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Evaluation of False + ve Cases & Diagnostic Accuracy of Abbotts CELL-DYN RUBY for Diagnosis of Malaria Parasite

Mirza Asif Baig

MD (Pathology) Former Asst. professor BLDUs ShriB.M.Patil medical college, Hospital &Research centre Bijapure, Karnataka, India

Abstract: ***Background :Malaria is a parasitic disease world wide distributed with a high morbidity and mortality. A rapid and accurate method is needed to detect the presence of malaria parasites in blood. Automatically generated alert could improve the detection capacity of these instruments and potentially expand their clinical utility in malaria diagnosis. Methodology &Results : The total Sample size was 275 cases. The blood samples were run in abbott's Cell-Dyn Ruby. The Sensitivity = 94.90% , Specificity = 95% , Positive predictive value = 97% & Negative predictive value = 91%Conclusion : Cell-Dyn Ruby has high sensitivity & specificity in detection of malarial parasite & even can be utilized in therapeutic monitoring of malaria patients. The flag / Alert ATYP DEP does not always means malaria infection because Ruby can give some false positive results due to certain drugs & few false negative results. The accuracy for malaria diagnosis may vary according to species, parasite load, immunity and clinical context where the method is applied. The Alert/ flag ATYP DEP should be used in conjunction with Platelet count , frequency of atypical depolarization &Clinical symptoms. This combined correlation can yield a high diagnostic accuracy & probably give 100% sensitivity & specificity.***

Keywords: Automated analyzer, Sensitivity, Specificity, Haemozoin, Malaria Parasite

1. Introduction

Every year, more than 500 million people are infected with malaria and 2.5 million malaria patients die from the disease. Main problem in controlling malaria is the limited access to effective diagnosis and treatment in endemic areas. The classical method for detecting malaria parasites is the examination of Giemsa stained thick and thin blood smears, which is labourous time-consuming and importantly, limited by light microscopy. [1]

When the level of parasitaemia is low,diagnosis by Giemsa-stained blood smears requires long periods of scanning by Pathologists (Moody 2002)[2]. This problem persists even with the advent of several alternative methods such as rapid dipstick tests (Iqbalet al. 1999, 2000)[3]

Mendelow and Coetzer (1999) showedthat automated hematology analyzers, such as the Cell-Dyn 3500 (CD3500), detect malaria during the routine fullblood counts. Malarial pigments ingested by phagocytescause the phagocytosing cells to also depolarize lightand thereby triggering cellular events in regions of the instrument's scatter plot, which are distinct from theeosinophil population (Hanscheidet al. 2000)[4]. Theappearance of cellular events, classified as monocytes (depicted as purple dots), occurs above the separation line between neutrophils and eosinophils in the eosinophil area(green dots), and is then used as an indicator for thepotential presence of malaria.[5]

CELL-DYN Ruby gives an Alert /flag " ATYP DEP " (Atypical Depolarisation) events

Detected in lobularity 90 deg. & Granularity 90 deg. Scatter data , such Alert can be seen in few other conditions which gives false + ve for Malaria parasite

The main Objective of this study is to:

- Asses False +ve Misdiagnosed cases of Malaria Parasite given by CELL-DYN Ruby
- Evaluate the Diagnostic Accuracy of CELL-DYN for MP

2. Methodology

The present study was conducted in tertiary referral hospital. Sample Size = 275

This study includes a total of 175Malaria Parasite positive cases diagnosed on Giemsa stained thick & thin smears &PCR. 100 normal blood samples from healthy individuals were taken as control .The p value, Sensitivity ,Specificity, + ve predictive value & -vepredictive value were calculated by standard statistical formulae

Special emphasis was give on False +ve Cases of Malaria parasite

M.P False Positivity

Few CBC samples run on CELL-DYN Ruby showed the Aert/ Flag " ATYP DEP " ie Atypical depolarization suggesting Malaria positivity. This samples were thoroughly & meticulously scanned for M.P by thick & thin smear & PCR but these samples were found to be M.P negative. These samples were labelled as False +ve for M.P

Reason for False +Ve M.P on Cell-Dyn Ruby

Few neonatal CBCs displayed ATYP DEP Alert / flag, so these cases were examined for Giemsa Stained thick & thin smears which were negative for Malaria parasite. To our surprise we found that these smears showed yellowish refractile Granules & these neonates were receiving sulfonamide & its derivatives as medications. It was concluded that sulfonamide drugs formed insoluble yellow

granules which interefered with the Light scattering mechanism of Ruby which was misinterpreted as malarial pigment & so a flag of ATYP DEP was noted

M.P False Negativity

When the Parasitic Density is low , cell-Dyn Ruby fails to recognizes the Malaria parasite

