. From medical center records, the researchers gathered information on the number and percentage of inappropriate Pap smears. The standard method of performing a Pap smear underwent two modifications in their approach. Firstly, they replaced the use of jelly with normal saline for lubricating the speculum. Secondly, instead of using a single glass slide for the smear, they used two glass slides. The findings indicate that the implementation of the modified technique with saline lubrication and two glass slides resulted in notable reduction in the proportion of inadequate Pap smears over the study period. The decrease from 4.71% to 0.33% suggests that this approach has the potential to improve the quality and accuracy of Pap smears, thereby minimizing the number of inadequate results. Nuel et al. [26] proposed an innovative approach for counting and recording data in microscopy using an electronic expansion of a mechanical counter. A time counter is used in his approach, which includes the counting process itself by measuring the time between two count button presses. Analyze the data with a hidden semi-Markov model to find the locations of the HPFs and the number of leukocytes and parasites per HPF. The microscopist's pauses and hesitations during the reading process are incorporated into this model, which takes into account the known distribution of leukocytes in a single HPF. Expert annotations are used to calibrate hyperparameters and parameter estimates made with the expectation maximization algorithm.

Dogbevi et al. [27] have introduced a novel approach for creating thin whole blood smears using pumpless microfluidics, which addresses the limitations associated with manually prepared smears. Their method involves the utilization of microfluidic design with channel columns strategically placed and an amphiphilic silicone material. The amphiphilic polysilane additive was mixed at various concentrations to enhance the silicone's functionality. The microfluidic device's inlet, outlet, and two internal components all had column sections integrated into them. Preventing channel collapse, enhancing flow dynamics, and facilitating uniform cell distribution are just a few of the many functions performed by these pillars. A blood sample was introduced into the channel at the channel's entrance, and the flow time and stop time were recorded as part of the experiment. A thin, uniform blood smear was successfully produced using microfluidic chips loaded with 0.3 milliliters of blood and prepared with 5% by weight of a surface-modifying additive. Jaso and

others 28] simplified the reporting procedure for peripheral blood smears by introducing an online synoptic reporting system. This system utilizes a comprehensive knowledge base consisting of 150 report templates that encompass a wide range of pathologic findings. Accessible through the Internet, this program allows users to navigate the system and select relevant attributes from predefined drop-down lists. By inputting specific findings of the case at hand, users can retrieve a shortlist of report templates that match those particular findings. The implementation of this synoptic reporting system has demonstrated several benefits. It has proven effective in reducing typographic errors, resulting in improved accuracy and enhanced quality of the generated reports. Additionally, the system has contributed to reduction in turnaround time, facilitating faster report generation and subsequent patient management.

Nowak et al. [29] have introduced a portable mechanical device called Inkwell, designed for creating high-quality thin blood smears in field settings. Inkwell offers several advantages, including low cost, independence from electricity, and minimal training requirement. The device operates based on the passive dissipative dynamics of a spiral spring coupled with an air dashpot featuring a tunable valve. This innovative mechanism allows for the production of constant velocity smears at a predetermined angle. Inkwell enables the generation of high-quality blood smears with precise control over cell density. Inkwell has demonstrated its capability to produce thin blood smears with more than 12 million individually distinguishable red blood cells on a single slide. The device's design utilizes a 17 cents plastic syringe and a spring, which can be manufactured with precision and printed using a standard 3D printer. This approach results in an overall unit cost of just a few dollars when produced in large quantities. McDermott et al. [30] have introduced two open design devices, namely a mechanical device and an electronic device, that can be fabricated using 3D printers and assembled using readily available tools. This open hardware approach offers research laboratories the flexibility to construct their own devices, leading to potential advancements in various aspects of laboratory research. By embracing open-source 3D-printed devices, laboratories can overcome barriers associated with cost and availability, thus promoting scientific progress and research collaborations. The democratization of laboratory equipment through open hardware approaches has the potential to revolutionize research practices, particularly in resource-constrained settings.

3. Background study

In recent years, various rapid diagnostic test kits have been developed and utilized for the detection of Dengue virus. These kits employ different approaches and target specific components of the virus or the immune response.

