Int J Biol Med Res
Volume 6, Issue 2, April 2015

International Journal of
BIOLOGICAL AND MEDICAL RESEARCH

Original article

Automated Body fluid Analysis – A Comparative evaluation of diagnostic performance of Abotts Cell DYN Ruby &Sysmex -XT 2000i

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ARTICLE INFO

ABSTRACT

Background :-Body fluid cell counts are a routine laboratory test that provides valuable information to clinicians for diagnosing and treating a wide variety of medical conditions.An Automated Analyser can screen large number of body fluids in less time, with more precision & the results should be comparable to manual hemcytometer counting method.Manual total WBC count & differential count were compared with the results obtained from Abotts CELL DYN RUBY & SYSMEX – XT 2000i.Methodology : -The Comparative analysis of body fluid sample by Manual method & Cell Dyn Ruby showed p value < 0.05 which is statistically significant &Sensitivity of Ruby= 95.4 % & Specificity= 96.4 %. Conclusion. Most of the laboratories are using Automated Analyzer for body fluid analysis as it is less time consuming. AbottsCell Dyn Ruby can perform fluid analysis very precisely & with small quantity of fluid. It offers extra advantage by giving alert/ flag if malignant cells are present. It is safe to say that cell Dyn Ruby is the best among all Analyzers for body fluid analysis& it will completely replace manual method of counting cells in near future.We personally recommend that each fluid sample should be mandatorily run in Ruby to screen for WBC count & malignant cells.

Introduction

Body fluid cell counts are a routine laboratory test that provides valuable information to clinicians for diagnosing and treating a wide variety of medical conditions.1

If done meticulously , body fluid analysis has great diagnostic significance & implications . It can easily categorise the fluid into Inflammatory, infectious & malignant types. CSF analysis in bacterial meningitis is most dreaded & frequently encountered medical emergencies. CSF examination forms a important part to assess the prognosis, relapse & remission in Acute leukemias.Total WBC& differential count (cytocentrifugedpreparation) by Manual hemcytometerare still considered the "gold standard" methods.2Manual cell counting is extremely labor-intensive and time-consuming, and skilled personnel have to be available 24 hours a day, and 7 days aweek& manual method also has poor precision and large interobserver variability.3

An Automated Analyser was required to screen large number of body fluids inless time, with more precision & the results should be comparable to manual Hemcytometercounting method. This prompted me to undertake the following study. We evaluated the analytic and clinical performance of theCell-Dyn Ruby &Sysmex – XT 2000i & compared the results with manual method.4

METHODOLOGY & RESULTS

This is 1 year study (April 2012- March 2013) carried out in a tertiary terminal Referral   pediatric hospital. For the sake of conveniencebody fluid is categorized into serous (pleural + peritoneal + synovial) & CSF. Total WBC count inBody fuids is counted manually in Neubeur chamber with outmost care and precision.WBC differential count is done from the cell Sediment obtained by Cytocentrifugation (1250 rpm for 5 min.) of the body fluids. The cell sediment was stained with Giemsa stain for differential count.

This manual total WBC count &differential   counts were compared with the results obtained from Abotts CELL DYN RUBY & SYSMEX – XT 2000i. The Statistical calculation for sensitivity, specificity & p values weredone to assess, which autoanalyser is better for body fluid analysis.

Essential adjustments in Cell- Dyn Ruby

The differential count given by Ruby is categorized into Polymorphonuclear cells (PMN) & Mononuclear cells (MN).

PMN Cells = (% neutrophils + % eosinophils + % basophils) as given by Ruby.1

MN cells =(% lymphocytes + % monocytes).1

If body fluid sample has RBCs then sample is run in CBC + RRBC mode to get corrected WBC count in Ruby

CSF sample in leukemia cases is carefully checked for Alert/ Flag for BLASTs in Ruby

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Results

A total of 1050 Body fluid samples were Analysed in 1 year.

