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Original Research Article

Evaluation of Accuracy of Cell Count in Ascitic Fluid in Automated Cell Counter and Comparison with Manual Count

Kusum Agrawal[1], Sandhya Gulati[2], Gaurav Gupta[3], Nidhi Sharma[4], Ajay Yadav[5]

[1]Final year Resident, [2]Professor, [4]Assistant professor, [5]Professor & Head, Department of Pathology,
[3]Associate Professor, Department of Gastroenterology,
S.M.S. Medical College & Hospital, Jaipur, Rajasthan, India.

ABSTRACT

Introduction: Correct cell enumeration and differential analysis of body fluids are important in the diagnosis and management of several diseases. Currently, microscopic analysis is still considered the "gold standard". The introduction of automated analysis has reduced interoperator variability, improved turnaround time and precision. The present study was designed to determine the shelf life and appropriate anticoagulant for automated cell counter and to compare manual and automated cell count in ascitic fluid.

Methods: We examined total 250 ascitic fluid samples. Total and differential cell counting of each sample has been conducted with the Sysmex XT-4000i and the manual method (Neubauer chamber). Linearity, carryover, precision and correlation were also assessed.

Results: The precision analysis of random sample with high cell count indicated that the Sysmex XT-4000i demonstrated good precision for RBC, WBC, MN#, PMN# at 0 and 6 hour in all vials resulted in CVs <0.012%. Carryover effect was negligible for both WBC and RBC count in ascitic fluid. It never exceeded 0.180%. Sysmex XT-4000i showed significant positive correlation for WBC count in plain vial (r=0.984, p<0.001s), EDTA vial (r= 0.998, p<0.001s), PT vial (r=0.958, p<0.001s) at 0 hour and those kept at 4°c and reanalyzed at 6 hour.

Conclusion: With some limitations, total and differential cell counts in ascitic fluid can be reliably determined using the Sysmex XT-4000i instrument.

Keywords: Ascitic fluid, Neubauer chamber, Sysmex XT-4000i, cell count.

***Correspondence to:**

Dr. Kusum Agrawal,
Final Year Resident, Department of Pathology,
S.M.S. Medical College & Hospital, Jaipur, Rajasthan, India.

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INTRODUCTION

Body fluid cell counts are a routine clinical laboratory test that provides valuable information for diagnosing and treating various medical conditions.[1] Manual microscopy, mostly based on total cell count and cytocentrifuged differential count is still considered the gold standard for body fluid analysis. Manual microscopy carries several important technical and analytical drawbacks such as inherent complexity, reduced turnaround time, the need for skilled and specialized personnel along with large interobserver variability and low precision.[2]

The use of automated hemocytometric analyzers has gained acceptance as an alternative method to manual microscopy for initial screening of abnormalities in BFs.[3] Automation offers potential benefits of improved accuracy, precision, laboratory efficiency and cost effectiveness.[4]

Sysmex XT-4000i is the fully automated analyzer developed by Sysmex containing a body fluid specific mode. The analyzer applies proven impedance and fluorescence flow cytometry ensuring an accurate body fluid count. It provides expanded sensitivity and linearity; WBC and RBC counts reportable to 3 decimal places. Seven body fluid reportable parameters are: WBC-BF, RBC-BF, TC-BF, MN%, MN#, PMN%, PMN#. No sample pretreatment, no additional reagents and no additional quality control material required. It has benefits of improved productivity, decreased turnaround time and decreased manual technical intervention.[5]

The aims of our study were to determine the shelf life and appropriate anticoagulant for automated cell counter and comparison of automated cell count with manual count.

MATERIAL AND METHODS

Study design: This was a laboratory based validational analytic type of observational study conducted in HLA and Advanced Hematology Lab, Department of pathology, SMS Medical College, Jaipur, Rajasthan.

Study population: All cases presenting with ascites from June 2014 to December 2015 were studied. We examined 250 ascitic fluid samples for our study.

Inclusion criteria: Freshly obtained all ascitic fluid samples.

Exclusion criteria: Those patients who did not give consent, there is delay in transport of samples.

METHODOLOGY

Ascitic fluid collection: Ascitic fluid was collected by abdominal paracentesis under aseptic conditions.Ascitic fluid sample was

collected in plain tubes, in tubes containing EDTA as anticoagulant and in tubes containing Na-citrate as anticoagulant.