1. IgG & IgM Combo Devices: These kits detect both Immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies produced by the immune system in response to Dengue virus infection. The presence of these antibodies indicates a current or past infection.
2. NS1 Combo Device Antigen and IgG/I: These kits combine the detection of the NS1 antigen, a protein produced by the Dengue virus, with the detection of IgG antibodies. This combination allows for the identification of both early-stage infections (through NS1 antigen detection) and past infections (through IgG antibody detection).
3. Immuno quick Dengue Fever IgG and IgM: Similar to the IgG & IgM combo devices, these kits specifically target the detection of Dengue-specific IgG and IgM antibodies. They provide a rapid and convenient method for diagnosing Dengue infections.
4. NS1 Antigen Strip: This type of kit focuses solely on the detection of the NS1 antigen, which is an early indicator of Dengue virus infection. The presence of NS1 antigen in the bloodstream suggests an active infection.
5. Panbio Dengue Early Rapid Kit: This kit is designed to provide early detection of Dengue virus infections by targeting the NS1 antigen. It offers a rapid and reliable method for identifying acute Dengue cases.
6. Panbio Dengue Duo Cassette: Similar to the Panbio Dengue Early Rapid Kit, this kit combines the detection of NS1 antigen with the detection of Dengue-specific IgG and IgM antibodies. It allows for comprehensive testing and differentiation between early and late-stage infections.

These rapid diagnostic test kits offer a convenient and efficient means of detecting Dengue virus infections in a timely manner. They provide quick results, often within minutes, and are useful in both clinical settings and field-based screening programs. These kits play a crucial role in the early diagnosis and management of Dengue

infections, enabling prompt medical interventions and appropriate public health measures.

3.1 Blood smears Vs Kits

IgG & IgM Combo Devices typically use a type of blood smear known as a thin blood smear for dengue virus detection. In this technique, a small drop of blood is placed on a glass slide, and another slide is used to spread the blood into a thin, even layer. The smear is then allowed to air dry and can be further processed for testing. The thin blood smear provides a suitable substrate for the detection of IgG and IgM antibodies specific to the dengue virus. These antibodies can bind to viral antigens present in the blood sample, and their presence can be visualized using various detection methods. The use of thin blood smears allows for better visualization and examination of individual blood cells and the detection of specific antibodies associated with dengue infection. The results obtained from the IgG & IgM Combo Devices can help in the diagnosis and management of dengue virus infections by providing information about the patient's immune response to the virus.

The NS1 Combo Device Antigen and IgG/I tests typically utilize a type of blood smear called a whole blood smear for dengue virus detection. In a whole blood smear, a small amount of blood is placed directly on a glass slide without any prior processing or preparation steps. The blood is then spread evenly across the slide using a spreading tool or another slide. This results in a smear that contains both the cellular components of the blood as well as any viral antigens present in the blood sample. The whole blood smear is then subjected to the testing process, which may involve the application of specific antibodies or reagents to detect the NS1 antigen and/or IgG antibodies associated with dengue virus infection. The presence or absence of these components is then analyzed and interpreted to determine the dengue virus status of the individual being tested. For the Immunoquick Dengue Fever IgG and IgM test, the type of blood smear typically used is a whole blood smear. For the NS1 antigen strip test, the type of blood smear typically used is a whole blood smear. Similar to other dengue diagnostic tests, a small amount of blood is collected and placed directly on a glass slide without any prior processing or preparation. The blood is then spread evenly across the slide to create a thin, uniform layer using a spreading tool or another slide.

The Panbio Dengue Early Rapid Kit typically uses a type of blood smear known as a serum or plasma smear. To prepare the serum or plasma smear, a small amount of the patient's blood is taken and left to clot. The blood sample is centrifuged after it has clotted to separate the liquid portion, which contains serum or plasma, from the blood's cellular components. After that, a glass slide with the resulting serum or plasma is carefully transferred. The serum or plasma smear contains the fluid component of the blood, devoid of red blood cells, white blood cells, and platelets. This type of smear is specifically used for detecting the presence of dengue antigens or antibodies in the serum or plasma sample using the Panbio Dengue Early Rapid Kit. The Panbio Dengue Duo Cassette typically uses a type of blood smear known as a whole blood smear. To prepare the whole blood smear, a small drop of blood is collected from the patient using a lancet or a similar device. The blood drop is placed directly onto the sample well or application area of the Dengue Duo Cassette. The device is designed to handle whole blood samples without the need for additional processing steps. The whole blood smear contains all components of blood, including red blood cells, white blood cells, platelets, and plasma. The Panbio Dengue Duo Cassette is specifically designed to detect dengue antigens or antibodies present in the whole blood sample, providing rapid diagnostic results for dengue virus infection.

3.2 Blood smears-design problems

Blood smears can sometimes encounter issues related to visualization and clarity, leading to potential failures in obtaining accurate and reliable results.

