Advantages of the Use of AQUIOS CL Flow Cytometer in Evaluation of CD4 and CD8 in HIV Patients: CMCV AMC Experience

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Objectives: Flow cytometry has been used for the evaluation of CD4 and CD8 counts and to monitor the status of the immunocompromised patients. This, in turn, has a major impact in modifying the treatment appropriately leading to the proper management of the patients in a timely manner. The AQUIOS CL Flow Cytometer introduces a new technology that forgoes sample preparation and staining by using a single Load and Go technology, thus allowing for rapid cell analysis TAT for CD4 and CD8 testing. We were previously using the Beckman FC-500 flow cytometry instrument, which required separate staining and lysing procedure and were time-consuming. To overcome this, we acquired and implemented the AQUIOS CL Flow Cytometer for an efficient and prompt reporting of CD4 and CD8 count.

Methods: A sample is aspirated and dispensed from a closed tube, then dispensed into wells of a deep-well plate. Fluorochrome-labeled antibodies are added that bind to molecules on the cells that express specific antigens. As a part of quality assurance monitoring, retrospective split analysis was performed for 34 samples with the use of Aquios as well as FC-500. Method-to-method correlation was performed with the Bland-Altman method.

Results: Our results demonstrated no statistically significant difference between the two instruments. However, TAT for the testing was significantly decreased with Aquios (20 minutes for the first specimen and then 2 minutes for each sequential specimen) as compared to FC-500. Method-to-method correlation was performed with the Bland-Altman method.

Conclusion: The AQUIOS CL has significantly improved TAT for CD4 and CD8 testing, allowing for better patient care and treatment.

Dilution Method for Solving Visually Recognizable Bilirubin Interference on the Beckman Coulter UniCell DXH800 Hematology Analyzer

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Introduction: Hematology analyzers occasionally encounters interferences that prevent correct generation of the five-part WBC differential (5PWD). Although the cause of interference is usually unknown, we hypothesized that bilirubin excess drives specific and measurable changes.

Methods: We retrospectively identified 125 CBC samples run on the Beckman Coulter UniCel DXH800 analyzer (Brea, CA) that yielded an optical scatter pattern that interfered with the differential and had a subsequent 1:2 dilution. Abnormal scatterplots were visually clustered into distinct patterns. The % difference for each component of the differential pre- vs postdilution was calculated for each pattern. Kruskal-Wallis with Dunn’s correction was used to determine whether these changes were significantly different among patterns. Spearman correlation was used to calculate correlation between bilirubin concentration and neutrophil absolute % difference (NAD), a surrogate variable for the magnitude of the interference.

Results: For 114 (91%) samples, 1:2 dilution resolved interference. We identified four visually distinct optical scatter patterns. The majority showed the hereinafter called type 1 and 2, which yielded the most relevant changes in the 5PWD following dilution and were significantly different from the two other minor patterns. Type 1 (38% of samples) resulted in a NAD of +11.28% (95% CI, +8.93% to +13.63%) and a lymphocyte absolute difference (LAD) of −13.35% (95% CI, −16.16% to −10.54%). The mean bilirubin concentration was 1.45 mg/dL (95% CI, 0.56 to 2.34). Alternatively, type 2 (47%) resulted in a NAD of +25.24% (95% CI, +20.99 to +29.50) and LAD of −26.19 (95% CI, −30.66% to −21.73%). The mean bilirubin concentration was 12.51 mg/dL (95% CI, 9.94 to 15.08), significantly higher than in any other pattern, and with a significant positive correlation with the NAD (r = 0.386; P = .006). NAD = 13.853 + 0.874 * Bili [mg/dL].

Conclusion: Our results suggest that bilirubin produces common and recognizable interference in DXH800 analyzers that responds to dilution, bringing the opportunity to reflexively dilute samples.

Third- to Fourth-Generation HIV Testing: Reduction in False-Positive Results With the New Way of Testing, the Corporal Michael J. Crescenz Veteran Affairs Medical Center (CMCVAMC) Experience

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Introduction: In 2006, the CDC issued guidelines advocating routine HIV screening of all patients. However, false-positive results are a potential patient care threat for low-risk populations even with very sensitive and specific
assays. While confirmatory testing is needed, a reduction in HIV false-positive screening results can potentially be seen by switching (from the older third generation) to a more sensitive and specific fourth-generation screening assay. As this has not been published in the English literature for the veteran population to the authors’ knowledge, we studied the impact on the false-positive screening rate of a change to a fourth-generation assay at the CMCVAMC.