WBC count was determined by both traditional method with a light microscope in a manual counting chamber (Neubauer chamber) and also with automated cell counter (Sysmex XT-4000i). Both total leukocyte count and differential cell count were determined. Samples were analysed at 0 hour and then stored at 4°c and reanalyzed at 6 hours.

Manual method

Neubauer counting chamber were used to perform manual RBC and nucleated cell counts. RBC count was determined by counting the cells in the standard 5 small squares in the centre square on each side of the chamber and the nucleated cells were counted in the 4 larger corner squares on each side of the chamber. The standard Neubauer calculation formula was used to determine the number of cells per cubic mm.[6]

Automated cell counter method

For WBC count by the automated method, the samples in plain vial, EDTA vial, Na-citrate vial were run in the automated hematology analyzer Sysmex XT- 4000i and values were noted. Automated system module Sysmex XT-4000i uses direct current (DC) and radiofrequency impedance for RBC count and fluorescence flow cytometry for WBC.

Precision

Within run precision of the Sysmex XT-4000i instrument for both high and low cell counts were analyzed ten times consecutively within a day. The results were expressed as the CV% at different mean cell concentrations.

Carryover

A sample with a high cell count (H1, H2, H3) was measured 3 times followed by three measurements of sample with a low cell count (L1, L2, L3). Carryover% was calculated as follows: {(L1-L3) / (H3-L3)} ×100.[3]

Linearity

This was verified for total WBC count, mononuclear cells, polymorphonuclear cells and RBC count by running a ascitic fluid five times in dilution. Then results of cell counts compared with expected cell counts.

Statistical analysis

Pearson's correlation coefficient were calculated between WBC and differential counts generated by Sysmex XT-4000i hematology analyzer and those determined by manual microscopic method. Agreement between the methods was determined using Passing-Bablok regression analysis.

Table 1a: Precision Analysis Of Random Sample With High Cell Count In (Plain Vial) At 0 And 6 Hour

	PLAIN VIAL AT 0 HOUR						PLAIN VIAL AT 6 HOUR					
SAMPLE RUN	S-WBC	S-RBC	MN#	PMN#	MN%	PMN%	S-WBC	S-RBC	MN#	PMN#	MN%	PMN%
1	1.151	0.009	0.478	0.67	41.5	58.5	0.627	0.008	0.361	0.266	57.6	42.4
2	1.125	0.009	0.448	0.68	39.8	60.2	0.644	0.008	0.328	0.316	50.9	49.1
3	1.142	0.008	0.467	0.68	40.9	59.1	0.623	0.009	0.346	0.277	55.5	44.5
4	1.066	0.009	0.417	0.65	39.1	60.9	0.614	0.009	0.322	0.292	52.4	47.6
5	0.999	0.009	0.376	0.62	37.6	62.4	0.727	0.01	0.359	0.368	49.4	50.6
6	0.966	0.009	0.369	0.6	38.2	61.8	0.702	0.009	0.33	0.372	47	53
7	1.014	0.008	0.375	0.64	37	63	0.739	0.009	0.362	0.377	49	51
8	0.994	0.009	0.374	0.62	37.6	62.4	0.811	0.009	0.357	0.454	44	56
9	0.959	0.009	0.366	0.59	38.2	61.8	0.895	0.01	0.405	0.49	45.3	54.7
10	0.962	0.009	0.353	0.61	36.7	63.3	0.855	0.009	0.365	0.49	42.7	57.3
MEAN	1.04	0.01	0.40	0.64	38.66	61.34	0.72	0.01	0.35	0.37	49.38	50.62
SD	0.08	0.00	0.05	0.03	1.63	1.63	0.10	0.00	0.02	0.08	4.86	4.86
CV%*	0.01	0.00	0.00	0.00	2.65	2.65	0.01	0.00	0.00	0.01	23.61	23.61

***CV=Coefficient of variation**

Table 1b: Precision Analysis Of Random Sample With High Cell Count In (EDTA Vial) At 0 And 6 Hour