1. Inadequate Sample Preparation: Improper preparation of the blood smear, such as uneven distribution of the blood sample or improper spreading technique, can result in unevenly distributed cells and inconsistent thickness throughout the smear. This can lead to difficulties in visualizing and identifying specific cell types or abnormalities.
2. Cell Distortion: During the process of making a blood smear, cells may become distorted or damaged, affecting their morphology and making it challenging to interpret the slide accurately. Overly forceful spreading or excessive pressure during the smear preparation can cause cell deformation.
3. Staining Artifacts: The staining process is crucial for enhancing the contrast and visualization of cells on a blood smear. However, issues such as over- or under-

staining, improper staining techniques, or the presence of staining artifacts can impact the clarity of the smear. These artifacts may obscure cell details or create false structures, leading to difficulties in interpretation.

4. Excessive Cell Density: If the blood smear contains an excessively high concentration of cells, it can lead to overlapping and crowding of cells, making it difficult to differentiate individual cells and assess their characteristics accurately.
5. Poor Microscopy Techniques: Inadequate microscopy techniques, including improper focusing, incorrect lighting conditions, or inexperienced observers, can contribute to poor visualization and clarity of blood smears. These factors can affect the ability to identify and analyze cells accurately.

Addressing these issues requires attention to proper sample preparation techniques, careful staining procedures, quality microscopy equipment, and skilled personnel trained in blood smear examination. Regular quality control measures, including slide assessments and proficiency testing, can also help identify and address visualization and clarity problems in blood smears.

3.3 Why glass strip blood smears

Glass strip blood smears are an alternative method that has been proposed to overcome some of the problems associated with conventional blood smears.

1. Enhanced visualization: Glass strip blood smears offers improved visualization compared to conventional smears. The use of transparent glass as a substrate provides a clear background, allowing for better contrast and visibility of cells and other components on the slide. This enhanced visualization can aid in the accurate identification and analysis of cellular morphology and abnormalities.
2. Clarity and minimal staining artifacts: Glass strip smears have the advantage of producing minimal staining artifacts. The smooth and flat surface of the glass strip facilitates even spreading of the blood sample, leading to consistent cell distribution and thickness across the slide. This reduces the occurrence of smearing-related distortions or artifacts, resulting in clearer and more reliable images.
3. Standardization and reproducibility: Glass strip blood smears offer better standardization and reproducibility compared to conventional smears. The use of pre-made glass strips ensures consistent slide preparation, including the angle and pressure applied during the smear process. This standardization helps reduce variability between slides and enhances the reliability of the results.
4. Convenience and ease of use: Glass strip blood smears are designed to be user-friendly and convenient. The pre-made glass strips eliminate the need for manual preparation of slides, saving time and effort in the laboratory. Additionally, glass strip smears can be easily handled, transported, and stored, making them practical for various laboratory settings and even field applications.
5. Long-term stability: The smears have demonstrated long-term stability, maintaining image quality and preventing fungal colonization even after extended storage periods. This ensures that the smears can be stored and archived for future reference or quality control purposes without degradation in image clarity or integrity.

Overall, glass strip blood smears offer advantages in terms of enhanced visualization, reduced staining artifacts, standardization, convenience, and long-term stability. By addressing the challenges associated with conventional smears, glass strip smears contribute to more reliable and accurate microscopic examination of blood samples.

4. Proposed methodology

4.1 Process of conventional blood smears

Blood smears play a crucial role in working with blood and studying blood diseases, particularly in regions with limited resources where manual preparation of smears is common practice. The World Health Organization's basic microscopy learner's guide provides a recommended method for creating manual thin blood smears. Let's explore the steps involved:

- Blood droplet placement: A small drop of blood is placed onto a glass slide, typically obtained from a finger prick or venous blood sample.
- Spreading the blood: Another glass slide, known as a spreader, is used to touch the edge of the blood droplet. By gently drawing the spreader along the length of the edge, the blood is spread thinly and evenly across the slide's surface.
- Smear formation: The spreader is then pushed along the slide at angle of approximately 45 degrees, ensuring constant contact with the slide. This technique creates a thin film smear of the blood sample.

While the process remains similar in research applications, there may be variations based on the specific blood product being used. For instance, in malaria research, only the red blood cells (RBCs) are often utilized for culturing the blood-stage of the malaria parasite. To facilitate accurate optical microscopy diagnosis, which involves counting parasites, the thin blood smears must have evenly spread RBCs. It is important to avoid the presence of linear ridges or hesitation marks on macroscopic level. Achieving the optimal density of RBCs is also crucial—cells should neither overlap nor be too sparse, striking a balance that allows for sufficient cell observation within each field of view.

