Validation of Horiba Medical Pentra XL/XLR and MicrosemiCRP Malaria Flag Performance derived from Algorithmic Data-Mining Techniques

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1. Abstract

Introduction:

Blood cell counter screening for the presence of malaria has been investigated for >20 years. Efficiency reported has varied by methodology and study design. Several manufacturers have introduced malaria flagging on high range instruments with reports claiming sensitivity and specificity >80%. However the economic reality is malaria endemic areas typically utilize low and medium range instruments. We applied contemporary computer machine-learning techniques to develop flagging algorithms for detection of malaria using two low to middle range Horiba Medical blood counters. The validation included Dengue fever patients, which have similar clinical symptomatology as malaria.

Method:

290 blood specimens were serially analyzed the following instruments and testing modes: MicrosemiCRP (CBC 3 part diff + CRP modes) and Pentra XLR (5 part Diff or fluorescent reticulocyte analysis modes). Specimen selection used three diagnostic categories confirmed by antigen testing: healthy, malaria positive, dengue fever positive. Malaria confirmed cases were further speciated into P. vivax (N=103) and P. falciparum (N=29) and microscopically scored the predominate development stage (schizoints, ring or amoeboid). Normal (N=70) and Dengue cases (N=87) were considered to be malaria negative samples for determination of sensitivity, specificity, negative and positive Predictive values, correct classification rate and positive and negative likelihood ratios. Datamining techniques were used to identify those instrument parameters that showed significant differences between malaria from normal in a training set of 550 cases (232 malaria positive) and the flagging algorithms developed included weighting of parameters showing higher discriminating power. The number of parameters selected from those tested for the malaria suspect flag varied by instrument (MicrosemiCRP, 59 of 886; PXLR DIFF, 412 of 25,994; PXLR Retic, 402 of 26,395) with the datamining and machine-learning techniques. Samples were analyzed with instrument software integrating the malaria suspect flag with results compared to the confirmatory testing.

Results:

Method	Sensitivity	Specificity	Correct Class	NPV	PPV	NLR	PLR
PXLR DIFF	0.871	0.803	83.33%	0.782	0.884	0.16	4.41
PLXR Retic	0.816	0.822	81.93%	0.846	0.789	0.22	4.58
Micros emiCRP	0.853	0.77	80.93%	0.865	0.756	0.19	3.76
Micros emiDiff	0.428	0.815	63.64%	0.624	0.665	0.7	2.31

2. Background

Malaria is one of the most common communicable diseases in the world, affecting the populations in all tropical regions. Malaria is a life threatening disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. About 3.2 billion people – almost half of the World's population are at risk of malaria. Malaria is the most common cause of morbidity in Africa. Malaria is a major public health problem with WHO estimates of 207 million cases of malaria occurred globally in 2012 and 6,270,000 deaths.

Several hematology blood analyzers have reported the ability to detect the presence of malaria infection using various parameters with varying efficiency. Abbott analyzers utilized light depolarization and nucleic acid fluorescent dyes (1-3), Beckman Coulter instruments utilized off-line algorithms developed from cell positional or size parameters (4-6) and Sysmex likewise has utilized cell size parameters in the white blood cell matrix and changes in the fluorescence of red cell or reticulocyte matrix for their malaria flag (7-12, copied as well by Mindray). In common to all these methods of malaria detection, either performed as an automated flag or by user interpretation of scattergram patterns, is that less than four parameters are utilized in the detection of blood changes associated with malaria infection.

HORIBA Medical chose to develop an automated malaria suspect flag using datamining techniques to examine all parameters generated by the MicrosemiCRP, Pentra XL and Pentra XLR instruments to determine those most useful in distinguishing malaria from both healthy and non-malarial infections. Using these contemporary machine learning techniques applied to over 500 patient specimens including not only malaria positive and healthy cases, but also a group of patients with Dengue fever, which has a clinical presentation similar to malaria (same signs and symptoms). From these data files 412 of 25,994 parameters for the PXL and PXLR in CBC+Diff mode, 402 of 26,395 for the PXLR in modified Retic mode, and 59 of 886 variables for the MicrosemiCRP were identified as having discriminatory power for malaria. From these useful parameters a flagging algorithm was developed and utilized in this validation study.