	EDTA VIAL AT 0 HOUR						EDTA VIAL AT 6 HOUR					
SAMPLE RUN	S-WBC	S-RBC	MN#	PMN#	MN%	PMN%	S-WBC	S-RBC	MN#	PMN#	MN%	PMN%
1	1.31	0.009	0.548	0.76	41.8	58.2	0.626	0.008	0.182	0.444	29.1	70.9
2	1.2	0.008	0.456	0.74	38.1	61.9	0.912	0.008	0.228	0.684	25	75
3	1.19	0.008	0.385	0.81	32.3	67.7	0.989	0.008	0.24	0.749	24.3	75.7
4	1.17	0.009	0.341	0.83	29.2	70.8	0.865	0.008	0.211	0.654	24.4	75.6
5	1.12	0.008	0.297	0.83	26.4	73.6	0.913	0.009	0.203	0.71	22.2	77.8
6	1.12	0.009	0.306	0.82	27.2	72.8	0.953	0.008	0.21	0.743	22	78
7	1.15	0.009	0.257	0.89	22.4	77.6	0.958	0.008	0.207	0.751	21.6	78.4
8	1.11	0.008	0.256	0.85	23.2	76.8	0.955	0.009	0.216	0.739	22.6	77.4
9	1.09	0.009	0.231	0.86	21.1	78.9	0.799	0.009	0.197	0.602	24.7	75.3
10	1.07	0.009	0.245	0.82	22.9	77.1	0.926	0.009	0.196	0.73	21.2	78.8
MEAN	1.153	0.009	0.332	0.821	28.460	71.540	0.890	0.008	0.209	0.681	23.710	76.290
SD	0.070	0.001	0.103	0.044	7.004	7.004	0.107	0.001	0.017	0.096	2.350	2.350
CV%	0.005	0.000	0.011	0.002	49.054	49.054	0.012	0.000	0.000	0.009	5.523	5.523

Table 1c: Precision Analysis Of Random Sample With High Cell Count In (PT Vial) At 0 And 6 Hour

	PT VIAL AT 0 HOUR						PT VIAL AT 6 HOUR					
SAMPLE RUN	S-WBC	S-RBC	MN#	PMN#	MN%	PMN%	S-WBC	S-RBC	MN#	PMN#	MN%	PMN%
1	0.93	0.008	0.379	0.56	40.6	59.4	0.884	0.007	0.343	0.541	38.8	61.2
2	0.97	0.008	0.385	0.58	39.9	60.1	0.897	0.008	0.339	0.558	37.8	62.2
3	1.03	0.007	0.397	0.64	38.5	61.5	0.994	0.008	0.349	0.645	35.1	64.9
4	1.04	0.008	0.353	0.69	34	66	0.804	0.006	0.281	0.523	35	65
5	1.04	0.007	0.369	0.68	35.3	64.7	0.964	0.007	0.325	0.556	31.8	68.2
6	1.02	0.007	0.324	0.69	31.9	68.1	0.98	0.007	0.332	0.582	32.8	67.2
7	1.02	0.007	0.322	0.7	31.4	68.6	0.811	0.007	0.321	0.634	38.4	61.6
8	0.96	0.008	0.307	0.66	31.8	68.2	0.895	0.008	0.354	0.672	39.2	60.8
9	0.98	0.007	0.322	0.66	32.9	67.1	0.855	0.008	0.347	0.575	30.5	69.5
10	1.05	0.007	0.32	0.73	30.5	69.5	0.84	0.008	0.327	0.701	34.5	65.5
MEAN	1.005	0.007	0.348	0.657	34.680	65.320	0.892	0.007	0.332	0.599	35.390	64.610
SD	0.040	0.001	0.033	0.054	3.729	3.729	0.068	0.001	0.021	0.060	3.083	3.083
CV%	0.002	0.000	0.001	0.003	13.906	13.906	0.005	0.000	0.000	0.004	9.505	9.505

Table 1d: Precision Analysis Of Random Sample With Low Cell Count In (EDTA Vial) At 0 And 6 Hour

