

AUTOMATED BLOOD ANALYSERS AND THEIR TESTING PRINCIPLES

- A COMPARATIVE STUDY

ABSTRACT:

Computerized hematology analyzers are the absolute most significant instruments in the present clinical laboratory. Hematology analyzers are the principal supplies in clinical labs. A very good quality, high-volume analyzers convey dependable red platelet checks, platelet tallies, and 5-section differentials of white platelets, recognizing lymphocytes, monocytes, neutrophils, eosinophils, and basophils. CELL-DYN Sapphire™ is a very good quality hematology analyzer made by Abbott Technologies. The UniCel® DxH 800 Analyzer is a quantitative, automated hematology analyzer developed for use in clinical laboratories for in vitro diagnostic screening of patient populations. XE-5000 is an automated hematology analyzer used in clinical laboratories for in vitro diagnostic. XE-5000 can examine and can give output for 67 parameters. The XN-2000 platform combines two investigative components that can be customized to suit a specific clinical application. Without the need for large track-based structures, costly stains, or reflexive testing, the ADVIA® 2120i Hematology Machine will fully simplify your hematology lab. Photometry is the method used in hemoglobin measurement. The traditional method for counting cells is electrical impulse, otherwise called as Coulter Principle. It is used in most hematology analyzers. In optical light scatter measurement, through a diluted blood spectrum a beam of laser light is passed and that is projected into the flow cell by hydrodynamic focusing. This review aimed to overview the working principle of various Hematological analyzers in common use.

Keywords: Hematology analyzer, Photometry, Impedance, Light scatter, flow cytometry, innovative technique.

INTRODUCTION:

Computerized hematology analyzers are the absolute most significant instruments in the present clinical laboratory. Due to the implementation of new physical rules for cellular analysis and the rapid development of algorithms, automated blood cell counters have undergone a significant technical evolution. The test for instrument makers is to build the scope of quantitative cell examination, yet to keep up throughput and limit cost per test by using suitable reflex testing. An essential interest for hematology analyzers is to limit the number of tests for which time-and-faculty escalated infinitesimal survey is required without increment of bogus negative outcomes. Hematology analyzers are the principal supplies in clinical labs. A very good quality, high-volume analyzers convey dependable red platelet checks, platelet tallies, and 5-section differentials of white platelets, recognizing lymphocytes, monocytes, neutrophils, eosinophils, and basophils. The first automated cell counter was introduced in the 1950s dependent on Coulter's electrical impedance standard in which cells got through an opening break in an electric circuit, demonstrating both the presence of a cell and the size of the cell(1).

In the 1970s, 7 parameters complete blood count (CBC) analyzers, 3 part differential leukocyte counters, and automated platelet counters were introduced to the market. Flow cell-based technologies have advanced in hematology analyzers, where cells are interrogated cell by cell using optical devices that can calculate more parameters than ever(2). In the 80s, 10 parameters CBC were produced by a single instrument. And in the 90s, using flow-cell techniques based on either electrical impedance the leukocyte differentials were advanced. For better laboratory optimization, the customer must recognize the capabilities and shortcomings of these analyzers. As a result, it is critical to disseminate this valuable knowledge obtained from hematology analyzers to both clinicians and laboratory physicians, as it can benefit patients by lowering the cost of diagnosis by preventing needless tests and may aid in timely management(3). In the present study, we will comparatively overcome about five hematological analyzers- CELL DYN Sapphire, UniCel DxH800, XE-5000, XN-2000, and ADVIA 2120i and its testing principles and its measuring technologies were indicated in [Table 1]. Our team has extensive knowledge and research experience that has translated into high quality publications (4).(5–18),(19–23)

CELL DYN SAPPHIRE:

CELL-DYN Sapphire™ is a very good quality hematology analyzer made by Abbott Technologies. It permits the cell population examination by monoclonal counter-acting agent fluorochromes practically equivalent to flow cytometry. FDA approves CELL-DYN Sapphire. CELL-DYN Sapphire is comparable with other blood analyzers such as Beckman Coulter LH750 and Bayer Advia in WBC and RBC parameters. It is able to provide information from monoclonal antibody examination and to classify leucocyte forms. A multi-focus assessment was attempted in European nations looking at CELL-DYN Sapphire™ against Abbott Cell-Dyn CD4000, Bayer Advia 120, and Beckman Coulter GenS. The assessment proposed comparable execution consistency for WBC checks and differentials, RBC boundary estimation, and platelet means tests with platelet tallies over $40 \times 10^9/L$ of every one of the four assessed analyzers. Notwithstanding, the exactness for platelet tallies with thrombocytopenia was moderately poor with proof of critical overestimations (Advia) and periodic huge disparities (all impedance and optical techniques) contrasted with the CD61 immune platelet count(24). Multidimensional cell classification using patented Mukti Angel Polarised Scatter Separation plus 3- color Fluorescent technology provides an analysis of both WBC count and differential count on the CELL-DYN Sapphire(25). Using red Fluorescence, the samples are analyzed for the presence of Nucleated Red Blood Cells(NRBCs). Multiple technologies were used to provide a high degree of accuracy for the platelet count. An examination assessing the execution of a wide scope of monoclonal antibodies with CELL-DYN Sapphire™ showed that the populaces segregation was like stream cytometry and the preparing techniques were effortlessly absorbed with insignificant preparation(26).

UniCel DxH800:

The UniCel® DxH 800 Analyzer is a quantitative, automated hematology analyzer developed for use in clinical laboratories for in vitro diagnostic screening of patient populations. Complete Blood Count (CBC), Leukocyte 5 Part Differential (Diff), Complete Reticulocyte (Retic), and Nucleated Red Blood Cell (NRBC) on entire blood, and total Nucleated Count (TNC) and Red Cell Count (RBC) on Body Fluids (cerebrospinal, serous, and synovial) are provided by the UniCel® DxH 800 Analyzer (BF)(27). In a study, the samples run on auto mode

using the common “CDR” and in the open mode of the Sapphire which is licensed by 2 scientists(28). The UniCel DxH 800 is an adaptable, completely computerized hematology analyzer framework equipped for examining up to 100 examples each hour and giving a CBC, 5-section WBC differential, NRBC check, and reticulocyte tally from 165 μ L of the test. The analyzer utilizes new volume, conductivity, and disperse boundaries for controlled stream cytometric investigation of WBC differential, reticulocytes, and NRBC location(29,30). Upgrades over past Beckman Coulter analyzers (counting the LH 780) incorporate an exclusive blood location configuration to limit test volume prerequisites, a strong state diluter plan that contains no squeeze valves, and new particular development with a front-stacking test track to encourage linkage of different DxH 800 instruments in a straight course of action(31).

XE-5000:

XE-5000 is an automated hematology analyzer used in clinical laboratories for in vitro diagnostic. XE-5000 can examine and can give output for 67 parameters(32). Using an optical detector block based on the fluorescence flow cytometry method using semiconductor lasers can analyze WBCs, reticulocytes, and nucleated RBCs. And by RBC detector using the Hydro-Dynamic Focusing method based on the fluorescence flow cytometry method RBCs and platelet counts are analyzed (33). The XE-5000 has a comprehensive clinical research menu for whole blood and body fluids, with precise, precise, and sensitive results. With its quick throughput, your doctors get quality, clinically important data to aid the administration and find, everything being equal. An extra advantage to the XE-5000 analyzer is the capacity to investigate persistent examples when just little volumes of the test are accessible. The fine mode (pre-weaken examination mode) requiring just 40 μ L of entire blood gives quality clinical information quickly and reliably. ‘Gold standard’ Fluorescence Flow Cytometry innovation and extraordinary boundaries as standard(34).

