CPAL
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Technical Bulletin
No. 158

March 13, 2017

Residual Leukocyte Testing of Leukoreduced Red Blood Cell and Platelet Products on the NanoEnTek ADAM rWBC
— New Assay —

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Ordering Information and Suggested Codes:
This test is provided as a quality control measure of leukoreduction practices for CPAL member blood donor centers. CPT and/or LOINC codes are not applicable to this test.

Effective Date: 3/27/2017

Performed: Monday through Friday, dayshift

Reference Range:
Not applicable

Specimen Requirements:

- 1 ml of a red blood cell, whole blood, or platelet product (0.5 mL minimum) aliquot
- RBC/Whole Blood products: store and ship samples at 1-8ºC
- Platelet products: store and ship samples at 1-8ºC or room temperature
- **ADAM testing must be completed within 48 hours of leukoreduction *and* within 24 hours of the post-leukoreduction sampling date and time.**

Background:
The presence of white blood cells (WBC) in blood and platelet products is associated with an increased incidence of febrile transfusion reactions, transmission of cytomegalovirus, and alloimmunization to HLA antigens in transfusion recipients. Leukoreduction, which is accomplished via the collection of blood products by apheresis or post-collection processing with special filters, can lower the WBC count to 5 x 10^6 per unit or below. This minimizes complications associated with transfusions.

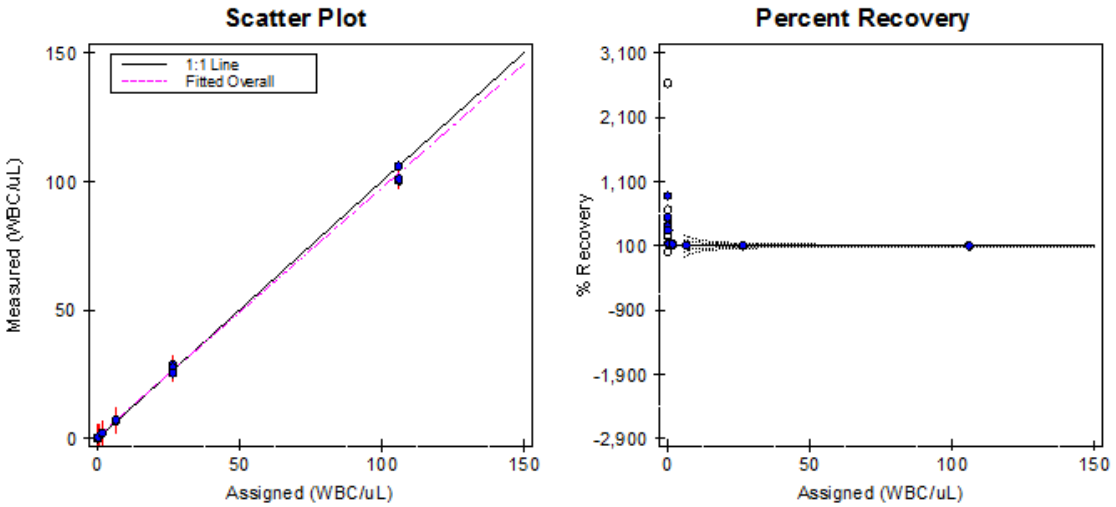
Principle of test:

The ADAM-rWBC system is an analyzing device and associated reagents that counts the number of residual leukocytes in a blood component for transfusion. It uses a technology based on fluorescence microscopy. Targeting the leukocyte, the ADAM-rWBC system uses a fluorescent dye (propidium iodide) to stain cellular components than contain DNA (WBC's). It then automatically counts them using LED optics and CCD detection. Automated cell counting eliminates user bias or subjective interpretation and is less labor-intensive than counting residual leukocytes using a Nageotte chamber. The ADAM-rWBC is less technique-dependent and less costly than flow cytometry.