	EDTA VIAL AT 0 HOUR						EDTA VIAL AT 6 HOUR					
SAMPLE RUN	S-WBC	S-RBC	MN#	PMN#	MN%	PMN%	S-WBC	S-RBC	MN#	PMN#	MN%	PMN%
1	0.08	0	0.073	0.00	94.8	5.2	0.194	0	0.147	0.047	75.8	24.2
2	0.08	0	0.076	0.01	92.7	7.3	0.191	0	0.131	0.06	68.6	31.4
3	0.07	0	0.066	0.00	95.7	4.3	0.201	0	0.153	0.048	76.1	23.9
4	0.08	0	0.072	0.01	92.3	7.7	0.182	0	0.136	0.046	74.7	25.3
5	0.09	0	0.083	0.01	90.2	9.8	0.207	0	0.137	0.07	66.2	33.8
6	0.09	0	0.061	0.02	71.8	28.2	0.206	0	0.132	0.074	64.1	35.9
7	0.09	0	0.077	0.01	86.5	13.5	0.221	0	0.134	0.087	60.6	39.4
8	0.10	0	0.086	0.02	82.7	17.3	0.162	0	0.144	0.018	88.9	11.1
9	0.12	0	0.109	0.01	94.8	5.2	0.238	0	0.141	0.097	59.2	40.8
10	0.13	0	0.12	0.01	90.9	9.1	0.247	0	0.142	0.105	57.5	42.5
MEAN	0.09	0.00	0.08	0.01	89.24	10.76	0.20	0.00	0.14	0.07	69.17	30.83
SD	0.02	0.00	0.02	0.01	7.33	7.33	0.03	0.00	0.01	0.03	9.75	9.75
CV%	0.00	0.00	0.00	0.00	53.67	53.67	0.00	0.00	0.00	0.00	95.08	95.08

RESULTS

Precision

The precision analysis of random sample with high cell count indicated that the Sysmex XT-4000i demonstrated good precision for RBC, WBC, MN#, PMN# at 0 and 6 hour in all vials resulted in CVs <0.012%.

Results of precision of low cell count were excellent in EDTA vial with CVs being 0% for RBC, TLC, MN#, PMN# at 0 and 6 hour. (Table 1a-1d)

Carryover

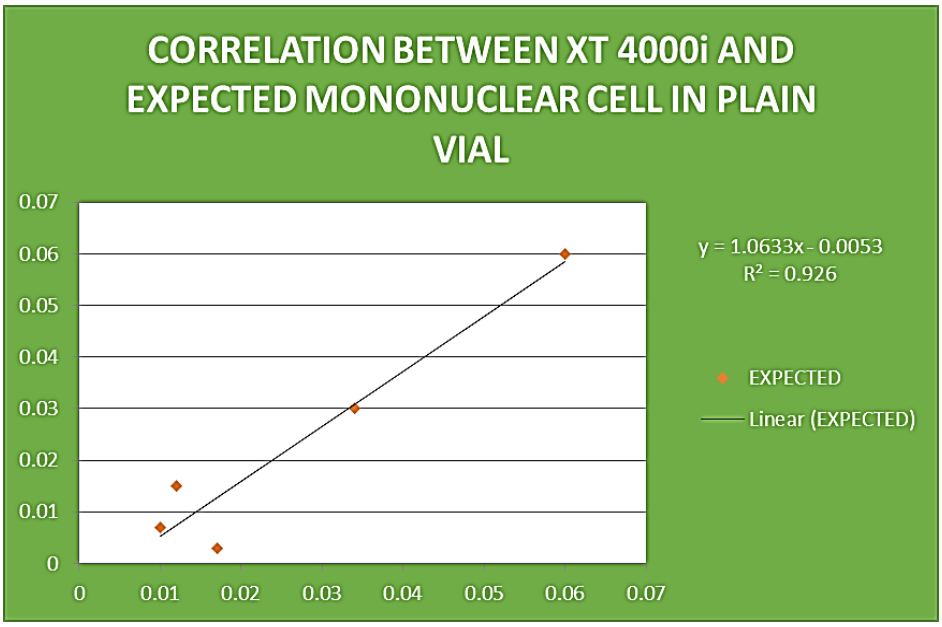
Carryover effect on the Sysmex XT-4000i BF mode was negligible for both WBC and RBC in ascitic fluid. It never exceeded 0.180% for WBC count. For RBC carryover results were 0% in all vials at 0 and 6 hour. (Table 2)