3. Results

Sample Size = 175+100 =275

Total number of Malaria Parasite(M.P) + ve cases diagnosed by Giemsastained Thick & thin Smears and PCR = 175

Total number of M.P +ve cases on CELL-DYN Ruby=166

False positive cases of MP given by Ruby = 05 (these cases were MP negative on PCR &Giemsa stained smears)

False negative cases given by CD Ruby due to low parasitemia = 09

Table 1: Statistical Analysis of Diagnostic Accuracy of CELL-DYN Ruby

	Disease	Non disease	
Positive test by CD Ruby	166 True positive	05 False positive	171
Negative test by CD Ruby	9 False Negative	95 True Negative	104
	175	100	275

Sensitivity = 94.90%
Specificity = 95%
Positive predictive value = 97%
Negative predictive value = 91%

4. Discussion

Mendelow and Coetzer (1999) showed that automated hematology analyzers, such as the Cell- Dyn 3500 (CD3500), detect malaria during the routine fullblood counts. Although current haematologyanalysers are not specifically designed to detect malaria-related abnormalities, most studies have found sensitivities that comply with WHO malaria-diagnostic guidelines, i.e. ≥ 95% in samples with > 100 parasites/µl[6,7]

Cell-Dynanalysers& detection of malaria pigment (haemozoin)[8]

The Cell-Dyn instruments use laser light scatter at various angles, the so called multiple-angle polarized scatter separation (MAPSS) for WBC analysis. MAPSS used to distinguish eosinophils from neutrophils based on the light depolarizing properties of their granules, but has also been found to detect haemozoin-containing monocytes and granulocytes .These malaria-related events usually appear in a scatter-plot with 90° side-scatter on the *x*-axis and 90° depolarized side-scatter on the *y*-axis, usually labelled as lobularity/granularity scatter-plot in the CD 3000 series or NEU-EOS in the CD 4000. Further studies confirmed that atypicaldepolarization is due to haemozoin-containing monocytes and neutrophils.

Cell-Dynanalyserscolour code events are

Purple dots : are probably due to depolarizing monocytes
Green dots :could be indicative of haemozoin-containing granulocytes
Blue dots : Abnormal depolarizing lymphocytes
Red & Black dots :Hemozoin containing parasites

Kramer et al , post-treatment study showed initial black dots changed to green dots during the treatment. This phenomenon may indicate that the decreased level of parasites was associated with green dots in the scatter plot. Moreover, the group of specimens exhibiting black dots showed significantly higher parasitaemia levels than the group with green dots

Table 2: Studies evaluating diagnostic performance of Cell-Dyn Ruby

Author	Sample size	M.P positive	Sensitivity	Specificity
Mendelow, 1999	224	95	72%	96%
Rathod[9]	523	135	62.2%	25.3%
Padial	411	40	72%	98%
Josephine,[10] 2005, Malaysia	889	20	100%	100%
De Langen,[11] 2006,	208	90	93%	97%
Hanscheid,[12] 2008,	368	152	96%	96%

Present study: is very well correlated with other studies

Sensitivity = 94.90%, Specificity = 95% , Positive predictive value = 97%

Negative predictive value = 91%

M.P False Positivity

Few CBC samples run on CELL-DYN Ruby showed the Aert/ Flag " ATYP DEP " ie Atypical depolarization suggesting Malaria positivity. This samples were thoroughly & meticulously scanned for M.P by thick & thin smear & PCR but these samples were found to be M.P negative. These samples were labelled as False +ve for M.P

Interesting fact :REASON FOR FALSE +VE M.P ON CELL-DYN Ruby:

Few neonatal CBCs displayed ATYP DEP Alert / flag, so these cases were examined for Giemsa Stained thick & thin smears which were negative for Malaria parasite. To our surprise we found that these smears showed yellowish refractile Granules & these neonates were receiving sulfonamide & its derivatives as medications. It was concluded that sulfonamide drugs formed insoluble yellow granules which interefered with the Light scattering mechanism of Ruby which was misinterpreted as malarial pigment & so a flag of ATYP DEP was noted

This is probably first study to discover the fact that Drugs& medicationcan cause atypical depolarization.

5. Conclusion

The latest Automated Hematology analyzer Abbott's Cell-Dyn Ruby has high sensitivity & specificity in detection of malarial parasite & even can be utilized in therapeutic monitoring of malaria patients. The flag / Alert ATYP DEP does not always means malaria infection because Ruby can give some false positive results due to certain drugs & few false negative results. The accuracy for malaria diagnosis may vary according to species, parasite load, immunity and clinical context where the method is applied.

The Alert/ flag ATYP DEP should be used in conjunction with the following parameters

1) Platelet count
2) frequency of atypical depolarization
3) Clinical symptoms

This combined correlation can yield a high diagnostic accuracy & probably give 100% sensitivity & specificity

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