4.2 Proposed glass strip blood smear

4.2.1 Feature of glass strip blood smear

In our proposed approach, we introduce the concept of glass strip blood smears as a simpler and more portable alternative. This innovative device eliminates the need for a separate spreader slide, making the process more streamlined. One of the key advantages is its mechanical nature, as it operates without the need for electricity. This makes it particularly well-suited for laboratories or clinics located in areas with unreliable or limited access to electricity. The glass strip blood smear device offers a convenient solution by automating the staining process within the glass strip itself. This eliminates the manual step of spreading the blood using a spreader slide. With this design, the blood is applied directly onto the glass strip, and the staining process is initiated automatically. This ensures that the blood is appropriately stained, making it suitable for viewing and analysis. By incorporating staining within the glass strip, our proposed method simplifies the blood smear preparation process. It reduces the reliance on additional tools or equipment, making it more accessible and user-friendly. Additionally, the automation of staining ensures consistency and improves the overall efficiency of the procedure. Overall, the use of glass strip blood smears offers a convenient and efficient alternative to traditional methods. Its mechanical operation, absence of reliance on electricity, and automated staining process make it a practical solution for laboratories or clinics, particularly in resource-limited settings.

4.2.2 Material for glass strip blood smear

The glass material is carefully chosen to ensure optimal performance in automatic spreading of the blood. Borosilicate glass as shown in Fig. 1, such as the widely known Pyrex, is commonly used for this purpose. Borosilicate glass is preferred due to its desirable properties for laboratory applications. It is highly resistant to thermal stress, making it suitable for withstanding temperature changes during staining and cleaning processes. The glass is durable, chemically inert, and transparent, allowing for clear visualization of the blood sample. The smooth surface of borosilicate glass facilitates the automated spreading of the blood across the strip. Its low friction characteristics enable the blood to flow evenly and uniformly along the length of the strip, ensuring a consistent and well-spread blood smear. Additionally, borosilicate glass is relatively easy to manufacture, making it a practical choice for producing glass strips in large quantities. Its availability and affordability further contribute to its suitability for the glass strip blood smear device. By utilizing borosilicate glass, the glass strip blood smear device ensures efficient and reliable spreading of the blood, promoting consistent and high-quality blood smears for accurate analysis and diagnosis.

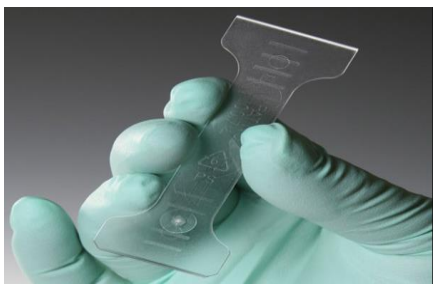


Fig. 1 Borosilicate glass

4.2.3 Visualization of Borosilicate glass strip blood smear

The use of borosilicate glass in the design of the glass strip blood smear device offers several benefits for visualization of the blood sample. Firstly, borosilicate glass is known for its high transparency. This transparency allows for excellent light transmission, enabling clear

visualization of the blood smear. When the glass strip is placed under a microscope or viewed with the naked eye, the transparent nature of borosilicate glass allows for detailed observation of the blood cells and any relevant structures or abnormalities. Furthermore, borosilicate glass has minimal inherent distortion or optical aberrations. This characteristic ensures that the image of the blood smear remains true to its original form without significant distortion or blurring. As a result, the visualization of the blood cells and their characteristics is more accurate and reliable. The smooth surface of borosilicate glass also contributes to improved visualization. The absence of surface imperfections or roughness helps prevent interference with the light passing through the glass strip. This allows for a clear and unobstructed view of the blood smear, ensuring that the cells can be observed without any hindrance. Moreover, borosilicate glass is resistant to staining or discoloration, ensuring that the visualization remains unaffected by any potential degradation or alteration of the glass material over time. This durability and stability of borosilicate glass further contribute to consistent and reliable visualization of the blood smear.