XN- 2000:

The XN-2000 platform combines two investigative components that can be customized to suit a specific clinical application. In its Rerun and Reflex design, it is changed into an amazing answer for fundamental and expanded testing(35). Since the two modules measure rack tests at the same time, it can oblige any example without easing back down. The modules cooperate consummately. What's more, you don't lose any valuable time, even with unusual examples(36).

ADVIA 2120i:

Without the need for large track-based structures, costly stains, or reflexive testing, the ADVIA® 2120i Hematology Machine will fully simplify your hematology lab. Siemens Healthineers adds Functional Automation to the hematology lab in this way. ADVIA technology utilizes peroxidase staining—the gold standard in differential testing(36,37). A secondary complete white cell count is used in the ADVIA Hematology Systems' main protocol as an independent quality management audit to ensure sample integrity(38).

Parameters	Instruments				
	Cell-Dyn Sapphire	DxH 800	Advia 2120i	XE- 5000	XN- 2000
Hemoglobin	Photometry	Photometry	Photometry	Photometry	Photometry
RBC count	Impedance/ Light scatter	Impedance	Light scatter	Impedance	Impedance
PLT count	Impedance/ Light scatter/ Ab based	Impedance	Light scatter	Impedance/ Light scatter/ Fluorescence	Impedance/ Light scatter/ Fluorescence
WBC count	Light scatter/ Fluorescence	Impedance	Light scatter/ Peroxidase	Impedance/ Light scatter/ Fluorescence	Light scatter/ Fluorescence
5- part differential	Light scatter/ Fluorescence	Impedance/ Conductivity/ Light scatter	Light scatter/ Peroxidase	Light scatter/ Fluorescence	Light scatter/ Fluorescence
Reticulocytes	Light scatter/ Fluorescence	Impedance/ Conductivity/ Light scatter	Light scatter	Light scatter/ Fluorescence	Light scatter/ Fluorescence
NRBC count	Light scatter/ Fluorescence	Impedance/ Conductivity/ Light scatter	Light scatter/ Peroxidase	Light scatter/ Fluorescence	Light scatter/ Fluorescence

Table 1: Measuring technology of various hematology analyzer

PHOTOMETRY:

Photometry is the method used in hemoglobin measurement. The photometry cyanmethemoglobin method depends on the principle of Darkin reagent reacts with hemoglobin in blood for cyanmethemoglobin formation and the color developed is measured by photometry at 550nm(39). Photometry is a technique that quantifies the concentration of the organic and inorganic mixture in a solution by determining the absorbed cells of the wavelength of light. Photometry is an integral segment of water quality management as they diagnose heavy, radionuclides, nitrates, phosphates, fluorides, pesticides, etc (40). Cell Dyn Sapphire, DxH 800, Advia 2120i, XE-5000, and XN-2000 use a photometry method for hemoglobin analysis.

IMPEDANCE:

The traditional method for counting cells is electrical impulse, otherwise called as Coulter Principle. It is utilized in most hematology analyzers. The blood will pass in between two electrodes through an aperture and at a time only one cell will pass through it. Some analyzers force the cells through the path using hydrodynamic focusing so the cells will take the path for the measurement of volume. The impedance will alter when a cell crosses through it(39). Cell volume is proportional to change in the impedance, which results in measuring the volume and the cell count. It is possible to count up to 10,000 cells per second, and a standard impedance analysis can be completed in less than a minute. Some analyzers use high-frequency current conductivity to determine the composition of WBC(41).

LIGHT SCATTER:

In optical light scatter measurement, through a diluted blood spectrum a beam of laser light is passed and that is projected into the flow cell by hydrodynamic focusing. The concentrated light is dispersed in different ways as each cell moves through, and photodetectors sense the signal, which is transformed into an electric pulse(42). The electrical signals are sent to a server where they are stored and analyzed. In the scattergram, cells with identical light scatter properties form a cluster that can be isolated with sophisticated software algorithms. The scale, shape, nuclear segmentation, cytoplasmic granulation, and inner complexity are all captured by the light that is

scattered and observed at a particular angle(43). In this present comparative review, we compared the testing principles of these five hematology analyzers but not about each of the working procedures and their complements.