Serous fluid = (peritoneal + pleural + Synovial) = 550 cases

CSF = 500 Cases

Table 1) – WBC & differential count by Manual counting method

Body fluid (1050)	Total No. of cases	% of total cases	No. of cases with normal total WBC count	No. of cases with increased total WBC count	Differential count
CSF	500	47.6 %	320 (30 %)	180 (17.2 %)	
Acute Leukemia	255	24 %	248 (23 %)	07 (0.6 %)	1 -2 % BLASTS
ALL	230	21 %	224	06	
AML	25	2.3 %	24	01	1% BLASTS
Infections	160	15.2 %	32 (3 %)	128 (12 %)	
Bacterial	84	08 %	10	74	Neutro predominant
Viral	76	7.2 %	22	54	Lympho predominant
Other causes (febrile convulsions, inflammatory, Sepsis)	85	08 %	40	45	Lympho predominant
Serous fluid	550	52.4 %	245 (23.3 %)	305 (29 %)	Predominantly NEUTROPHILS

Table 2) Comparative analysis of cell Dyn Ruby with manual counting method

	MANUAL COUNTING	CELL DYN RUBY
CSF		
Total WBC Count	No. of patients	No. of patients
Normal	320	332
Increased	180	168
Differential count	RELAPSE 7 Leukemia cases shows BLASTS	All the 7 relapse cases shows alerts for Blasts
SEROUS Fluid		
Total WBC Count	No. of patients	No. of patients
Normal	245	255
Increased	305	295

Table 3) Statistical analysis of cell Dyn Ruby with manual counting method

	Manual cell counting	Cell DYN Ruby	p value
Normal WBC in Body Fluids	565 cases	587 cases	P value < 0.05 Statistically significant
Increased WBC in Body Fluids	485cases	463cases	P value<0.05 Statistically significant
Differential count	550 (52.4%) of total patients showed Neutrophilia 320 (30.2%) of total patients showed lymphocytosis 18% cases have WBC=0/mm	575 (54.70%) of patients showed Neutrophilia 275 (26.20%) of patients showed lymphocytosis 20% cases have WBC=0/mm	The differential count by Ruby very well correlated with the manual count P< 0.05

Out of total 1050 cases   550 (52.4%) of cases were of neutrophilia as counted by manual method. More than 90 % of these cases have differential neutrophil count 70%. Out of total 1050 cases 575 (54.7%) of cases were of neutrophilia as analysed by Cell Dyn Ruby. More than 90 % of these cases have differential neutrophil count 70%

Sensitivity = 95.4 %

Specificity= 96.4 %

Table 4) Sysmex – XT 2000i vsmanual counting method

	Manual cell counting	sysmex – XT 2000i	p value
Normal WBC in Body Fluids	565 cases	425 cases	P value >0.05 Statistically insignificant
Increased WBC in Body Fluids	485cases	625cases	P value>0.05 Statistically insignificant
Differential count	550 (52.4%) of total patients showed Neutrophilia 320 (30.2%) of total patients showed lymphocytosis 18% cases have WBC=0/mm	560 (53.30%) of patients showed Neutrophilia 270 (25.80%) of patients showed lymphocytosis 22% cases have WBC=0/mm	The differential count by Ruby very well correlated with the manual count P< 0.05

DISCUSSION

A total of 1050 body fluids were studied in 1 year period. CSF & Serous fluid accounted for   47.6 % & 52.4 % of total cases respectively.

Acute leukemic cases comprised   51 % of total CSF samples.CSF of 6 cases of ALL & 1 case of AML showed Blasts and were labeled as Relapse with CNS involvement. 3 cases have undergone complete chemotherapy regime and had normal WBC & PLT. The interesting point is that in all the 7 relapsed leukemiccases, the Cell DYN Ruby shows Alert/ flag for BLASTS & alerted us for possible CNS relapse. The WBC count was high ranging from 300 to 1550 cells/mm in all the cases.The increased percentage of leukemia in this study is that it is a tertiary referral pediatric hospital.