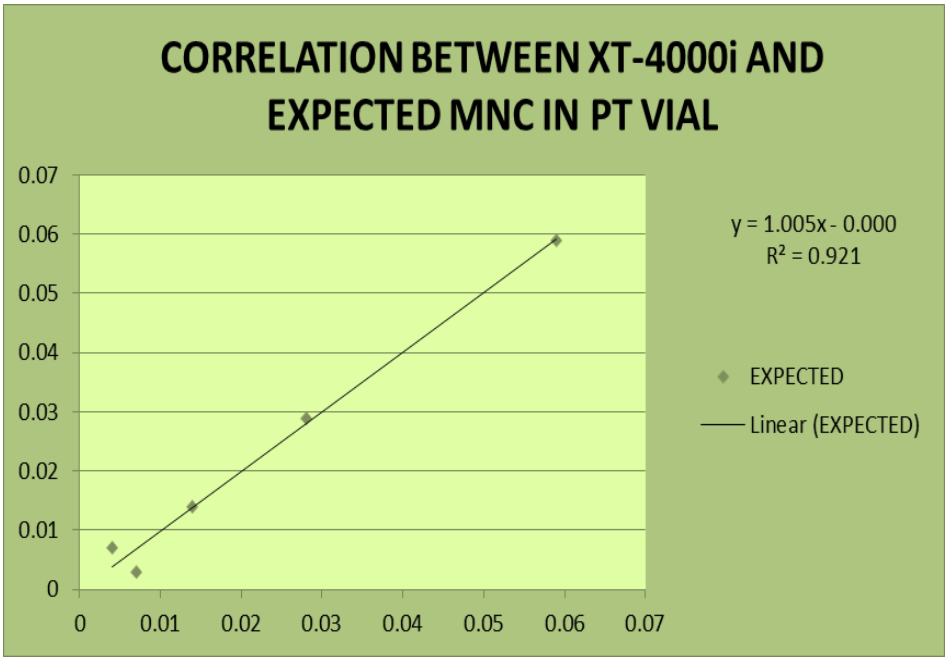
Linearity

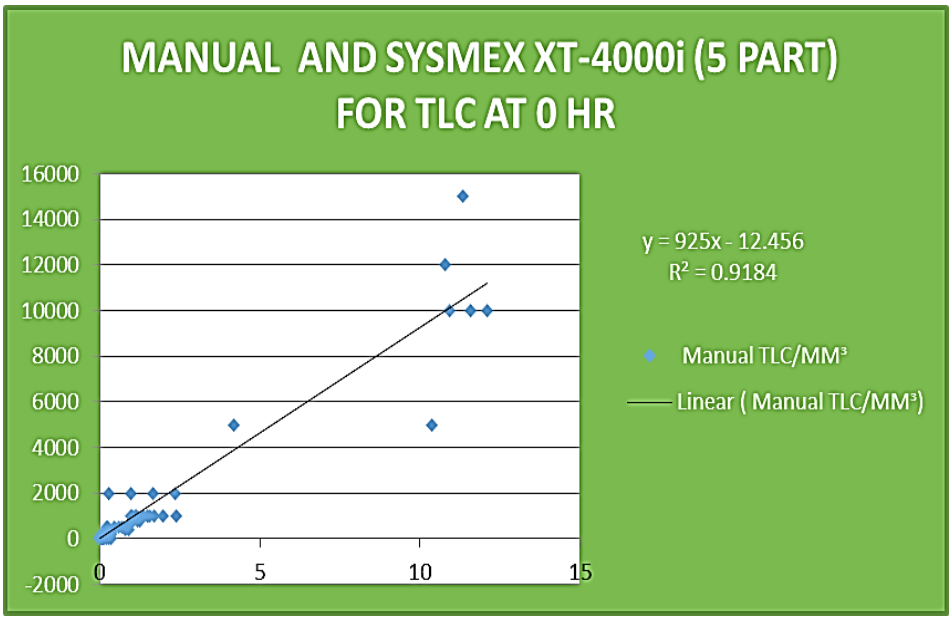
Sysmex XT-4000i presented an excellent correlation between expected and observed values (r=0.962) in plain vial and (r=0.960) in PT vial for MN. (Fig 1, 2)