CONCLUSION:

In this comparative review, we have overcome about five hematology analyzers- Cell Dyn Sapphire, DxH 800, Advia 2120i, XE-5000, and XN-2000 and its testing principles. The methods like photometry, impedance and light scatter are also explained.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCE:

1. Bruegel M, Nagel D, Funk M, Fuhrmann P, Zander J, Teupser D. Comparison of five automated hematology analyzers in a university hospital setting: Abbott Cell-Dyn Sapphire, Beckman Coulter DxH 800, Siemens Advia 2120i, Sysmex XE-5000, and Sysmex XN-2000 [Internet]. Vol. 53, Clinical Chemistry and Laboratory Medicine (CCLM). 2015. Available from: <http://dx.doi.org/10.1515/cclm-2014-0945>
2. Brundha MP, Pathmashri VP, Sundari S. Quantitative Changes of Red Blood cells in Cancer Patients under Palliative Radiotherapy-A Retrospective Study. Research Journal of Pharmacy and Technology. 2019;12(2):687–92.
3. Chhabra G. Automated hematology analyzers: Recent trends and applications [Internet]. Vol. 10, Journal of Laboratory Physicians. 2018. p. 015–6. Available from: http://dx.doi.org/10.4103/jlp.jlp_124_17
4. Anita R, Paramasivam A, Priyadharsini JV, Chitra S. The m6A readers and aberrations associated with metastasis and predict poor prognosis in breast cancer patients. Am J Cancer Res. 2020 Aug 1;10(8):2546–54.

5. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. *Hypertens Res.* 2020 Dec;43(12):1459–61.
6. Sivakumar S, Smiline Girija AS, Vijayashree Priyadharsini J. Evaluation of the inhibitory effect of caffeic acid and gallic acid on tetR and tetM efflux pumps mediating tetracycline resistance in *Streptococcus* sp., using computational approach. *Journal of King Saud University - Science.* 2020 Jan 1;32(1):904–9.
7. Smiline Girija AS. Delineating the Immuno-Dominant Antigenic Vaccine Peptides Against gacS-Sensor Kinase in *Acinetobacter baumannii*: An in silico Investigational Approach. *Front Microbiol.* 2020 Sep 8;11:2078.
8. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of csgA gene among ESBL strains of *A. baumannii* and targeting with essential oil compounds from *Azadirachta indica*. *Journal of King Saud University - Science.* 2020 Dec 1;32(8):3380–7.
9. Girija ASS. Fox3+ CD25+ CD4+ T-regulatory cells may transform the nCoV's final destiny to CNS! *J Med Virol* [Internet]. 2020 Sep 3; Available from: <http://dx.doi.org/10.1002/jmv.26482>
10. Jayaseelan VP, Ramesh A, Arumugam P. Breast cancer and DDT: putative interactions, associated gene alterations, and molecular pathways. *Environ Sci Pollut Res Int.* 2021 Jun;28(21):27162–73.
11. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. *Arch Oral Biol.* 2021 Feb;122:105030.
12. Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from *Ganoderma lucidum*: A computational study. *pharmaceutical-sciences* [Internet]. 2020;82(2). Available from: <https://www.ijpsonline.com/articles/targeting-nm23h1-mediated-inhibition-of-tumour-metastasis-in-viral-hepatitis-with-bioactive-compounds-from-ganoderma-lucidum-a-comp-3883.html>
13. Girija SA, Priyadharsini JV, Paramasivam A. Prevalence of carbapenem-hydrolyzing OXA-type β -lactamases among *Acinetobacter baumannii* in patients with severe urinary tract infection. *Acta Microbiol Immunol Hung.* 2019 Dec 9;67(1):49–55.
14. Priyadharsini JV, Paramasivam A. RNA editors: key regulators of viral response in cancer patients. *Epigenomics.* 2021 Feb;13(3):165–7.
15. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with *Murraya koengii* bio-compounds: An in-silico approach. *Acta Virol.* 2020;64(1):93–9.
16. Girija As S, Priyadharsini J V, A P. Prevalence of Acb and non-Acb complex in elderly

population with urinary tract infection (UTI). *Acta Clin Belg.* 2021 Apr;76(2):106–12.

