



**CPAL**

Central Pennsylvania Alliance Laboratory

# Technical Bulletin

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## **Residual Leukocyte Testing of Leukoreduced Red Blood Cell and Platelet Products on the NanoEnTek ADAM rWBC 2 — Platform Change —**

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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: 3/1/2019**

**Performed:** Monday through Saturday, dayshift

**Reference Range:**

Not applicable

**Specimen Requirements:**

- 1 ml aliquot of a red blood cell, whole blood, or platelet product (0.5 mL minimum)
- RBC/Whole Blood products: store and ship samples at 1-8°C
- Platelet products: store and ship samples at 1-8°C 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  $5 \times 10^6$  per unit or below. This minimizes complications associated with transfusions.

## Principle of test:

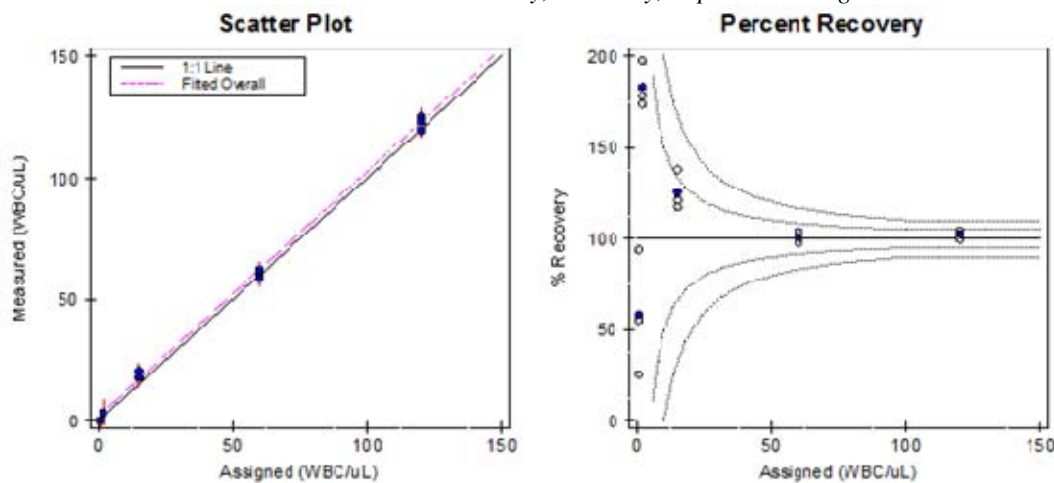
The ADAM-rWBC 2 system is an analyzing device and associated reagents that counts the number of residual leukocytes in a blood component for transfusion. It uses a technology based on fluorescence microscopy. Targeting the leukocyte, the ADAM-rWBC 2 system uses a fluorescent dye (propidium iodide) to stain cellular components that contain DNA (WBC's). It then automatically counts them using LED optics and CMOS 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 2 is less technique-dependent and less costly than flow cytometry.

## Validation Summary

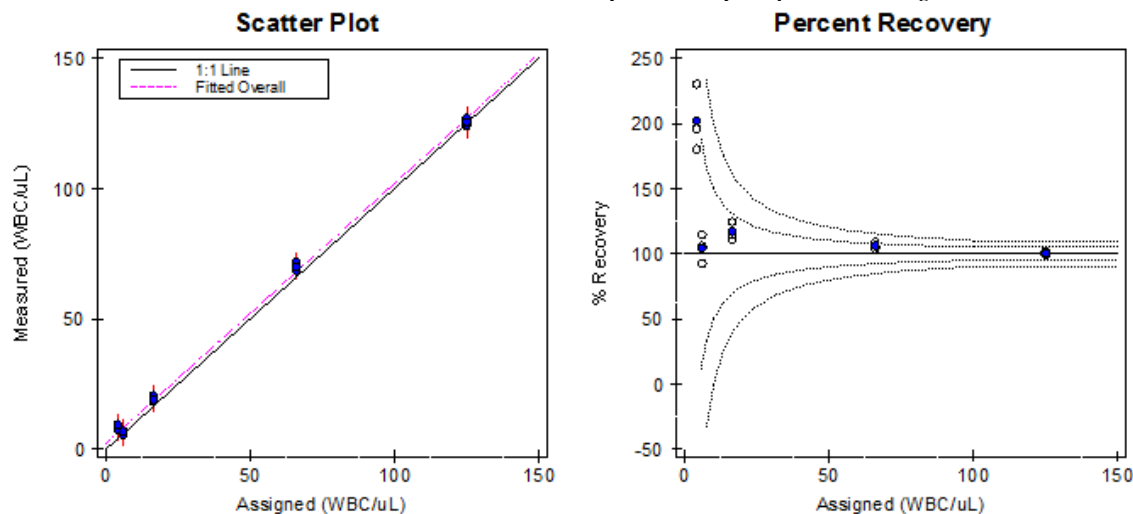
*Linearity, Accuracy, and Analytical Measurement Range:* Studies performed were found to be acceptable. Spiking and dilution studies were performed to verify NanoEnTek's stated analytical measurement range of 0 – 100 WBC/ $\mu$ L:

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

### Leukoreduced Platelet Linearity, Accuracy, Reportable Range



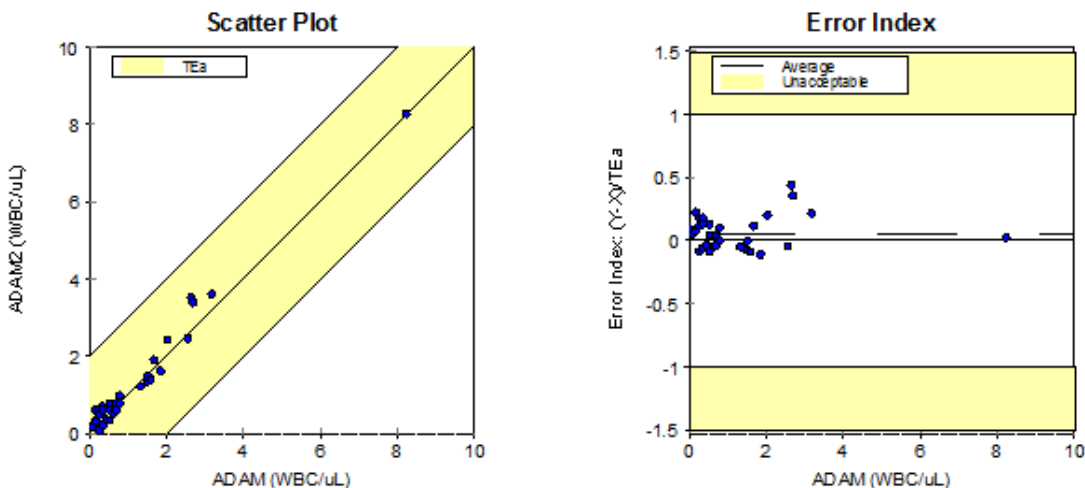
*Leukoreduced Red Blood Cell Linearity, Accuracy, Reportable Range*



*Method Comparison:*

Samples from each institution were tested and compared to CPAL’s current method of residual leukocyte enumeration on the ADAM r-WBC. This ensured that CPAL will provide comparable, accurate results using the ADAM rWBC 2. A minimum of 10 samples of each product type, anticoagulant, and filtration method were tested. In total, 35 RBC/WB units and 20 platelet units were tested.

