

EVALUATION OF DENGUE AND MALARIA SPECIATION SUSPECT FLAGS ON COMPACT 5 PART DIFFERENTIAL HORIBA MEDICAL BLOOD CELL COUNTER



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Introduction: Though spread by different subspecies of vector insects, Malaria and Dengue are frequently found to be coexistent in economically challenged endemic area of developing countries. Common symptomatology poses challenges to health care givers. Encouraged by the performance of a previous malaria flagging algorithm applications developed through contemporary computer machine-learning techniques on blood cell counter, Horiba decided to develop similar tools to screen for Dengue fever, as well as sub speciation of Malarial parasites, as a part of continual improvement endeavor. We evaluated in this study the performance of these flags on the HORIBA Yumizen H550 analyzer.

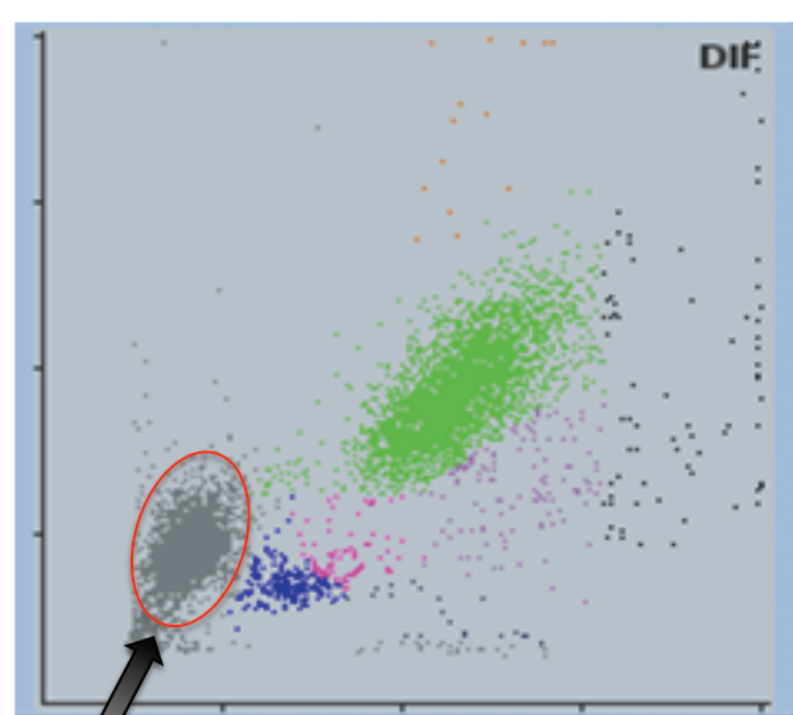
Background: Uncontrolled population growth, ill planned urbanization, inadequate resources for public health & overall lack of awareness have possibly been the chief causes for the resurgence of one of the most common viral disease, Dengue Fever, in tropical region & developing nations. With heavy unpredictable monsoons it has become one of the leading infectious diseases causing public health menace.

Though spread by different subspecies of vector insects, Malaria and Dengue are frequently found to be coexistent in economically challenged endemic area of developing countries. Besides mosquitoes as vectors, both diseases share similar incubation periods in humans of 5 to 6 day, along with similar signs & symptomatology of acute febrile illness, with or without myalgia, hepatosplenomegaly & thrombocytopenia. Absence of other classical findings pose challenges to clinicians, who constantly desire accurate & quick differentiation between these major public health concerns for better therapeutic management. Other than above mentioned common features, various studies have documented hematological parameter abnormalities like hemoglobin, hematocrit, MCHC, leukopenia, leukocyte population abnormalities, as well as biochemical parameter abnormalities including liver function tests.

Bhargava et al have suggested use of discriminant factors based on Volume, Conductivity and Scatter Properties of Leucocytes (VCS Technology) in Beckman Coulter analyzers for rapid diagnosis of Malaria & Dengue. Even after introduction of Dengue & Malaria PCR technology on a global platform its requirements of special instrumentation, time consumption & economic constraints have kept them out of reach of common individuals. The gold standard method of malarial parasite detection by peripheral blood smear examination, although most commonly utilized & economical, is known to require technical expertise and may need repetitive testing, thereby increasing turnaround time. Similarly for Dengue infection, screening tests detecting presence of disease specific antigen &/ or antibody testing, preferably by Elisa technique, requiring additional instrumentation, are being used as the mainstay for therapeutic interventions.

Encouraged by the performance of a previous malaria flagging algorithm applications developed through contemporary computer machine-learning techniques on blood cell counter ABX Pentra XLR (A five part differential counter with Reticulocyte counting technique) & MicrosemiCRP (a Three part differential blood cell counter with CRP), Horiba decided to develop similar tools to screen for Dengue fever, as well as assess possibility to carry out sub speciation of Malarial parasites, as a part of continual improvement endeavor.

