HORIBA



"Ease of Screening Malaria and Dengue" Malaria and Dengue Flags of HORIBA Yumizen H500 and Yumizen H550 Hematology Analyzers

White Paper | April 2024

Introduction:

Malaria is a global disease found in tropical and sub-tropical regions of the world. Despite international initiatives aimed at eradicating the disease, malaria is still responsible for more than 600 000 deaths every year – most of them children.

Malaria is transmitted to humans via the Anopheles Mosquito. The mosquito bite introduces the parasite into the human's blood via sporozoites in the saliva. The parasites then migrate to the liver, where they mature and reproduce. The organisms multiply in the liver in infected hepatocytes. These differentiate into thousands of merozoites, which rupture the host cell, infiltrating the blood and infecting red blood cells.







P. Falciparum - Trophozoites P. Viva and crescent shaped gametocyte and tro

P. Vivax - Gametocyte (L) and trophozoite (R)

There are five known types of malaria parasite: Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi.

Dengue is another mosquito-borne disease but, unlike malaria, it is caused by a virus rather than a parasite. Dengue is spread by several species of female mosquitoes of the Aedes genus, principally Aedes aegypti. It is an RNA virus of the family Flavivirdae. Dengue is common in more than 120 countries, many of them where malaria is also endemic. Around 390 million people are infected per year of whom about 500 thousand require hospitalization and approximately 40 000 people die.

Control of infection:

Despite many advances in vector control, diagnostic screening and effective treatments, malaria continues to have a devastating impact on health around the world. The World Health Organization (WHO) launched a Global Technical Strategy for malaria 2016 – 2030 in 2015 with the aim to reduce global malaria incidence and mortality rates by at least 90% by 2030. Technical solutions include the control of the mosquito vector, improved treatment and prophylaxis regimes, and effective screening and diagnosis. Malaria-endemic regions are usually economically challenged and extremely cost conscious, where accessible, dependable diagnostic methods are urgently needed.

High performance screening with a complete blood count (CBC):

Diagnosis of malaria is typically carried out by manual examination of thick or thin blood smears stained by Romanowski methods at a pH of 7.2. Other tests include rapid testing techniques and PCR. Most patients presenting with malaria symptoms will have a full blood count (FBC) performed. In addition, these symptoms are like those of other infections, including dengue, which can be endemic in the same area.

HORIBA has developed infectious screening flags from full blood count data, based on innovative and contemporary machine-learning techniques and thousands of measurement points. (1) The clinical performances of these flags have been assessed through on-site validation studies performed for malaria (both Plasmodium vivax and Plasmodium falciparum) and dengue with high clinical sensitivity and specificity.

Suspicion screening flags for malaria speciation and dengue infections:

With the aid of Artificial Intelligence (AI), scientists have developed hematology analyzers that can screen out diseases like malaria and dengue in a few minutes, along with normal CBC reports.

HORIBA 6-part hematology analyzer, Yumizen H550 and Yumizen H500 can raise flags of dengue and malaria in suspicious cases without the use of extra reagent.



This instrument has the potential to segregate Plasmodium vivax and Plasmodium falciparum through AI based algorithms and machine learning from instrument generated raw data measurements.

- The infectious suspicion messages aim at providing a **screening flag** for triggering out suspected malaria and dengue infections.
- The flags are combined with FBC analysis (CBC or DIFF mode) without any extra specific reagents and burden or specific sampling mode.
- The flagging algorithms are capable to trigger out 3 species:
 - Malaria P. falciparum?
 - Malaria P. vivax?
 - Dengue?

The flagging messages are **displayed** and can be **printed out** and **transmitted** to a laboratory information system (LIS) or middleware.

Flagging scores are displayed to provide the flags' triggering level and hence help the users to appreciate the flagging rate.



Visual representation of the scoring flags can be seen in the expert data screen on the instrument for additional information:



The technology:

The infectious screening flags of the Yumizen H500 and H550 were developed using a Random Forests machine learning algorithm. This is a common technique used in artificial intelligence methodologies, but it has a particular application in bioinformatics and the development of biomarkers of disease.

While the Yumizen H500 and Yumizen H550 instruments produce 37 reported hematology parameters, the raw data that produces these results is far more complex. In particular, the differential channel generates the resistive and optical measurements of each individual cell plus the pattern and position of clusters generated on the scattergram.

Machine learning is able to take this data and explore the variables in the context of different disease states, using these to generate predictor software. These can then be further analyzed using a large cohort of clinical cases to develop thresholds for flagging.