*Leukoreduced Red Blood Cell Residual WBC Count, ADAM r-WBC vs ADAM r-WBC 2*



**Key Statistics**

Average Error Index 0.06  
 Error Index Range -0.11 to 0.44  
 Coverage Ratio --

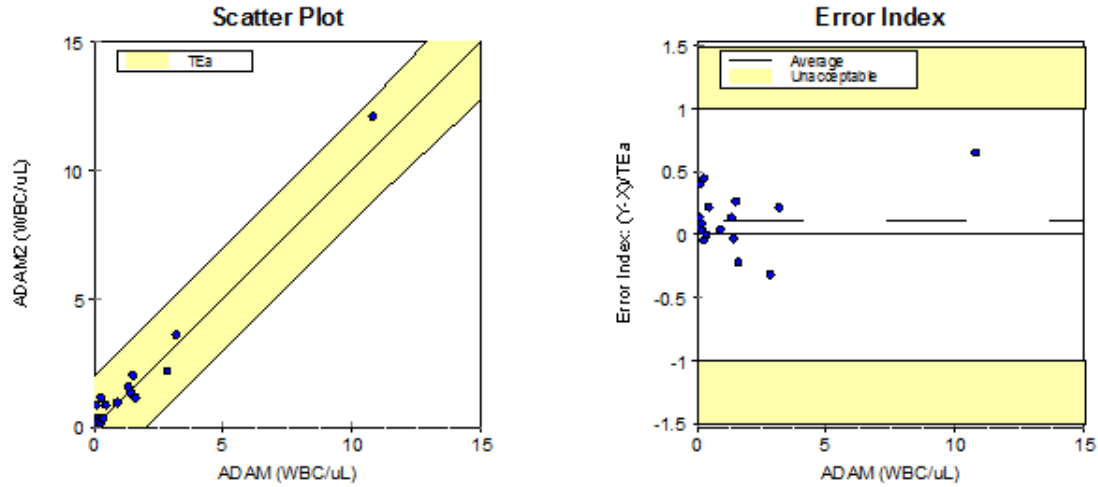
**Evaluation Criteria**

Allowable Total Error 2 WBC/uL (conc) or 15%  
 Reportable Range 0 to 0 WBC/uL

**Deming Regression Statistics**

$Y = \text{Slope} * X + \text{Intercept}$   
 Correlation Coeff (R) 0.9862  
 Slope 1.040 (0.979 to 1.102)  
 Intercept 0.065 (-0.050 to 0.180)  
 Std Error Estimate 0.264  
 N 35 of 35

*Leukoreduced Platelet Residual WBC Count, ADAM r-WBC vs. ADAM r-WBC 2*



**Key Statistics**

Average Error Index 0.11  
 Error Index Range -0.31 to 0.65  
 Coverage Ratio --

**Evaluation Criteria**

Allowable Total Error 2 WBC/uL (conc) or 15%  
 Reportable Range 0 to 0 WBC/uL

**Deming Regression Statistics**

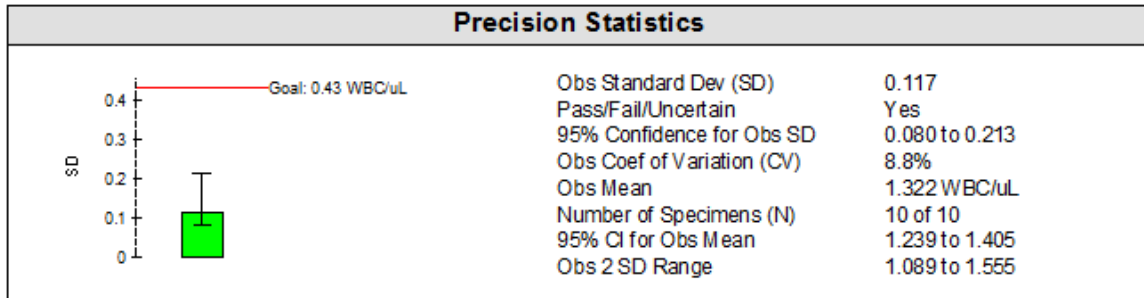
$Y = \text{Slope} * X + \text{Intercept}$   
 Correlation Coeff (R) 0.9890  
 Slope 1.091 (1.011 to 1.171)  
 Intercept 0.114 (-0.102 to 0.329)  
 Std Error Estimate 0.404  
 N 20 of 20