We evaluated in this study the performance of these flags on the HORIBA Yumizen YH 550 analyzer, a new platform for the low and middle range market.



Malaria infected RBCs in YH550 WBC scattergram

Materials and methods: A total number of 1436 patients, who visited Dr. Dharap's Diagnostic Centre, Mumbai, India for routine complete blood count examination, during July to October 2018 were studied. Residual blood specimens were serially analyzed on Horiba Yumizen H550 analyzer. Local ethical standards were followed; however, informed consent was not required, as study was done by anonymizing retained blood samples without any extra charges & not communicating results of the study to the clinician, so as to affect the diagnostic and therapeutic management of the patient. Besides the primary patient selection criteria of fever, diagnostic microscopic blood smear examination for malarial parasite and confirmation by antigenic testing for Malaria and Dengue NS1 antigen were performed for all cases.

For screening of Malaria cases, Rapid Malaria Antigen detection test kits manufactured by SD Biosensor Healthcare Pvt. Ltd., Gurugram, India using monoclonal anti-P. falciparum HRP-II (0.75+/- 0.15 µg) with monoclonal anti-P. vivax pLDH(0.75+/- 0.15 ug) respectively to detect presence of P. falciparum & P. vivax related antigen. For screening & diagnosis of Dengue, RecombiLISA NS1 Antigen test utilizing pairs of specific polyclonal & monoclonal anti-dengue antibodies of all four serotypes (DEN1, 2, 3, 4) and analytical sensitivity of 0.3ng/ml for type 2 NS1 antigen, manufactured by CTK Biotech, Inc., United States of America was used.

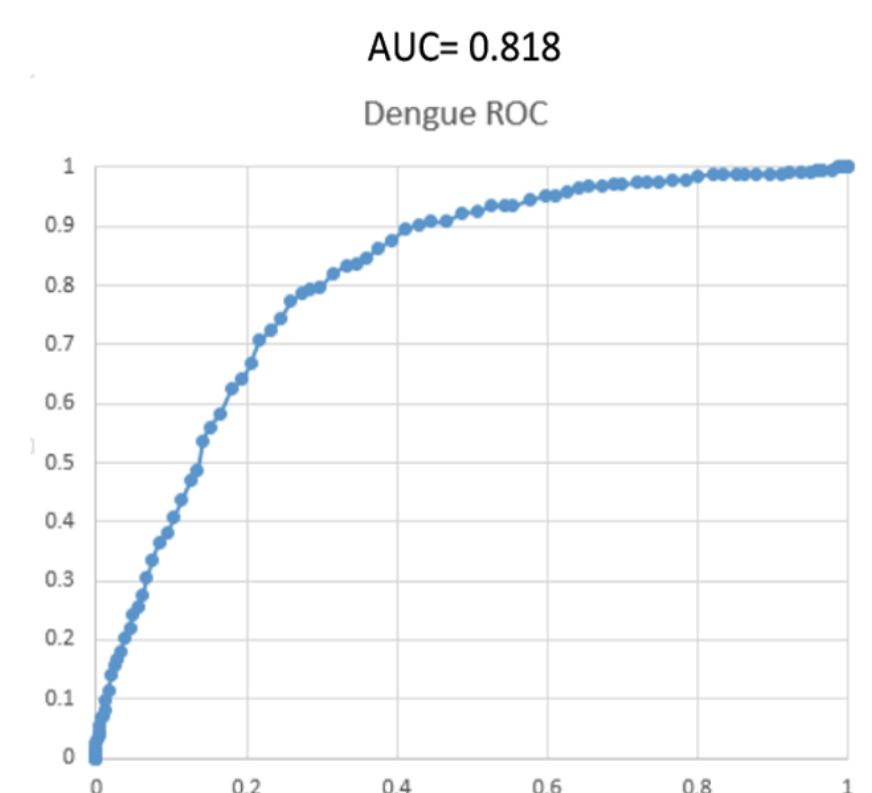
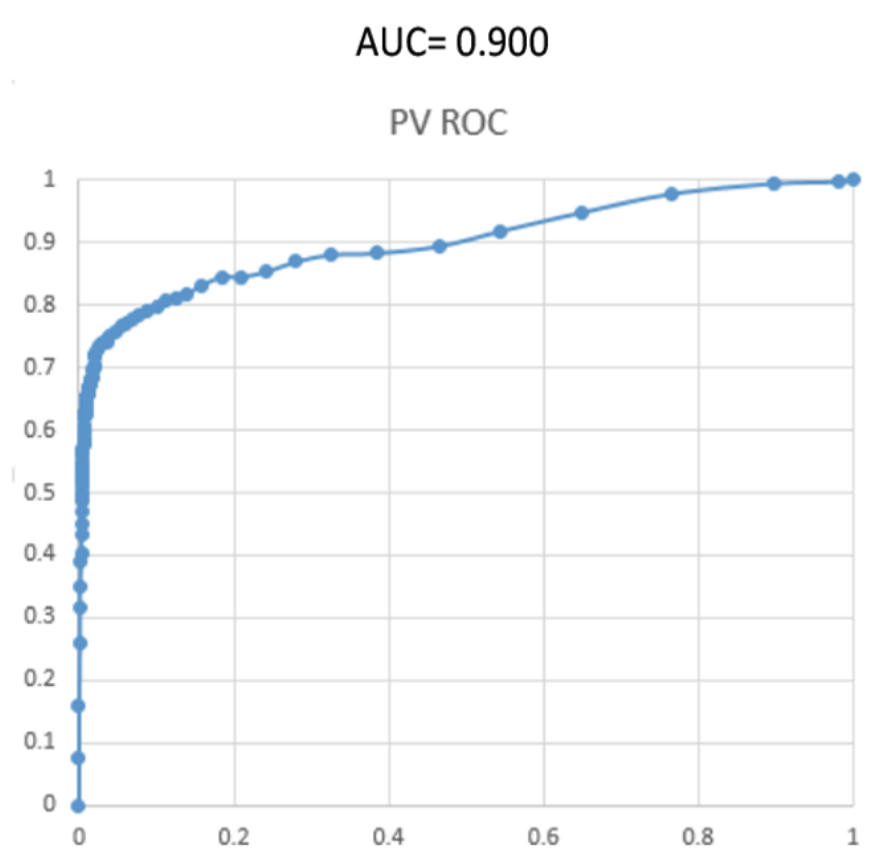
All the samples were identified & recruited for evaluation with screening/ diagnostic testing & microscopic review by an experienced pathologist. Samples were grouped as Normal, Malaria positive & Dengue positive cases. Malaria cases were further classified by high magnification microscopic review of 2,000 red cells by an experienced pathologist for speciation, dominant life cycle forms and parasite density. Persons for routine health check-up without history of any specific ailments were considered for the Normal or control group (N = 60). Samples were analyzed (N=1424, Vivax Positive N=184, Falciparum Positive N=21, Dengue fever N=213, other Pyrexia N=933) with prototype integrated instrument software comparing flagging results obtained with confirmatory testing.