Table 2: CARRYOVER Analysis

	PLAIN VIAL				EDTA VIAL				PT VIAL			
	AT 0 HOUR		AT 6 HOUR		AT 0 HOUR		AT 6 HOUR		AT 0 HOUR		AT 6 HOUR	
RUN NO.	WBC	RBC	WBC	RBC	WBC	RBC	WBC	RBC	WBC	RBC	WBC	RBC
H1	1.151	0.009	0.627	0.008	1.312	0.009	0.626	0.008	0.934	0.008	0.884	0.007
H2	1.125	0.009	0.644	0.008	1.197	0.008	0.912	0.008	0.965	0.008	0.897	0.008
H3	1.142	0.008	0.623	0.009	1.193	0.008	0.989	0.008	1.032	0.007	0.994	0.008
L1	0.062	0.000	0.183	0.000	0.077	0.000	0.194	0.000	0.063	0.000	0.144	0.000
L2	0.059	0.000	0.082	0.000	0.082	0.000	0.191	0.000	0.088	0.000	0.127	0.000
L3	0.071	0.000	0.086	0.000	0.069	0.000	0.201	0.000	0.099	0.000	0.142	0.000
CARRY OVER %	-0.008	0.000	0.180	0.000	-0.055	0.000	-0.008	0.000	-0.038	0.000	0.002	0.000


CORRELATION BETWEEN XT 4000i AND EXPECTED MONONUCLEAR CELL IN PLAIN VIAL
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
y = 1.0633x - 0.0053
R² = 0.926
EXPECTED
Linear (EXPECTED)


CORRELATION BETWEEN XT-4000i AND EXPECTED MNC IN PT VIAL
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
y = 1.005x - 0.000
R² = 0.921
EXPECTED
Linear (EXPECTED)


MANUAL AND SYSMEX XT-4000i (5 PART) FOR TLC AT 0 HR
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
0
5
10
15
y = 925x - 12.456
R² = 0.9184
Manual TLC/MM³
Linear (Manual TLC/MM³)

Correlation studies

A significant positive perfect correlation existed between WBC (/mm³) count measured through Neubauer chamber and Sysmex XT-4000i (y=925x-12.456, r=0.958, p<0.001) in plain vial at 0 hour by using Pearson's correlation coefficient. (Fig 3)

For RBC correlation, a ROC curve of RBC count was constructed. The area under curve (AUC) was found to be 0.926 (95% confidence interval =0.892 to 0.960). The P value was <0.05. This shows a statistically significant difference. The best cut off value for RBC count for predicting RBC Positivity was 0.005 units (Sensitivity 97.4% and specificity 71.5%, maximum youdan index 0.689) was determined with SE 0.017. (Fig4)

Sysmex XT-4000i showed significant positive correlation for WBC count (10^3/µl) in plain vial (r=0.984, p<0.001s), EDTA vial (r=0.998, p<0.001s), PT vial (r=0.958, p<0.001s) at 0 and those kept at 4°c at 6 hour. So by keeping the sample at 4°c we can increase the shelf life of ascitic fluid. (Table 3)

TABLE 3: Correlation Of Total WBC Count By Manual Method And Sysmex XT-4000i (5 PART)

	SYSMEX XT-4000i						MANUAL METHOD
	PLAIN VIAL		EDTA VIAL		PT VIAL		
	0 HOUR	6 HOUR	0 HOUR	6 HOUR	0 HOUR	6 HOUR	
NO. OF SAMPLES	250	249	250	250	246	248	250
MEAN	0.49	0.003	0.765	0.74	0.535	0.586	445.720
STANDARD DEVIATION	1.741	0.021	2.7	2.663	1.988	2.763	1680.788
r	0.984		0.998		0.958		0.958
R SQUARE	0.968		0.996		0.918		0.918
P VALUE	<0.001S		<0.001S		<0.001S		<0.001S

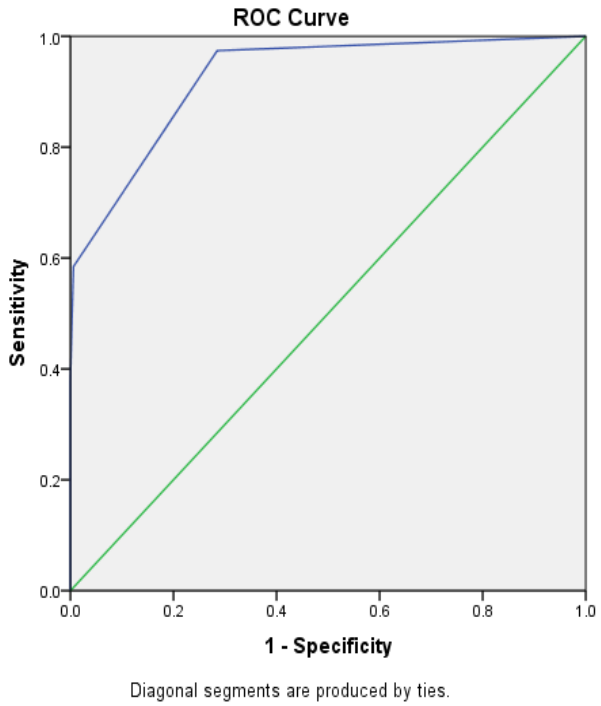


Fig 4: ROC Curve For The Optimal Cut Off Value Of Sysmex XT-4000i (5 Part) For The RBC Count By Manual Method