17. Anchana SR, Girija SAS, Gunasekaran S, Priyadharsini VJ. Detection of gene in carbapenem-resistant strains and targeting with biocompounds. *Iran J Basic Med Sci.* 2021 May;24(5):690–8.
18. Girija ASS, Shoba G, Priyadharsini JV. Accessing the T-Cell and B-Cell Immuno-Dominant Peptides from *A.baumannii* Biofilm Associated Protein (bap) as Vaccine Candidates: A Computational Approach. *Int J Pept Res Ther.* 2021 Mar 1;27(1):37–45.
19. Arvind P TR, Jain RK. Skeletally anchored forsus fatigue resistant device for correction of Class II malocclusions-A systematic review and meta-analysis. *Orthod Craniofac Res.* 2021 Feb;24(1):52–61.
20. Venugopal A, Vaid N, Bowman SJ. Outstanding, yet redundant? After all, you may be another *Cholutedca* Bridge! *Semin Orthod.* 2021 Mar 1;27(1):53–6.
21. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. *Clin Oral Investig.* 2019 Sep;23(9):3543–50.
22. Varghese SS, Ramesh A, Veeraiyan DN. Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students. *J Dent Educ.* 2019 Apr;83(4):445–50.
23. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of *Streptococcus mutans*, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial [Internet]. Vol. 24, *Clinical Oral Investigations*. 2020. p. 3275–80. Available from: <http://dx.doi.org/10.1007/s00784-020-03204-9>
24. Timothy CN, Samyuktha PS, Brundha MP. Dental pulp Stem Cells in Regenerative Medicine--A Literature Review. *Research Journal of Pharmacy and Technology.* 2019;12(8):4052–6.
25. Ermens AAM, J J M, Krockenberger M, Van WIJK EM. New erythrocyte and reticulocyte parameters on CELL-DYN Sapphire: analytical and preanalytical aspects [Internet]. Vol. 34, *International Journal of Laboratory Hematology*. 2012. p. 274–82. Available from: <http://dx.doi.org/10.1111/j.1751-553x.2011.01391.x>
26. Müller R, Mellors I, Johannessen B, Aarsand A, Kiefer P, Hardy J, et al. European Multi-Center Evaluation of the Abbott Cell-Dyn Sapphire Hematology Analyzer [Internet]. Vol. 12, *Laboratory Hematology*. 2006. p. 15–31. Available from: <http://dx.doi.org/10.1532/lh96.05041>
27. Zhang P, Tang H, Chen K, Chen Y, Xu D. Biological variations of hematologic parameters determined by UniCel DxH 800 hematology analyzer. *Arch Pathol Lab Med.* 2013 Aug;137(8):1106–10.