Results

Overall results show a significant AUC for these flags. An optimal cut-off of 0.31 was identified for the Vivax flag.

Malaria Vivax Flag									
cutoff		0.5		REF					
Test Flag	N	NEG	POS	Test Flag	N	NEG	POS	Test Flag	NPV
	2846	2478	368		2846	2478	368		0.950
	NEG	TN	FN		2580	2452	128		0.950
	POS	FP	TP		265	36	240		0.902
	Runs	NEG	POS		2846	2478	368		0.950
Runs	2846	2478	368	2846	2478	368	2846	2478	368
Specificity	0.990	0.852	94.59%	Specificity	0.990	0.852	94.59%	Specificity	0.990
Youden	0.642	62.2	0.352	Youden	0.642	62.2	0.352	Youden	0.642

Dengue Flag									
cutoff		0.5		REF					
Test Flag	N	NEG	POS	Test Flag	N	NEG	POS	Test Flag	NPV
	2846	2478	368		2846	2478	368		0.950
	NEG	TN	FN		2580	2452	128		0.950
	POS	FP	TP		265	36	240		0.902
	Runs	NEG	POS		2846	2478	368		0.950
Runs	2846	2478	368	2846	2478	368	2846	2478	368
Specificity	0.979	0.720	94.59%	Specificity	0.979	0.720	94.59%	Specificity	0.979
Youden	0.700	35.0	0.286	Youden	0.700	35.0	0.286	Youden	0.700



Febrile Patients : Results are similar for this subset of patients where these flags are useful in real-life

Malaria Vivax Flag									
cutoff		0.31		REF					
Test Flag	N	NEG	POS	Test Flag	N	NEG	POS	Test Flag	NPV
	2684	2316	368		2684	2316	368		0.950
	NEG	TN	FN		2373	2270	103		0.957
	POS	FP	TP		311	46	265		0.892
	Runs	NEG	POS		2684	2316	368		0.950
Runs	2684	2316	368	2684	2316	368	2684	2316	368
Specificity	0.980	0.720	94.45%	Specificity	0.980	0.720	94.45%	Specificity	0.980
Youden	0.700	35.3	0.286	Youden	0.700	35.3	0.286	Youden	0.700