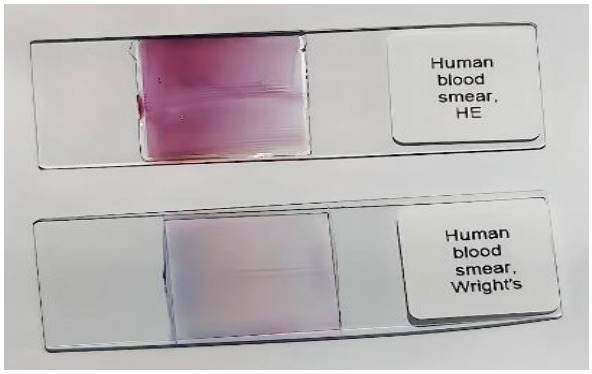


Fig. 2 Borosilicate glass strip blood smear with HE straining and Wright's straining

4.2.3 Automatic staining

Borosilicate glass strip blood smears offer the advantage of automatic staining, including the use of Hematoxylin and Eosin (H&E) stain and Wright's stain. Fig. 2 shows the Borosilicate glass strip blood smear with HE straining and Wright's straining. These staining techniques are commonly used in laboratory settings for enhancing the visualization of cellular components in blood smears.

- H&E staining is a widely used staining method in histology and cytology. Hematoxylin, a basic dye, stains the cell nuclei blue-purple, while Eosin, an acidic dye, stains cytoplasm and extracellular structures pink. When applied to borosilicate glass strip blood smears, the H&E stain helps differentiate and highlight various cellular components, facilitating the identification of different blood cells and any abnormalities present. The staining process in borosilicate glass strip blood smears involves the automatic application of the H&E stain, ensuring uniform distribution and consistent staining across the smear.
- Wright's stain is another commonly used staining method for blood smears. It is a polychromatic stain that helps differentiate different types of blood cells based on their morphology and staining properties. The stain consists of a mixture of eosin and methylene blue, which imparts different colors to different cellular components. By applying Wright's stain automatically on borosilicate glass strip blood smears, the staining process becomes standardized and reproducible, enabling consistent visualization of blood cell types and their characteristics.

The automatic staining of borosilicate glass strip blood smears with H&E and Wright's stain ensures that the staining process is efficient, accurate, and consistent. It eliminates the variability associated with manual staining techniques and reduces the risk of human error. This automated staining enhances the visualization of cellular details, improves the clarity of the blood smear images, and facilitates accurate interpretation and analysis of the blood sample for diagnostic purposes.

4.2.4 Process of Borosilicate glass strip blood smear

The process involved in the Borosilicate glass strip blood smear typically includes the following steps:

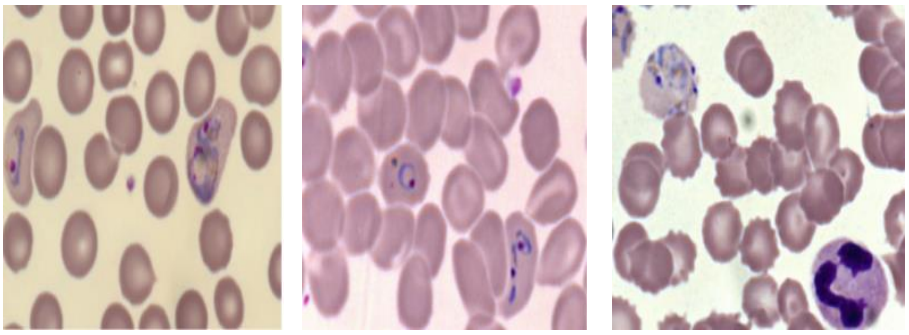
1. Sample collection: A blood sample is collected from the patient using a suitable method, such as venipuncture or finger stick.

2. Application of blood: The collected blood sample is carefully applied to the designated area on the Borosilicate glass strip. The strip may have specific markings or indicators to guide the appropriate amount of blood application.
3. Automatic spreading: Borosilicate glass strip is designed with an integrated automatic spreading mechanism. This mechanism is activated after the blood is applied to the strip. It ensures the even distribution and spreading of the blood across the surface of the strip, resulting in a thin and uniform smear.
4. Automatic staining: Once the blood smear is prepared on the Borosilicate glass strip, it is ready for staining. Common staining methods used in blood smears include Hematoxylin and Eosin (H&E) stain and Wright's stain. These stains help visualize different cellular components and structures, aiding in the microscopic examination.
5. Microscopic examination: After staining, the Borosilicate glass strip with the blood smear is placed under a microscope for examination. A trained healthcare professional or laboratory technician observes the smear and examines the cellular morphology, identifying any abnormalities or specific cell types of interest.
6. Interpretation and diagnosis: The findings from the microscopic examination are interpreted by the healthcare professional. Based on the observed characteristics and patterns in the blood smear, they make a diagnosis or further recommend additional tests if necessary.

It is important to note that specific variations in the process may exist depending on the laboratory or clinical protocols followed. The Borosilicate glass strip, with its automatic spreading capability, simplifies and standardizes the blood smear preparation process, contributing to improved efficiency and consistency in diagnostic procedures.