3. Methods

290 patient samples, selected from diagnostic groups of normal, malaria positive or Dengue Fever positive, were included in the study with each sample run in duplicate

Rapid antigen diagnostic tests manufactured by SD – Bio Standard Diagnostics Pvt Ltd; Gurgaon, India were used to screen for Plasmodium falciparum (histidine rich protein II), Plasmodium vivax (Plasmodium lactate dehydrogenase), and Dengue Fever (NS1 Antigen &/ IgM Antibody)

All malaria cases were confirmed by microscopy and scored for the predominant life cycle forms (trophozoites, schizoints, gametocytes)

Blood samples were analyzed in duplicate on a Horiba Medical Pentra XLR instrument (CBC 5 part differential counts and thiazole orange based reticulocyte analysis) using both the CBC DIFF mode and again with a modified (decreased) fluorescence gain Retic modes and on a MicrosemiCRP instrument (CBC 3 part differential counts and whole blood C reactive protein) in both CBC+Diff mode or CBC+Diff+CRP modes

Results statistically analyzed for the efficiency, sensitivity, specificity, positive Youden J index, correctness of classification, positive predictive value and negative predictive value of the malaria suspect flag compared to the usual laboratory methods for malaria confirmation.

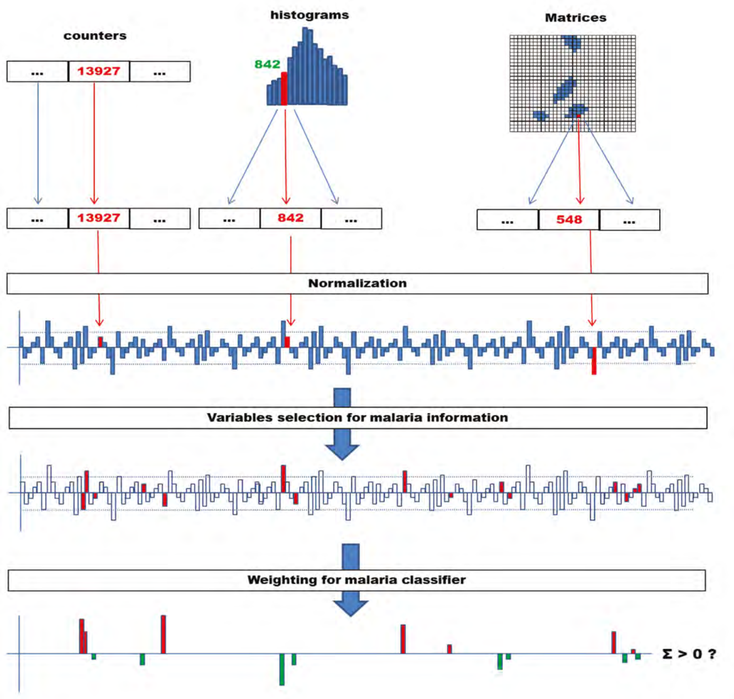

Figure 1. Summary of datamining process. All parameters measured and generated by the blood counter instrument, including counts, histogram information; and matrix plot positions are compared for each diagnostic category (normal; malaria positive and Dengue fever). Differences between the diagnostic groups are normalized for both negative and positive differences. Variables with discriminating ability are identified and further weighted in the flagging algorithm developed to classify malaria as distinct from healthy individuals and Dengue fever patients.

4. Results

A		Reference Method		
N =	285	NEG	POS	
	157	128		
Test Flag	NEG	TN	FN	NPV
	285	252	33	0.884
	POS	FP	TP	PPV
	285	62	223	0.782
	Runs	314	256	570
		Specificity	Sensitivity	CorrectClass
		0.803	0.871	83.33%
		Youden J	PLR	NLR
		0.674	4.41	0.161

B		Reference Method		
N =	198	NEG	POS	
	70	128		
Test Flag	NEG	TN	FN	NPV
	162	129	33	0.796
	POS	FP	TP	PPV
	234	11	223	0.953
	Runs	140	256	396
		Specificity	Sensitivity	CorrectClass
		0.921	0.871	88.89%
		Youden J	PLR	NLR
		0.793	11.1	0.14

Table 1. Performance of malaria suspect flag on Pentra XLR using CBC and DIFF mode The number of true negative (TN), false negative (FN), true positive (TP) and false positive (FP) results are determined for flagging on duplicate analysis. Statistical analysis for negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (PLR); negative likelihood ratio (NLR), Youden J index with correct classification rate with specificity and sensitivity are good for both A, malaria vs. all negative samples (normal and Dengue fever) and B, malaria vs. normal samples.

