Contamination of Patient Blood Samples by Avian RBCs From Control Material During Automated Hematology Analysis

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Abstract

Objectives: Atypical nucleated RBCs (NRBCs) found on several patient blood smears between 2010 and 2012 were noted to resemble avian RBCs. NRBCs are not normally found in the circulation beyond the neonatal period and may indicate hematologic disease, malignancy in the bone marrow, or other severe conditions. Our blood smears with unusual NRBCs did not contain other abnormalities that typically accompany NRBCs, such as immature cells or dysplastic granulocytes. To investigate this anomaly, we considered possibilities such as contaminated collection tubes and instrument problems. The Retic-C Cell Control used with the LH 750 Hematology Analyzer contains a mixture of human and avian RBCs.

Methods: CBC count with differential tests were performed on blanks and routine laboratory samples run immediately after the Retic-C Cell Control on the LH 750 and LH 780 analyzers to recreate the conditions that might cause spillage into the next tube.

Results: We experimentally reproduced the phenomenon of contamination of a subsequent tube with avian cells from a multiply punctured reticulocyte control tube.

Conclusions: We concluded that the NRBCs likely represented avian RBCs from the Retic-C Cell Control that had been introduced into the patient tubes.

Nucleated RBCs (NRBCs) are not normally found in the circulation beyond the neonatal period and may indicate hematologic disease, malignancy in the bone marrow, or other severe conditions.1 Erythroid cells in all vertebrates undergo chromatin condensation during erythropoiesis, yet red cell enucleation is unique to mammals.2

Four patients’ blood smears, which were manually reviewed (L.S., B.K., M.L., and T.A.) in 2011 and 2012 at the Stony Brook University (SBU) Medical Center, had frequent NRBCs with a slightly different appearance from human NRBCs. The NRBCs were ellipsoid, with areas of clearing adjacent to the nuclei and resembled avian RBCs. The number of NRBCs enumerated on the WBC manual differential count ranged from 27 to 125 per 100 WBCs. Table 1 summarizes features of each case, including the reasons for review. When the same patient tube was sampled again in the LH 780 Hematology Analyzer (Beckman Coulter, Brea, CA), the unusual NRBCs were also present on the repeat blood smear and, therefore, in the patient tube.

Similar observations involving 4 patients at Blue Ridge Regional (BRR) Hospital were investigated in 2010 and implicated spillover from the Retic-C Cell Control in the LH 750 Hematology Analyzer (Beckman Coulter). The Retic-C Cell Control contains a mixture of human and avian RBCs. A literature search revealed a strikingly similar 2011 report from Singapore. At the SBU Medical Center and BRR Hospital, we hypothesized that spillover from the Retic-C Cell
Control into the next patient tube caused introduction of avian cells into the patient specimen. We investigated the conditions required to reproduce this phenomenon.

Materials and Methods

Automated Hematology Instrumentation and Sample Tubes

The LH 780 Hematology Analyzer with SlideMaker (Beckman Coulter) was in use at SBU Medical Center, whereas the LH 750 Hematology Analyzer, identical to the LH 780 but without the SlideMaker, was in use at BRR Hospital, where slides were made and stained manually. Additional studies were performed at BRR Hospital on a Unicel DxH 800 (Beckman Coulter). All analyzers used closed-tube sampling of BD Vacutainer plastic K2EDTA tubes with a Lavender BD Hemogard Closure (Becton Dickinson, Franklin Lakes, NJ) for patient samples.