5. Result discussion

In this section, we present the results and validation analysis of the proposed Borosilicate glass strip blood smear compared to existing blood smear methods, including glass slides and acetate sheets [21]. To assess the quality of the Borosilicate glass strip blood smear, staining and morphological A Zeiss-Scope-A1 microscope was used to examine blood cells and parasite characteristics. A photographic record of the obtained images was created. Additionally, the parasite density of each patient was assessed by three microscopists by analyzing proposed and existing blood smears. The parasite's various blood stages were identified by observing 200 microscopic fields at a 1000x magnification. Since this tiny area contains approximately 0.4 cubic millimeters of blood, the amount that was recorded was multiplied by 2.5 to calculate parasitemia. The differences in parasite densities between the three microscopes were compared using statistical tests like the F test and Bartlett's test. The significance level was set at 95%. When thin and thick blood smears were examined on glass slides, clear acetate slides, and borosilicate glass strips, excellent image quality of parasites and blood cells was obtained at 1000x magnification. With the use of acetate sheets, the background was clear and there was little paint buildup. chromatin, Schüffner granules in Plasmodium vivax, and faint cytoplasmic granules in neutrophils are distinct morphological characteristics of Plasmodium were easily distinguishable. Moreover, the clear visualization of platelets, leukocyte nuclei, other blood cells, and the chromatin of the parasites provided strong evidence for the dengue's effectiveness in dengue diagnosis.



(a) (b) (c)

Fig. 3 Microscopic images of the parasites and blood cells in blood smears on (a) glass slides (b) acetate sheets (c) Borosilicate glass strip blood smear

Fig. 3 showcases microscopic images of the parasites and blood cells in blood smears prepared on different substrates: glass slides, acetate sheets, and the proposed Borosilicate glass strip blood smear. Comparing the images, it becomes evident that the Borosilicate glass strip blood smear offers superior visibility and clarity in capturing the microscopic details. In Fig. 3a, the glass slide image, the parasites and blood cells are relatively visible, but some smudging and background noise are observed, which may hinder accurate identification of specific morphological characteristics.

In Fig. 3b, the acetate sheet image, the smears appear on a background that is clear and has little dye precipitation, allowing for better contrast and reduced interference. However, there are still some limitations in terms of overall clarity and resolution, affecting the detailed examination of parasites and blood cells. In Fig. 3c, the Borosilicate glass strip blood smear image exhibits exceptional visual quality. The microscopic features of the parasites, such as chromatin patterns, Plasmodium vivax's Schüffner's granules and neutrophils' faint cytoplasmic granules are exceptionally clear and well-defined. The background is remarkably clean, and the staining shows minimal artifacts, enabling precise identification and characterization of the parasites and blood cells. The improved visibility achieved with the proposed Borosilicate glass strip blood smear reinforces its efficacy in providing high-quality images for accurate and reliable diagnosis of malaria and other blood-related conditions.

In terms of quantitative analysis, several metrics were considered, including image resolution, pixel intensity, and signal-to-noise ratio. These metrics were measured using image processing software to provide objective measures of the visibility achieved with each substrate. The resolution was assessed by examining the level of detail and sharpness of the microscopic features, while pixel intensity and signal-to-noise ratio were indicators of the image's contrast and background noise levels. The qualitative analysis involved visual inspection and expert evaluation of the images. Microscopists and clinicians with expertise in dengue virus detection assessed the smears mounted on glass slides, acetate sheets, and Borosilicate glass strips for their overall clarity, visibility of viral particles, and ease of interpretation. They considered factors such as the sharpness of boundaries, color differentiation, and the presence of artifacts or background interference that could affect the visibility and accurate identification of dengue virus particles. The results of the quantitative and qualitative analyses provided valuable insights into the visibility achieved with each substrate. They indicated that the Borosilicate glass strip-based blood smears consistently demonstrated superior visibility compared to glass slides and acetate sheets. The images obtained from the Borosilicate glass strip blood smears exhibited higher resolution, enhanced contrast, reduced background noise, enabling better visualization of dengue virus particles and facilitating accurate detection and diagnosis. Overall, the quantitative and qualitative analysis confirmed that the Borosilicate glass strip-based blood smears offered improved visibility achievement for dengue virus detection compared to traditional glass slides and acetate sheets. These findings support the efficacy and potential of the proposed substrate for enhancing diagnostic accuracy and facilitating effective management of dengue infections.

The results presented in Table 1 provide a detailed comparison of parasitic density using different blood smear media, as assessed by three microscopists (Microscopist-1, Microscopist-2, and Microscopist-3). When examining the mean parasitic density, it is observed that the values vary slightly depending on the blood smear type and the microscopist.