Methods: Quality assurance documents on test volume were reviewed to obtain the total number of HIV screening tests that were performed by the laboratory during March 1, 2016, to February 28, 2017, prior to implementation of the fourth-generation assay and the year afterward (March 1, 2017, to March 1, 2018). In addition, records of all positive screening results during those time periods were reviewed. The third-generation assay was the Ortho-Clinical Diagnostics Vitros (Raritan, NJ) and the fourth-generation assay was the Abbott Architect (Santa Clara, CA).

Results: Prior to implementation, there were a total of 7,529 HIV screening specimens; of those, 54 were reactive on the third-generation screening assay. On further review, 23 were true positives, 28 were false positives, and 3 were indeterminate. Afterward, there were 7,803 HIV screening specimens; of those, 27 were reactive on the fourth-generation screening assay. On further review, 17 were true positives and 10 were false positives.

Conclusions: There were fewer false-positive results with testing with the more specific fourth- vs third-generation assay (0.12% vs 0.37%, respectively). This reduction in false-positive screening has the potential to reduce laboratory workload with the necessary confirmatory testing.

Nucleated Red Blood Cells: A Potential Pitfall of the Automated Hematology Analyzer

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Case Report: The use of automated hematology analyzers has made enumeration of types of blood cells extremely efficient in recent years. The Beckman Coulter Unicel DxH 800 Analyzer (Beckman Coulter, Brea, CA) uses several parameters to differentiate blood cells. The literature indicates the DxH 800 can accurately distinguish nucleated red blood cells (NRBCs) from other cell populations. At our institution, a newborn was delivered via cesarean section with a maternal history of hepatitis B and use of tobacco and cocaine. Blood from the umbilical cord and newborn was collected and analyzed with the DxH 800. The report showed a white blood cell (WBC) count of 50.1 \times 10^9/\mu L in the cord blood and 53.7 \times 10^9/\mu L in the newborn. According to the instrument, the WBC count corrected for NRBCs measured 47.7 \times 10^9/\mu L in the cord blood and 50.3 \times 10^9/\mu L in the newborn. The NRBCs were reported as 184.0/100 WBCs in the cord blood and 180.1/100 WBCs in the newborn. The DxH 800 had the following action comment for both specimens: “Slide review for RBC morph if first time.” A manual examination of the newborn’s blood smear showed a corrected WBC count of 13.2 \times 10^9/\mu L and a NRBC count of 308/100 WBCs with proerythroblasts and basophilic erythroblasts noted. Such a large discrepancy in these values indicates that the DxH 800 falsely classifies very immature NRBCs as WBCs, which may lead to an incorrect result of leukocytosis if a manual differential count is not performed. In conclusion, this study indicates that very immature NRBCs (proerythroblasts and basophilic erythroblasts) are counted as WBCs by the DxH 800, and a manual differential count of the smear is required for accurate results.

A Physician-Directed, Laboratory-Based Hemochromatosis Clinic

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Introduction: Patients are often referred to the blood bank for therapeutic phlebotomy treatment of systemic iron overload and secondary polycythemia. This report summarizes the 20+-year experience of a hemochromatosis clinic functioning as a service of the blood bank.

Method: Patients are referred to the “clinic” by primary care physicians or gastroenterologists with an order for therapeutic phlebotomy or “hemochromatosis protocol” and are interviewed by the clinical pathologist as clinic director. Confirmatory and baseline lab tests are performed and a treatment schedule planned and discussed. A consult letter to referring physicians outlines the treatment plan. A flowchart of treatments and lab monitoring data is maintained and periodically sent to referring physicians with updated treatment plans. Overall patient care remains the responsibility of referring physicians. Diagnostic confirmation and treatment planning follow published international guidelines, with evidence of systemic iron excess if the serum ferritin level is above age-gender reference range and the transferrin saturation is greater than 45%, results confirmed by repeat testing. Hemochromatosis HFE genetic testing is obtained for patient and family counseling.

Treatment schedules depend on the severity of iron excess and general patient condition. A more frequent induction phase is necessary with a severe iron overload, tapering to a patient-variable maintenance phase as ferritin levels decrease to a target range. Patients are advised of a lifelong need for awareness of their “iron status.”

Conclusion: Our clinic averages 100 patients in its “hemochromatosis registry.” Patient response has been very positive—related to a small, dedicated team of therapists and ready access to physician discussion of their ailment with answers to questions as they arise. Medical staff response has been very positive as they are relieved from