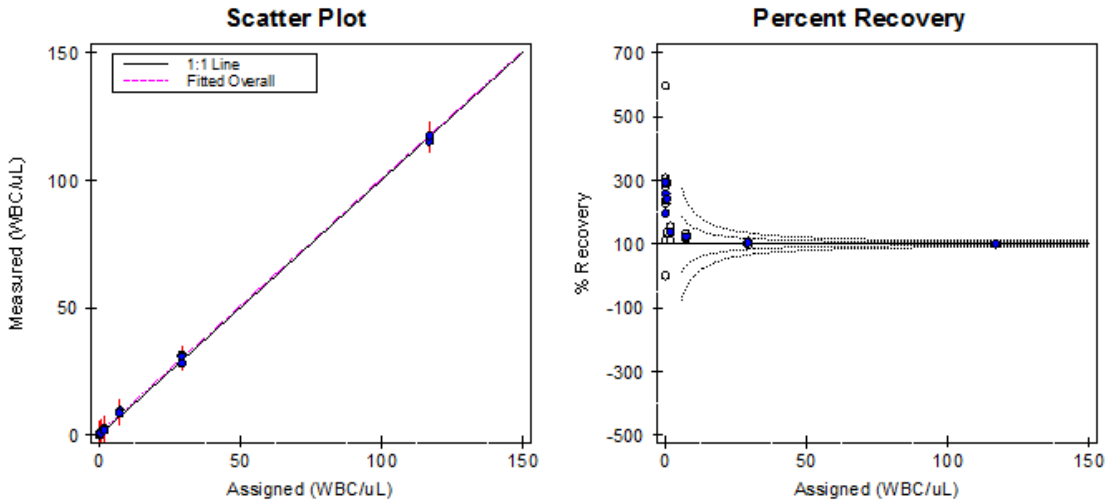
Validation Summary

Linearity, Accuracy, and Analytical Measurement Range: Studies performed were found to be acceptable. Spiking and dilution studies were performed to verify NanoEnTek's stated analytical measurement range of 0 – 100 WBC/µL:

Leukoreduced Platelet Linearity, Accuracy, Reportable Range



Leukoreduced Red Blood Cell Linearity, Accuracy, Reportable Range

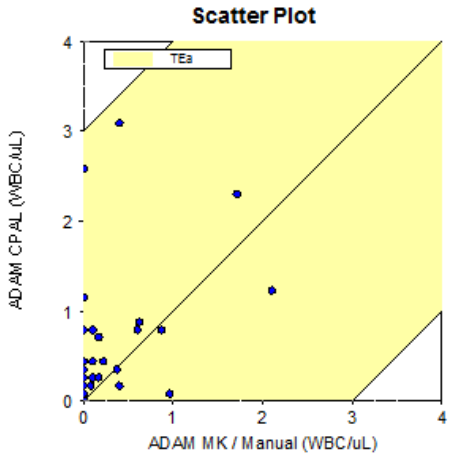


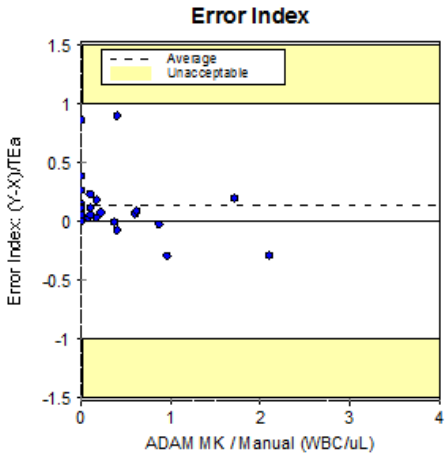
	Analyte		Linearity	Accuracy	Rep Range	Precision
	LR-PLT		Pass	Pass	Pass	--
▶	LR-RBC		Pass	Pass	Pass	--

Method Comparison:
Samples from each institution were tested and compared to their current method of residual leukocyte enumeration to ensure that CPAL will provide comparable, accurate results using the ADAM rWBC. Two institutions use Nageotte Chamber counts; the third institution sends testing to Miller Keystone Blood Center, which utilizes a NanoEnTek ADAM r-WBC for testing. In total, 35 RBC units and 32 platelet units were tested.