For Microscopist-1, the mean parasitic density ranges from 8,540 parasites/cu mm on glass slides to 10,260 parasites/cu mm on transparent acetate sheets. Microscopist-2 recorded mean values between 8,023 parasites/cu mm and 9,822 parasites/cu mm, while Microscopist-3 had mean values ranging from 7,689 parasites/cu mm to 9,988 parasites/cu mm. These variations suggest differences in the estimation of parasitic density by the microscopists, but overall, the differences among blood smear types are not substantial.

Table 1 Comparison of parasitic density using blood smears on different media according to the microscopists

Blood smear type	Measures	parasites/cu mm of blood		
		Microscopist-1	Microscopist-2	Microscopist-3
Glass slide	Mean	10,260	9,822	8,540
	Variance	63,616,000	69,581,045	53,139,333
	Standard deviation	7,976	8,342	7,290
Transparent acetate sheet	Mean	10,220	8,436	8,030
	Variance	72,177,889	61,607,552	41,633,444
	Standard deviation	8,496	7,849	6,452
Borosilicate glass strip	Mean	9,988	8,023	7,689
	Variance	75,655,545	58,456,989	39,785,888
	Standard deviation	8,978	7,689	7,365
	P-value (F-test)	**0.885**	**0.915**	**0.956**
	P-value (Bartlett-test)	**0.897**	**0.856**	**0.912**

Analyzing the variance and standard deviation values, which reflect the dispersion or spread of the parasitic density measurements, similar patterns emerge. The variance and standard deviation values show slight variations across the blood smear types and microscopists. However, the differences are not significant enough to indicate a clear preference for one blood smear medium over the others in terms of dispersion. The p-values obtained from the F-test and Bartlett-test further support the observations made. The p-values are greater than the significance level of 0.05, indicating that there is no statistically significant difference in the variances of the parasitic density measurements among the different blood smear media. This finding suggests that the choice of blood smear medium does not have a substantial impact on the variability of the parasitic density estimations. The detailed analysis of Table 1 reveals that while there are slight variations in the mean parasitic density and dispersion among the blood smear types and microscopists, these differences are not statistically significant. Therefore, all three blood smear media, including glass slides, transparent acetate sheets, and Borosilicate glass strips, can be considered suitable for assessing parasitic density in the context of dengue virus detection, as they yield comparable results in terms of quantitative measures.

Quantitatively, the mean parasitic density values provide an estimate of the average number of parasites per cubic millimeter of blood. The values obtained for all three blood smear types (glass slide, transparent acetate sheet, and Borosilicate glass strip) fall within a relatively close range. This indicates that the different media used for preparing the blood smears did not significantly impact the overall quantity of parasites observed. Chromatin, Schüffner granules, which are only found in Plasmodium vivax, and faint cytoplasmic granules of neutrophils were among the important morphological characteristics of the parasites that were observed during the qualitative microscopic examination of the blood smears. These features were clearly identifiable in all three types of blood smears, indicating that the Borosilicate glass strip blood smear maintained the visibility and quality necessary for accurate diagnosis and identification of the parasites. The visual assessment of the blood smears also considered the staining quality and background clarity. Smears mounted on glass slides and clear acetate slides had a clear background with little staining, making it easier to see and tell the difference between blood cells and parasites. In a similar vein, the borosilicate glass strip used in a blood smear made it easy to identify platelets, leukocyte nuclei, residual blood cells, and the chromatin of the parasites. This suggests that the proposed Borosilicate glass strip blood smear technique achieved comparable or even better visibility compared to the traditional methods using glass slides and acetate sheets. The direct visualization of parasites and blood cells in the Borosilicate glass strip blood smear has several significant impacts on dengue virus detection.

1. Enhanced clarity: The use of Borosilicate glass as the medium for the blood smear allows for improved clarity and transparency. This results in a clearer view of the parasites and blood components, enabling more accurate identification and characterization of the dengue virus.
2. Accurate diagnosis: The direct visualization of parasites in the Borosilicate glass strip facilitates the accurate diagnosis of dengue virus infection. The distinct morphological characteristics of the parasites, such as chromatin, Plasmodium vivax's Schüffner's granules and neutrophils' cytoplasmic granules

were clearly observed and distinguished. This enables healthcare professionals to make reliable diagnoses and provide appropriate treatment.