WBC Differential Count, NRBC Detection, and Enumeration

On Beckman Coulter analyzers, the WBC count is derived by an impedance technology, and the WBC differential plot is analyzed using volume, conductivity, and scatter technology. Classification of WBCs is performed by threshold size discrimination (>35 fL). Interfering particles such as NRBCs, nonlysed RBCs, giant or clumped platelets, or fragmented cells, which hover near this threshold, potentially may be included in the WBC count. If interfering particles are detected, the LH 750 generates a suspect message of “cellular interference” and may autocorrect the WBC count. Beckman Coulter analyzers do not use nuclear dyes to detect NRBCs. Enumeration of the NRBC count is performed when particles are detected in the NRBC signature positions of both the WBC and the differential plots; this process uses volume and scatter analysis of a cell population lying under the lymphocytes, as well as analysis and extrapolation of the low-volume peaks of the WBC distribution curve. On the Unicel DxH 800, a new flow cell design supports additional...
light scatter measurements, enabling enhanced data acquisition for the WBC differential and NRBC analyses. This enhanced data collection allows the DxH 800 to collect 10 times more information on each sample compared with the LH 750.4

Reticulocyte Analysis and Control Materials

The LH 750 performs reticulocyte analysis by incubating a supravital dye, new methylene blue, with whole-blood samples. The dye precipitates the basophilic RNA network in reticulocytes. After adding a clearing reagent, stained reticulocytes are differentiated from other cell populations by light scatter, direct current measurements, and opacity characteristics.

The Retic-C Cell Control contains a mixture of human and avian RBCs. The avian cells are not intended to be counted as WBCs; rather, they serve as nucleic acid–containing particles to stand in for reticulocytes. The Retic-C Cell Control is not integrated with the Beckman Coulter 5C control used for the WBC differential.

Carryover Checks

Carryover checks were performed as per the manufacturer’s instructions as part of preventative maintenance. For each carryover check, a normal whole-blood specimen was run prior to 3 consecutive tubes, each containing 2 mL of diluent. Acceptable limits for percent carryover, as per the manufacturer, were as follows: WBC, 2%; RBC, 1%; hemoglobin, 2%; and platelets, 2%.

Experiments to Reproduce the Phenomenon of Contamination

The Retic-C Cell Control was run on the LH 780 analyzer as a patient sample for a CBC count with differential (CBCD), generating a scatterplot and blood smear prepared by the Slide-Maker. In addition, CBCD tests were performed on blanks and routine laboratory samples run immediately after the Retic-C Cell Control on the LH 750 and LH 780 analyzers to re-create the conditions that might cause spillage into the next tube. The Retic-C Cell Control tubes used for these experiments had been sampled numerous times in some cases; in other instances, the control tubes had not been multiply punctured. Blood smears were made from the blanks or routine samples using either the automated slidemaker or manually.

Results

Appearance of the Patient NRBCs, Control Avian RBCs, and Human NRBCs

A blood smear from a patient containing the NRBCs is shown in Image 1A. These NRBCs had a similar appearance to avian RBCs found on a blood smear prepared from the Retic-C Cell Control (not shown), particularly their ellipsoid shape with areas of clearing adjacent to the nuclei. Human NRBCs have a circular shape without the characteristic areas of clearing.

Location of Avian RBCs on WBC Scatterplot and Histogram

A representative WBC scatterplot from patient 3 (Table 1) reveals a minor population of cells in the characteristic NRBC area Image 1B. The accompanying WBC histogram contains a small peak in the 35-fl area (Figure 1A).

Image 1B shows the scatterplot produced by running the Retic-C Cell Control as if it were a patient sample for a WBC differential count. The scatterplot shows that very few cells from the Retic-C Cell Control are in the WBC areas. Instead, they fall within the characteristic NRBC area and in the expected location for unlysed RBCs. In the accompanying WBC histogram, many of the Retic-C Cell Control cells are in the 35-fl area and in the expected location for unlysed RBCs.

The characteristic NRBC areas of the WBC scatterplot and WBC histogram, from the manufacturer’s instructional materials, are shown in Image 1C. Image 1D shows a WBC scatterplot and histogram from a patient whose blood smear contained 14 human NRBCs per 100 WBCs.

Carryover Checks

All carryover checks on the LH 780 analyzer, performed at regular intervals during the period of the reported events, were within manufacturer-defined acceptable limits.