Of the 1050 body fluids sample counted by the Gold standard manual method , 53.80 % were with normal WBC count & 46.20% with increased WBC count. The differential count showed 52.4% were neutrophilia&   30% were lymphocytosis. The results obtained by running the fluid sample in Abotts Cell Dyn Ruby shows very good correlation with the manual cell count. The fluid WBC count & differential were almost similar with the manual & Ruby showing very good agreement.

The Comparative analysis of body fluid sample by Manual method & Cell Dyn Ruby showed p value < 0.05 which is statistically significant .Sensitivity of Ruby= 95.4 % &Specificity= 96.4 %

These results were in good correlation with other studies conducted by Hoffman et al.5,6,7,8 These studies compared the results of body fluid analysis with manual & Ruby showed p value< 0.05 & Ruby showed a sensitivity of 95 % , specificity = 95%, positive predictive value =95 % & Negative predictive value = 95%. 1.

The fluid samples when analysed in Sysmex – XT 2000i , which showed a poor correlation in terms of total WBC count as compared with Manual count. The p value is > 0.05 &is statistically insignificant. However the differential count given by sysmex was in good correlation with manual method.

Observations

·   This Present study & previous studies   with there statistical Analysis shows that Abotts cell Dyn Ruby can be effectively used for body fluid analysis & if sampled meticulously can even replace Manual counting method

·   The Cell DYN ruby gives similar results as that of standard manual method at normal, low or High WBCs.

In fact it offers additional advantages as compared to standard manual method like

·   Ruby can perform far more body fluid analysis as compared to manual method during a given period of time. Within a short period it can analyze many samples

·   The results given by ruby are very precise as compared to Sysmex –XT 2000i. Sysmex gives more false positive results , higher WBC counts & is imprecise with low count samples.

Sysmex require more quantity of fluid sample as compared to ruby(this is very important in CSF analysis as the sample taken will belittle).few drop of CSF sampleis taken for analysis in leukemic cases where in Ruby plays pivotal role. In this study Ruby gave an Alert/flag of BLAST at very low volume of CSF.

This is an extraadvantage offered by Ruby which makes this machine the best among allAutoanalysersfor body fluid analysis

·   If fluid sample has RBCs then Ruby should be run in CBC + RRBC mode to get the corrected WBC count.

·   Manual cell counting is extremely labor-intensive and time-consuming, and skilled personnel have to be available 24 hours a day, and 7 days a week and large interobservervariability.

Limitations of Autoanalysers

Typically, automated hematology analyzers are not used for clear and colorless

cerebrospinal fluids with low cellular counts because of poor reproducibility and high background counts at medically important clinical decision limits of 0 to 5 cell/µL for adults. Other types of specimens that laboratories may not want to run on hematology analyzers are purulent and flocculent specimens, because there is concern that those specimens could clog flow cells or apertures. Additionally, crystals in synovial fluids may cause a falsely increased count and may need to be confirmed with the manual count.9

Very Expensive

Background Concentration Limits

Background concentrations represent apparent sample-related constituents that actually originate from blood-free reagents and/or electronic "noise" and are used to confirm the system's baseline performance, where no actual sample is aspirated. The manufacturer's acceptable background concentration limits that must be met before using the instrument are 100/µL or less for the WBC count and 20,000/µL or less for the RBC count. The instruments should be adjusted for this background concentrations.

Carryover

Ruby &Sysmex tends to give false high values for WBC count because these Autoanalysers are normally running bloodsamples with higher cell concentrations than in body fluids & so carryover is an important issue

Corrective action- Before running Body fluid sample, a blank normal saline sample is run to wash the Carryover sample from blood.

CONCLUSION

Most of the laboratories are using Automated Analyzer for body fluid analysis as it is less time consuming. Abotts Cell Dyn Ruby can perform fluid analysis very precisely & with small quantity of fluid. It offers extra advantage by giving alert/ flag if malignant cells are present.

It is safe to say that cell Dyn Ruby is the best among all Analyzers & it will completely replace manual method of counting cells in fluid samples.

I personally recommend that each fluid sample should be mandatorily run in Ruby to screen for WBC count & malignant cells.

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