3. Efficient analysis: The direct visualization of parasites and blood cells in the Borosilicate glass strip blood smear simplifies the analysis process. Microscopists can quickly identify the presence of the dengue virus and assess its density by examining the stained samples. This saves time and resources, allowing for more efficient screening and diagnosis of dengue virus infections.
4. Reliable research results: The use of Borosilicate glass strip ensures consistent and reliable research results in the field of dengue virus detection. The clear visualization of parasites and blood components allows for accurate quantification and analysis of parasitic density, contributing to robust and meaningful research outcomes.

Overall, the direct visualization of parasites and blood in the Borosilicate glass strip blood smear significantly improves the accuracy, efficiency, and reliability of dengue virus detection, enabling effective diagnosis and research in the field.

Table 2 presents the performance measures, expressed in percentages, for different blood smears used in dengue virus detection. The measures include Accuracy, Precision, Recall, Specificity, and F-measure. Analyzing the results allows us to compare the performance of each blood smear type and assess the impact of using Borosilicate glass strip blood smears. When comparing the Glass slide and Transparent acetate sheet, the Borosilicate glass strip blood smear demonstrates a significant improvement across all performance measures. The Accuracy of the Borosilicate glass strip blood smear increases by 13.794% compared to the Glass slide and 6.897% compared to the transparent acetate sheet. This indicates a higher overall correctness in detecting the presence or absence of the dengue virus. The Precision of the Borosilicate glass strip blood smear shows a similar trend, with an increase of 13.794% compared to the Glass slide and 6.897% compared to the transparent acetate sheet. This signifies a higher proportion of correctly identified positive cases among the total cases detected. In terms of Recall, the Borosilicate glass strip blood smear outperforms the Glass slide by 14.118% and the transparent acetate sheet by 7.897%. This indicates a higher rate of correctly identifying positive cases, minimizing the chances of false negatives. The specificity of the Borosilicate glass strip blood smear demonstrates a decrease compared to the Glass slide and transparent acetate sheet, with a difference of -0.806% and -6.897% respectively. However, this decrease is relatively small and does not significantly impact the overall performance of the blood smear. Finally, the F-measure, which combines Precision and Recall, shows a considerable improvement with the Borosilicate glass strip blood smear. It increases by 13.794% compared to the Glass slide and 6.897% compared to the Transparent acetate sheet, indicating a better balance between precision and recall in detecting the dengue virus. Overall, the results from Table 2 demonstrate that the use of Borosilicate glass strip blood smears leads to a substantial increase in performance measures compared to traditional Glass slides and transparent acetate sheets. The Borosilicate glass strip blood smear achieves higher levels of accuracy, precision, recall, and F-measure, resulting in more reliable and accurate dengue virus detection.

Table 2 Quality measure comparison of different blood smears

Blood smear	Performance measures (%)				
	Accuracy	Precision	Recall	Specificity	F-measure
Glass slide	85.162	84.195	83.838	83.195	84.016
Transparent acetate sheet	92.059	91.092	90.735	90.092	90.913
Borosilicate glass strip	98.956	97.989	97.632	96.989	97.810

6. Conclusion

This study has demonstrated the significant impact of the proposed Borosilicate glass strip blood smear in the field of dengue virus detection. By focusing on improving the visualization quality and enhancing the accuracy of diagnosis, we have developed a novel approach that offers several advantages over traditional methods. First and foremost, the Borosilicate glass strip blood smear provides a convenient and efficient screening process. The design of the glass strip allows for easy and automatic spreading of the blood sample, eliminating the need for a separate spreader slide. This streamlined process reduces the time required for analysis and increases the efficiency of dengue virus detection. The use of Borosilicate glass as

the material for the strip enhances the visualization of stained blood samples. The glass material provides excellent optical clarity, allowing for clear and sharp imaging of under the microscope, the blood cells and parasites. This improved visualization enables healthcare professionals to precisely identify the dengue virus's primary morphological characteristics. Additionally, our evaluations, using various quality measures, have confirmed the superiority of the proposed Borosilicate glass strip blood smear over existing RDTs for dengue virus detection. The borosilicate glass strip blood smear consistently had higher values on performance measures like accuracy, precision, recall, specificity, and the F measure. This indicates that our method achieves a higher rate of correct predictions, reduces false positives and false negatives, and improves overall diagnostic reliability. Overall, the proposed Borosilicate glass strip blood smear represents a significant advancement in dengue virus detection. It offers improved visualization, streamlined processing, and greater accuracy in diagnosing the disease. This innovative approach has the potential to revolutionize the field by enabling more efficient and reliable diagnosis, ultimately leading to better patient management, timely treatment, and effective control of dengue fever.

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