A		Reference Method		
N =	171	NEG	POS	
	70	101		
Test Flag	NEG	TN	FN	NPV
	155	129	26	0.832
	POS	FP	TP	PPV
	187	11	176	0.941
	Runs	140	202	342
		Specificity	Sensitivity	CorrectClass
		0.921	0.871	89.18%
		Youden J	PLR	NLR
		0.793	11.1	0.14

B		Reference Method		
N =	96	NEG	POS	
	70	26		
Test Flag	NEG	TN	FN	NPV
	136	129	7	0.949
	POS	FP	TP	PPV
	56	11	45	0.804
	Runs	140	52	192
		Specificity	Sensitivity	CorrectClass
		0.921	0.865	90.63%
		Youden J	PLR	NLR
		0.787	11.1	0.15

Table 2. Performance of malaria suspect flag on Pentra XLR using CBC and DIFF mode The statistical analysis for negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), Youden J index; correct classification rate; specificity and sensitivity are equally good for both A, P. vivax vs. all negative samples (normal and Dengue fever) and B, P with falciparum vs. normal samples.

A		Reference Method		
N =	285	NEG	POS	
	157	128		
Test Flag	NEG	TN	FN	NPV
	305	258	47	0.846
	POS	FP	TP	PPV
	265	56	209	0.789
	Runs	314	256	570
		Specificity	Sensitivity	CorrectClass
		0.822	0.816	81.93%
		Youden J	PLR	NLR
		0.638	4.58	0.223

B		Reference Method		
N =	198	NEG	POS	
	70	128		
Test Flag	NEG	TN	FN	NPV
	164	117	47	0.713
	POS	FP	TP	PPV
	232	23	209	0.901
	Runs	140	256	396
		Specificity	Sensitivity	CorrectClass
		0.836	0.816	82.32%
		Youden J	PLR	NLR
		0.652	4.97	0.22

Table 3. Performance of malaria suspect flag on Pentra XLR RETIC mode is equally good for both A, malaria vs. all negative samples (normal and Dengue fever) and B, malaria vs. normal samples. However, the modified reticulocyte analysis mode did not improve performance over the CBC + DIFF mode.

A		Reference Method		
N =	215	NEG	POS	
	87	128		
Test Flag	NEG	TN	FN	NPV
	156	123	33	0.788
	POS	FP	TP	PPV
	274	51	223	0.814
	Runs	174	256	430
		Specificity	Sensitivity	CorrectClass
		0.707	0.871	80.47%
		Youden J	PLR	NLR
		0.578	2.97	0.18

B		Reference Method		
N =	215	NEG	POS	
	87	128		
Test Flag	NEG	TN	FN	NPV
	188	141	47	0.75
	POS	FP	TP	PPV
	242	33	209	0.864
	Runs	174	256	430
		Specificity	Sensitivity	CorrectClass
		0.81	0.816	81.40%
		Youden J	PLR	NLR
		0.627	4.3	0.23

Table 4. Performance of malaria suspect flag on Pentra XLR using CBC + DIFF mode (A) and PXLR using RETIC mode (A) show equally good distinction between malaria vs. Dengue fever samples.

A		Reference Method		
N =	257	NEG	POS	
	141	116		
Test Flag	NEG	TN	FN	NPV
	252	218	34	0.865
	POS	FP	TP	PPV
	262	64	198	0.756
	Runs	282	232	514
		Specificity	Sensitivity	CorrectClass
		0.773	0.853	80.93%
		Youden J	PLR	NLR
		0.626	3.76	0.19

B		Reference Method		
N =	186	NEG	POS	
	70	116		
Test Flag	NEG	TN	FN	NPV
	158	124	34	0.785
	POS	FP	TP	PPV
	214	16	198	0.925
	Runs	140	232	372
		Specificity	Sensitivity	CorrectClass
		0.886	0.853	86.56%
		Youden J	PLR	NLR
		0.739	7.47	0.16

Table 5. Performance of malaria suspect flag on MicrosemiCRP using CBC + 3-part DIFF + CRP mode. The statistical performance are equally good for both A, malaria vs. all negative samples (normal and Dengue fever) and B, malaria vs. normal samples.