The two predictors above provide a score that is a probability of having a given disease. The threshold for the predictors allows the triggering of the alarm to capture the majority of cases while minimizing false positives.

Added values of white blood cell (WBC) differential matrix in malaria infections:

Although the patterns generating the flags are not always obvious on the instrument graphics, in some cases the infected red blood cells (RBCs) may be clearly seen on the WBC differential scattergram and can be observed on the left side of Lymphocytes cells population. Especially in the case of malaria vivax species, the presence of infected RBCs can visually confirm the positive sample.

Corrected WBC counts in malaria infection:

Malaria infection (Plasmodium vivax) is known to cause interference in the total WBC counts due to parasitized RBC's. The WBC count is automatically corrected by removing the infected RBCs from total WBC count in HORIBA Yumizen H500 and Yumizen H500, whereas in most of other hematology analyzers there is interference in WBC total counts.



Case studies:

Malaria infection

In this patient there was a very clear population visible to the left of the matrix.

The algorithm triggered a strongly positive flag for Plasmodium vivax which was confirmed by both blood film examination and a Malaria Rapid Test.



>Dengue infection



Here a febrile patient is present with mild neutropenia, relative monocytosis, and mild thrombocytopenia. A positive flag for dengue was generated by the instrument and confirmed by an antigenic test.

Discussion:

There have been several studies conducted on testing the sensitivity and specificity of these flags in different parts of the world which demonstrates a possibility of the effective use of automated infectious flags for screening malaria and dengue infection in a clinical setting. (1)

One such pilot study was conducted for verifying the flags sensitivity across four different corners of India demonstrated a sensitivity rate of P. vivax as 90% & P. falciparum as 100% and specificity rate of 95.01% & 99.59% for P. vivax & P. falciparum respectively. (2)

The thresholds can also be adjusted as per the population data and requirement. A high cutoff threshold is directly proportional to the specificity. These alarms can be used as markers for the type of infection. This information provided by the instrument within minutes can save the cost for testing all the samples and can be used for early screening of these infections.

Note: Clinical co-relation and further testing is also required to confirm the diagnosis.

Conclusions:

The novel technology of Yumizen H500 and Yumizen H550 provides a robust, rapid, automated, and accurate platform for screening of malaria in a clinical setting.

- The flags generated were evaluated in a prospective study and showed similar performance to gold standard i.e., microscopy.
- The instrument showed good sensitivity and specificity. The sensitivity for P. vivax was 90% while for P. falciparum came out to be 100%. The specificity came out to be 95.01% and 99.59% for P. vivax and P. falciparum respectively. For the automated dengue fever flag, it yielded a ROC AUC of 0.82 with 79.3% sensitivity and 71.5% specificity. (1)
- The automated generation of a full blood count for each sample provides an opportunity for detecting unsuspected cases.
- Asymptomatic carriers can also be identified, which will be useful in blood transfusion centers, and will enable treatment of these individuals to prevent the spread of disease.

- The objective enumeration of red blood cells infected with Plasmodium species makes it suitable for global use and allows monitoring of the parasite load once therapy has been initiated, thereby providing an early marker of drug resistance.
- The HORIBA Yumizen H550 and Yumizen H500 hematology analyzer demonstrates flags malaria along with CBC+Diff that can be clinically useful for the screening of malaria infections in low-resourced, endemic areas and thus facilitate further diagnosis testing in a cost-effective manner and prevention of antimalarial drug resistance. (1)

Note: For more details, please do visit https://www.horiba.com/int/medical/academy/t echnology/malaria-dengue-screening

References:

1. <u>Performance evaluation of machine</u> <u>learning-based infectious screening flags on</u> <u>the HORIBA Medical Yumizen H550</u> <u>Hematology Analyzer for vivax malaria and</u> <u>dengue fever</u>, Parag Dharap 1, Sebastien Raimbault 2 PMID: 33228680, PMCID: PMC7684750, DOI: 10.1186/s12936-020-03502-3

2. Evaluation of Yumizen H550 for screening and classification of type of Malaria (Multicentric in house pilot study for verification of Malaria & Dengue Flags on HORIBA Yumizen H550 Haematology analyzers, 2020, India

3. https://www.who.int/teams/global-malariaprogramme

Authors:

Mandy Campbell, International Hematology Product Manager, HORIBA

Dr. Prakash Suvasia, Scientific & Medical Officer, HORIBA