DISCUSSION

Laboratory assessments of ascitic fluid is an essential part of disease diagnosis and follow up; therefore accurate and rapid results are very important.[3]

In the present study, chronic liver disease was the most common cause of ascites comprising 94.4% of cases. The present study included 199 (79.6%) male and 51 (20.4%) female patients. Male to female ratio was 3.9:1. In our study cases of ascites were highest in 40 to 49 years age group.

The present study evaluated the performance of the Sysmex XT-4000i in terms of correlation, precision, carryover and linearity analysis.

For precision analysis the body fluid sample assayed ten times. The precision analysis of random sample with high cell count indicated that the Sysmex XT-4000i demonstrated good precision for RBC, WBC, MN#, PMN# at 0 and 6 hour in all vials resulted in CVs <0.012%.

Results of precision of low cell count were excellent in EDTA vial with CVs being 0% for RBC, TLC, MN#, PMN# at 0 and 6 hour. In the study done by Giuseppe Lippie et al[7] the imprecision was excellent, being always lower than 11%.

Our results show negligible carryover between samples with high and low cell counts. The carryover was always lower than 0.180% for WBC. For RBC carryover results were 0% in all vials at 0 and 6 hour. To avoid carryover, Sysmex XT-4000i has incorporated an automatic rinse cycle after every measurement. This was corresponding to studies done by Giuseppe Lippi G et al[8] and Buoro S et al[2] in which carryover was always lower than 0.2%.

Linearity was verified for RBC count, WBC count, MN and PMN by running a fluid five times in dilution. Sysmex XT-4000i presented an excellent correlation between expected and observed values (r=0.962) in plain vial and (r=0.960) in PT vial for MN.

Sysmex XT-4000i showed significant positive correlation for WBC count (10^3/µl) in plain vial (r=0.984, p<0.001s), EDTA vial (r=0.998, p<0.001s), PT vial (r=0.958, p<0.001s) at 0 and those kept at 4°c at 6 hour. So by keeping the sample at 4°c we can increase the shelf life of ascitic fluid.

A significant positive perfect correlation existed between WBC (/mm³) count measured through Neubauer chamber and Sysmex XT-4000i (y=925x-12.456, r=0.958, p<0.001) in plain vial at 0 hour by using pearson correlation coefficient so plain vial is the best vial for ascitic fluid cell count. This was corresponding to studies done by Kersie L et al[9] in which Pearson's correlation coefficient for WBC parameter was 0.95 for serous fluid.

Our study is limited by that we did not find a statistically significant correlation of MN% and PMN% in all vials between Sysmex XT-4000i and the Neubauer chamber. This is because of high incidence of macrophages, mesothelial, lymphoid and other tumoral cells in this kind of fluid sample that were not identified by Sysmex XT-4000i. In the study done by Sabrina Buoro et al[2] showed lack of correlation between MN-BF in Sysmex XE-5000 and lymphocytes in optical microscopy.

Sysmex XT-4000i presented non-significant positive correlation between expected and observed values of RBC,TLC, PMN in all vials.

The results of the present study confirm that the Sysmex XT-4000i exhibits additional advantages over manual microscopy, which are typical of automated flow cytometric analysis and thereby include higher throughout, reduced turnaround time, reduction of sample volume, no need of sample preparation as well as optimal analytical performances. So Sysmex XT-4000i may be reliably used for routine analysis of body fluids.

CONCLUSION

In conclusion, despite some discrepancies compared to manual chamber count, the Sysmex XT-4000i BF mode is sufficiently accurate, supported by technical reasons such as fully automated sample preparation (i.e, mixing, aspirating, diluting) as well as optimal analytical performances (i.e, extended linearity, acceptable imprecision, low carryover). The Sysmex XT-4000i is not a substitute for manual microscopy; nevertheless it can substantially reduce the number of samples submitted for microscopy.

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