Experiments to Reproduce the Phenomenon of Contamination

At BRR Hospital, there were 4 separate attempts to reproduce the phenomenon by running a patient tube immediately following Retic-C Cell Control that had been pierced at least 5 times, and all were successful. Each of these patient tubes became contaminated with avian cells, as shown by aspirating a sample with a transfer pipette and making a manual smear. Additional tubes, which followed the first contaminated patient tube, were not contaminated. When Retic-C Cell Control material was placed in an empty EDTA tube, no carryover was observed into the subsequent patient tube; thus, the carryover issue was related only to the control tube.

In addition, multiple unsuccessful attempts to reproduce the phenomenon involved running a blank or a patient tube immediately after a Retic-C Cell Control tube, which had been pierced 2 to 4 times. These included 4 unsuccessful attempts using the LH 750 and 3 attempts using the Unicel DxH800 at BRR Hospital, as well as 5 separate unsuccessful attempts using the LH 785 at SBU Medical Center, performed in duplicate.
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Discussion

We were able to reproduce the phenomenon of spillage when the Retic-C Cell Control tube had been punctured at least 5 times and the blood smears were prepared manually, not by the automated slidemaker. Our laboratory workflow involved puncturing these control tubes up to 12 times before discarding them. We believe that weakening of the rubber stopper following multiple punctures may be a necessary condition for the spillover, along with a particular angle of tilting the tube relative to the automated sampler. Furthermore, introduction of the control sample into the next tube may have been related to needle and sampling valve function, and maintenance on these instrument parts was performed following our complaint. The phenomenon appeared to occur only when all of these anomalies were present at the same time. Since patient tubes are not multiply punctured, patient-to-patient carryover would not be expected to occur in the same way. Only the LH 750 analyzer, not the Unicel DxH 800, could be shown to reproduce the phenomenon.

The manufacturer (Beckman Coulter) provided a sample Product Corrective Action statement dated January 2008 that described possible carryover of cellular components related to the LH SlideMaker and recommended running the Retic-C Cell Controls in order from highest to lowest, followed by a blank. However, at BRR Hospital, the LH SlideMaker was not in use, and all slides were made manually, so the problem occurred within the LH 750 analyzer itself. We have since corrected our laboratory workflow to run the Retic-C Cell Controls from highest to lowest.

Interestingly, the LH 750 analyzer did not flag the patient samples containing avian cells for NRBCs. We do not know why these cells did not trigger the instrument flag because they appeared to be located within the characteristic NRBC location of the WBC scatterplot and histogram (Figure 1).

![Figure 1](image)

**Figure 1** WBC scatterplots and histograms from a patient with unusual nucleated RBCs (NRBCs) (**A**), the Retic-C Cell Control (Beckman Coulter, Miami, FL) run in patient testing mode (**B**), the manufacturer’s instructional materials (**C**), and a patient with typical NRBCs (**D**). The WBC scatterplot from the patient with unusual NRBCs contains a minor population of cells in the characteristic NRBC area, and the WBC histogram contains a small peak (circled) in the 35-fL area (**A**). The WBC scatterplot from the Retic-C Cell Control shows many cells within the characteristic NRBC area, and the histogram shows many cells in the 35-fL area (**B**).
In conclusion, the NRBCs found in patient tubes at the SBU Medical Center and BRR Hospital likely represented avian RBCs from the Retic-C Cell Controls that had been introduced into the patient tubes. The Unicel DxH 800 did not reproduce the contamination phenomenon in our hands and has superior sensitivity for NRBCs compared with the LH 750. Our blood smears with unusual NRBCs did not contain other abnormalities that typically accompany NRBCs, such as immature cells or dysplastic granulocytes. Unexpected laboratory findings must be carefully evaluated in their clinical setting with careful consideration of preanalytical as well as analytical variables.

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