Discussion: Two data points, one for the RBC comparison and one for PLT comparison, were excluded from each method comparison. In both instances, the comparator method was the Nageotte Chamber count. Per Dzik, et al, this was a potential expected variance due to higher variability and lower sensitivity within the Nageotte Chamber counting method. All other Method Comparisons were found to be acceptable when evaluated using EP Evaluator.

Leukoreduced Platelet Residual WBC Count, All Methods vs. CPAL ADAM





Key Statistics

Average Error Index	0.13
Error Index Range	-0.29 to 0.90
Coverage Ratio	--

Evaluation Criteria

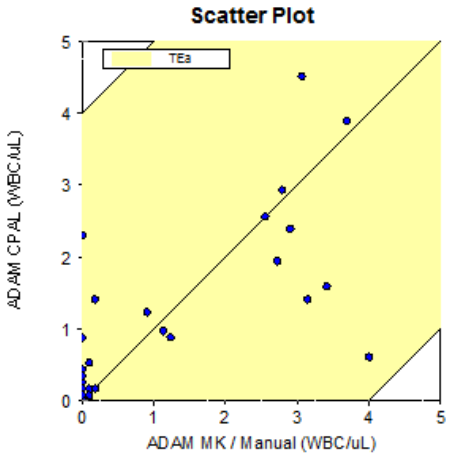
Allowable Total Error	3 WBC/uL (conc) or 15%
Reportable Range	--

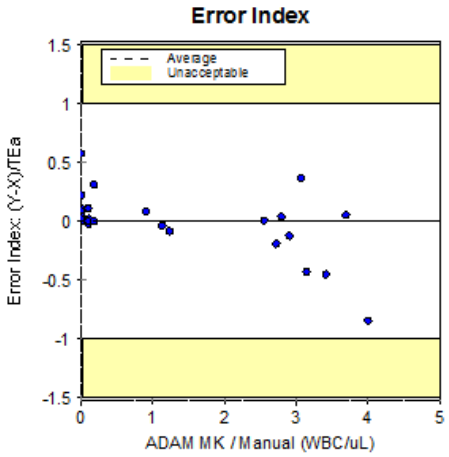
Deming Regression Statistics

Y = Slope * X + Intercept

Correlation Coeff (R)	0.3606
Slope	2.464 (1.567 to 3.362)
Intercept	-0.029 (-0.548 to 0.490)
Std Error Estimate	1.223
N	31 of 32

Leukoreduced Red Blood Cell Residual WBC Count, All Methods vs. CPAL ADAM





Key Statistics

Average Error Index	0.00
Error Index Range	-0.85 to 0.57
Coverage Ratio	--

Evaluation Criteria

Allowable Total Error	4 WBC/uL (conc) or 15%
Reportable Range	--

Deming Regression Statistics

Y = Slope * X + Intercept

Correlation Coeff (R)	0.7422
Slope	0.809 (0.593 to 1.024)
Intercept	0.183 (-0.175 to 0.542)
Std Error Estimate	0.843
N	34 of 35

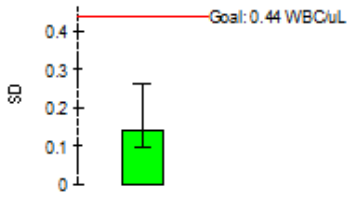
Precision Studies:

Intra-assay Within Run Reproducibility

One leukoreduced red blood cell sample was prepared for testing three times and pooled. One leukoreduced platelet sample was prepared three times and pooled. Each pool was tested 10 times.