28. Bain BJ. *Blood Cells: A Practical Guide*. John Wiley & Sons; 2008. 488 p.
29. Kim SJ, Kim Y, Shin S, Song J, Choi JR. Comparison Study of the Rates of Manual Peripheral Blood Smear Review From 3 Automated Hematology Analyzers, Unicel DxH 800, ADVIA 2120i, and XE 2100, Using International Consensus Group Guidelines [Internet]. Vol. 136, *Archives of Pathology & Laboratory Medicine*. 2012. p. 1408–13. Available from: <http://dx.doi.org/10.5858/arpa.2010-0757-oa>
30. Hannah R, Ramani P, Brundha MP, Sherlin HJ, Ranjith G, Ramasubramanian A, et al. Liquid Paraffin as a Rehydrant for Air Dried Buccal Smear. *Research Journal of Pharmacy and Technology*. 2019;12(3):1197–200.
31. Group: ICFS in HW, International Council for Standardization in Haematology, Writing Group:, Briggs C, Culp N, Davis B, et al. ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting [Internet]. Vol. 36, *International Journal of Laboratory Hematology*. 2014. p. 613–27. Available from: <http://dx.doi.org/10.1111/ijlh.12201>
32. Boer K, Deufel T, Reinhoefer M. Evaluation of the XE-5000 for the automated analysis of blood cells in cerebrospinal fluid. *Clin Biochem*. 2009 May;42(7-8):684–91.
33. Buttarello M, Plebani M. Automated blood cell counts: state of the art. *Am J Clin Pathol*. 2008 Jul;130(1):104–16.
34. Urrechaga E, Borque L, Escanero JF. The role of automated measurement of red cell subpopulations on the Sysmex XE 5000 analyzer in the differential diagnosis of microcytic anemia. *Int J Lab Hematol*. 2011 Feb;33(1):30–6.
35. Schoorl M, Schoorl M, Chevallier M, Elout J, van Pelt J. Flagging performance of the Sysmex XN2000 haematology analyser [Internet]. Vol. 38, *International Journal of Laboratory Hematology*. 2016. p. 160–6. Available from: <http://dx.doi.org/10.1111/ijlh.12461>
36. Strobel E, Betten S. Novel aspects in the evaluation of an automated blood group analyser [Internet]. Vol. 21, *Transfusion Medicine*. 2011. p. 205–8. Available from: <http://dx.doi.org/10.1111/j.1365-3148.2010.01062.x>
37. Jain DK. Comparison of automated method and photometric cyanmethemoglobin method for haemoglobin estimation [Internet]. Vol. 5, *IP Journal of Diagnostic Pathology and Oncology*. 2020. p. 273–6. Available from: <http://dx.doi.org/10.18231/j.jdpo.2020.053>
38. Pintér E, László K, Schüsler I, Konderák J. The stability of quantitative blood count parameters using the ADVIA 2120i hematology analyzer [Internet]. Vol. 4, *Practical Laboratory Medicine*. 2016. p. 16–21. Available from: <http://dx.doi.org/10.1016/j.plabm.2015.12.001>
39. Briggs C, Longair I, Slavik M, Thwaite K, Mills R, Thavaraja V, et al. Can automated blood film analysis replace the manual differential? An evaluation of the CellaVision DM96

automated image analysis system. *Int J Lab Hematol*. 2009 Feb;31(1):48–60.

40. Borrmann S, Grobusch MP, Trinkle C, Oyakhirome S, Krishna S, Issifou S, et al. SHORT REPORT: EVALUATION OF A SIMPLE AND INEXPENSIVE PHOTOMETRIC DEVICE FOR THE MEASUREMENT OF HEMOGLOBIN [Internet]. Vol. 71, *The American Journal of Tropical Medicine and Hygiene*. 2004. p. 691–2. Available from: <http://dx.doi.org/10.4269/ajtmh.2004.71.691>
41. Bauer NB, Nakagawa J, Dunker C, Failing K, Moritz A. Evaluation of the impedance analyzer PochH-100iV Diff for analysis of canine and feline blood [Internet]. Vol. 41, *Veterinary Clinical Pathology*. 2012. p. 194–206. Available from: <http://dx.doi.org/10.1111/j.1939-165x.2012.00405.x>
42. Kim YR, Oever RV, Landayan M, Bearden J. Automated red blood cell differential analysis on a multi-angle light scatter/fluorescence hematology analyzer [Internet]. Vol. 56B, *Cytometry*. 2003. p. 43–54. Available from: <http://dx.doi.org/10.1002/cyto.b.10048>
43. de Jonge R, Brouwer R, Smit M, de Frankrijker-Merkestijn M, Dolhain RJEM, Hazes JMW, et al. Automated counting of white blood cells in synovial fluid. *Rheumatology* . 2004 Feb;43(2):170–3.