Dengue Flag									
cutoff		0.5		REF					
Test Flag	N	NEG	POS	Test Flag	N	NEG	POS	Test Flag	NPV
	2684	2316	368		2684	2316	368		0.950
	NEG	TN	FN		2373	2270	103		0.957
	POS	FP	TP		311	46	265		0.892
	Runs	NEG	POS		2684	2316	368		0.950
Runs	2684	2316	368	2684	2316	368	2684	2316	368
Specificity	0.980	0.720	94.45%	Specificity	0.980	0.720	94.45%	Specificity	0.980
Youden	0.700	35.3	0.286	Youden	0.700	35.3	0.286	Youden	0.700

Dengue Flag									
cutoff		0.5		REF					
Test Flag	N	NEG	POS	Test Flag	N	NEG	POS	Test Flag	NPV
	2684	2316	368		2684	2316	368		0.950
	NEG	TN	FN		2373	2270	103		0.957
	POS	FP	TP		311	46	265		0.892
	Runs	NEG	POS		2684	2316	368		0.950
Runs	2684	2316	368	2684	2316	368	2684	2316	368
Specificity	0.980	0.720	94.45%	Specificity	0.980	0.720	94.45%	Specificity	0.980
Youden	0.700	35.3	0.286	Youden	0.700	35.3	0.286	Youden	0.700

Dengue Flag									
cutoff		0.5		REF					
Test Flag	N	NEG	POS	Test Flag	N	NEG	POS	Test Flag	NPV
	2684	2316	368		2684	2316	368		0.950
	NEG	TN	FN		2373	2270	103		0.957
	POS	FP	TP		311	46	265		0.892
	Runs	NEG	POS		2684	2316	368		0.950
Runs	2684	2316	368	2684	2316	368	2684	2316	368
Specificity	0.980	0.720	94.45%	Specificity	0.980	0.720	94.45%	Specificity	0.980
Youden	0.700	35.3	0.286	Youden	0.700	35.3	0.286	Youden	0.700

Dengue Flag									
cutoff		0.5		REF					
Test Flag	N	NEG	POS	Test Flag	N	NEG	POS	Test Flag	NPV
	2684	2316	368		2684	2316	368		0.950
	NEG	TN	FN		2373	2270	103		0.957
	POS	FP	TP		311	46	265		0.892
	Runs	NEG	POS		2684	2316	368		0.950
Runs	2684	2316	368	2684	2316	368	2684	2316	368
Specificity	0.980	0.720	94.45%	Specificity	0.980	0.720	94.45%	Specificity	0.980
Youden	0.700	35.3	0.286	Youden	0.700	35.3	0.286	Youden	0.700

Malaria or Dengue Positive Patients : Results show little confusion of these flags between these pathologies

Malaria Vivax Flag									
cutoff		0.31		REF					
Test Flag	N	NEG	POS	Test Flag	N	NEG	POS	Test Flag	NPV
	826	458	368		826	458	368		0.950
	NEG	TN	FN		779	744	35		0.950
	POS	FP	TP		279	14	265		0.950
	Runs	NEG	POS		826	458	368		0.950
Runs	826	458	368	826	458	368	826	458	368
Specificity	0.989	0.720	85.84%	Specificity	0.989	0.720	85.84%	Specificity	0.989
Youden	0.690	23.6	0.289	Youden	0.690	23.6	0.289	Youden	0.690

Dengue Flag									
cutoff		0.5		REF					
Test Flag	N	NEG	POS	Test Flag	N	NEG	POS	Test Flag	NPV
	826	458	368		826	458	368		0.950
	NEG	TN	FN		779	744	35		0.950
	POS	FP	TP		279	14	265		0.950
	Runs	NEG	POS		826	458	368		0.950
Runs	826	458	368	826	458	368	826	458	368
Specificity	0.989	0.720	85.84%	Specificity	0.989	0.720	85.84%	Specificity	0.989
Youden	0.690	23.6	0.289	Youden	0.690	23.6	0.289	Youden	0.690

Dengue Flag									
cutoff		0.5		REF					
Test Flag	N	NEG	POS	Test Flag	N	NEG	POS	Test Flag	NPV
	826	458	368		826	458	368		0.950
	NEG	TN	FN		779	744	35		0.950
	POS	FP	TP		279	14	265		0.950
	Runs	NEG	POS		826	458	368		0.950
Runs	826	458	368	826	458	368	826	458	368
Specificity	0.989	0.720	85.84%	Specificity	0.989	0.720	85.84%	Specificity	0.989
Youden	0.690	23.6	0.289	Youden	0.690	23.6	0.289	Youden	0.690

Dengue Flag				
cutoff	0.5	REF		
	N	NEG	POS	
Test Flag	826	400	426	
	NEG	TN	FN	
	434	346	88	
	POS	FP	TP	
	392	54	338	
	Runs	NEG	POS	
	826	400	426	
		Specificity	Sensitivity	
		0.865	0.793	
		Youden	PLR	