Intra-assay Precision, Leukoreduced Platelet Sample

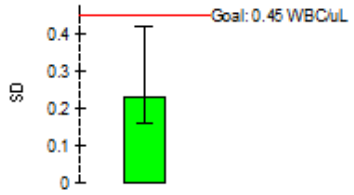
Precision Statistics



Obs Standard Dev (SD)	0.144
Pass/Fail/Uncertain	Yes
95% Confidence for Obs SD	0.099 to 0.263
Obs Coef of Variation (CV)	65.4%
Obs Mean	0.220 WBC/uL
Number of Specimens (N)	10 of 10
95% CI for Obs Mean	0.117 to 0.323
Obs 2 SD Range	-0.068 to 0.508

Intra-assay Precision, Leukoreduced Red Blood Cell Sample

Precision Statistics



Obs Standard Dev (SD)	0.230
Pass/Fail/Uncertain	Yes
95% Confidence for Obs SD	0.158 to 0.419
Obs Coef of Variation (CV)	33.4%
Obs Mean	0.688 WBC/uL
Number of Specimens (N)	10 of 10
95% CI for Obs Mean	0.524 to 0.852
Obs 2 SD Range	0.229 to 1.147

Inter-Assay Between Run Reproducibility

Due to the nature of a cellular-based assay and specimen stability of this test, testing a single sample across multiple days was not possible. As a replacement investigation, 5 leukoreduced platelet products and 5 leukoreduced red blood cell products were stained in triplicate and tested on the same day.

Donor Test	Replicate	WBC/uL	Mean	SD
PLT Donor 1	Rep 1	0.08	0.027	0.046
	Rep 2	0		
	Rep 3	0		
PLT Donor 2	Rep 1	0.44	0.44	0.27
	Rep 2	0.71		
	Rep3	0.17		
PLT Donor 3	Rep 1	0.26	0.35	0.09
	Rep 2	0.44		
	Rep 3	0.35		
PLT Donor 4	Rep 1	0.79	0.87	0.23
	Rep 2	1.13		
	Rep 3	0.7		
PLT Donor 5	Rep 1	0.79	0.7	0.15
	Rep 2	0.79		
	Rep 3	0.53		

Donor Test	Replicate	WBC/uL	Mean	SD
RBC Donor 1	Rep 1	2.56	2.41	0.51
	Rep 2	1.85		
	Rep 3	2.83		
RBC Donor 2	Rep 1	0.97	1.17	0.18
	Rep 2	1.23		
	Rep3	1.32		
RBC Donor 3	Rep 1	2.93	2.95	0.13
	Rep 2	3.09		
	Rep 3	2.83		
RBC Donor 4	Rep 1	3.89	3.22	0.82
	Rep 2	3.47		
	Rep 3	2.3		
RBC Donor 5	Rep 1	2.39	2.13	0.23
	Rep 2	2.04		
	Rep 3	1.95		

Between Run Reproducibility

High level and low level control materials for each analyte (leukoreduced platelet and leukoreduced red blood cell) were tested twice per day at two separate time intervals (AM and PM) across 20 days.

		Analyte	Sample	N	Mean	SD	CV
▶	●	LR-PLT	LR-PLT QC Hi	40 of 40	23.387 / 22.0	1.620 / 2.75	6.9% / 12.5
	●	LR-PLT	LR-PLT QC Lo	40 of 40	2.026 / 2.4	0.433 / 0.44	21.4% / 18.3
	●	LR-RBC	LR-RBC QC Hi	40 of 40	21.815 / 22.0	1.911 / 3	8.8% / 13.6
	●	LR-RBC	LR-RBC QC Lo	40 of 40	2.033 / 2.5	0.420 / 0.45	20.7% / 18.0

References

1. ADAM-rWBC Instruction Manual, NanoEnTek, 2013

2. ADAM-rWBC kit Instructions for Use, 10/2013

3. S. Dzik et al., A Multicenter Study Evaluating Three Methods for Counting Residual WBCs in WBC-reduced Blood Components: Nageotte Hemocytometry, Flow Cytometry, and Microfluorometry. Transfusion 40 (5), 513-520. 5 2000.