Instrument and Mode and Groups	Sensitivity	Specificity	Correct Class	NPV	PPV	NLR	PLR	Youden J
PentraXLR: CBC + 5DIFF malaria vs. all	0.871	0.803	83.33%	0.884	0.782	0.16	4.41	0.674
PentraXLR: CBC + 5DIFF malaria vs. normal	0.871	0.921	88.89%	0.796	0.953	0.14	11.10	0.793
PentraXLR: CBC + 5DIFF P. vivax vs. normal	0.871	0.921	89.18%	0.832	0.941	0.14	11.10	0.793
PentraXLR: CBC + 5DIFF P. falciparum vs. normal	0.865	0.921	90.63%	0.949	0.804	0.15	0.15	0.787
PentraXLR: CBC + 5DIFF malaria vs. Dengue	0.871	0.707	80.47%	0.788	0.814	0.18	2.97	0.578
PentraXLR: RETIC malaria vs. all	0.816	0.822	81.93%	0.846	0.789	0.22	4.58	0.638
PentraXLR: RETIC malaria vs. normal	0.816	0.836	82.32%	0.713	0.901	0.22	4.97	0.652
PentraXLR: RETIC P. vivax vs. normal	0.817	0.836	82.46%	0.760	0.878	0.22	4.97	0.653
PentraXLR: RETIC P. falciparum vs. normal	0.827	0.836	83.33%	0.929	0.652	0.21	5.03	0.663
PentraXLR: RETIC malaria vs. Dengue	0.816	0.810	81.40%	0.750	0.864	0.23	4.30	0.627
MicrosemiCRP: CBC+3DIFF+CRP malaria vs. all	0.853	0.773	80.93%	0.865	0.756	0.19	3.76	0.626
MicrosemiCRP: CBC+3DIFF+CRP malaria vs. normal	0.853	0.886	86.56%	0.785	0.925	0.16	7.47	0.739
MicrosemiCRP: CBC+3DIFF+CRP P. vivax vs. normal	0.851	0.886	86.62%	0.827	0.902	0.17	7.44	0.736
MicrosemiCRP: CBC+3DIFF+CRP P. falciparum vs. normal	0.875	0.886	88.27%	0.947	0.754	0.14	7.66	0.761
MicrosemiCRP: CBC+3DIFF+CRP malaria vs. Dengue	0.853	0.662	78.07%	0.734	0.805	0.22	2.52	0.515
MicrosemiCRP: CBC+3DIFF malaria vs. all	0.428	0.815	63.64%	0.624	0.665	0.70	2.31	0.243
MicrosemiCRP: CBC+3DIFF malaria vs. normal	0.428	0.929	60.15%	0.463	0.919	0.62	5.99	0.357
MicrosemiCRP: CBC+3DIFF P. vivax vs. normal	0.441	0.929	63.95%	0.533	0.900	0.60	6.18	0.370
MicrosemiCRP: CBC+3DIFF P. falciparum vs. normal	0.397	0.929	77.27%	0.788	0.697	0.65	5.55	0.325
MicrosemiCRP: CBC+3DIFF malaria vs. Dengue	0.428	0.720	54.17%	0.445	0.706	0.79	1.53	0.148

Table 6. . Performance summary of malaria suspect flag on Pentra XLR and MicrosemiCRP for all modes tested and comparison among the various clinical groups (All = normal + Dengue).

5. Conclusions

1. The malaria suspect flag developed with datamining techniques on the Pentra XLR, both in CBC + Diff and RETIC modes, and MicrosemiCRP in CBC + DIFF + CRP mode provide effective screening for malaria.
2. Reticulocyte analysis does not significantly improve the performance of malaria detection on Pentra XLR over routine CBC + Diff mode.
3. The MicrosemiCRP malaria flag requires the addition of C reactive protein measurement to achieve suitable performance of sensitivity and NPV.
4. The malaria flags also provide good distinction between malaria and Dengue fever and equal performance for both P. vivax and P. falciparum
5. Effective malaria suspect flags can be developed on low and middle range instruments and are more practical solutions for malaria endemic areas, which tend to be in economic developing countries.