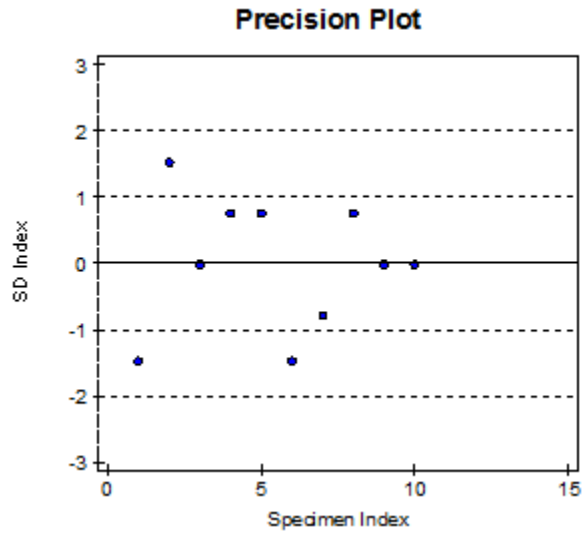
*Precision Studies:*

*Intra-assay Within Run Reproducibility*

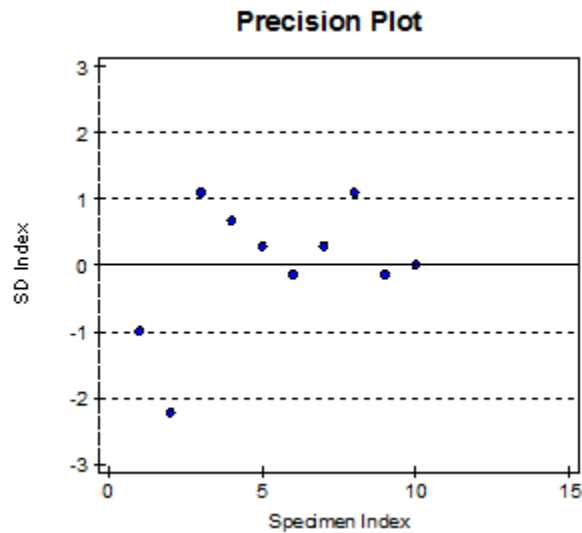
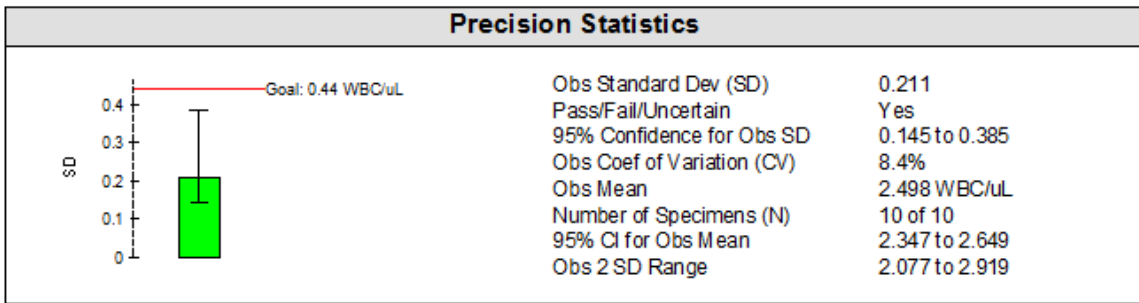
One leukoreduced red blood cell sample was prepared for testing three times and pooled. One leukoreduced platelet sample was prepared three times and pooled. Each pool was tested 10 times.

*Within Run Precision, Leukoreduced Red Blood Cell Sample*





*Within Run Precision, Leukoreduced Platelet Sample*



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	Acceptable Range	Acceptable?
RBC Donor 1	Rep 1	0.18	0.207	0.046	0.115 - 0.299	Yes
	Rep 2	0.18				
	Rep 3	0.26				
RBC Donor 2	Rep 1	0.36	0.45	0.085	0.283 - 0.62	Yes
	Rep 2	0.53				
	Rep3	0.46				
RBC Donor 3	Rep 1	0.62	0.683	0.055	0.573 - 0.793	Yes
	Rep 2	0.71				
	Rep 3	0.72				
RBC Donor 4	Rep 1	1.5	1.33	0.175	0.98 - 1.68	Yes
	Rep 2	1.15				
	Rep 3	1.34				
RBC Donor 5	Rep 1	0.62	0.583	0.148	0.287 - 0.879	Yes
	Rep 2	0.71				
	Rep 3	0.42				

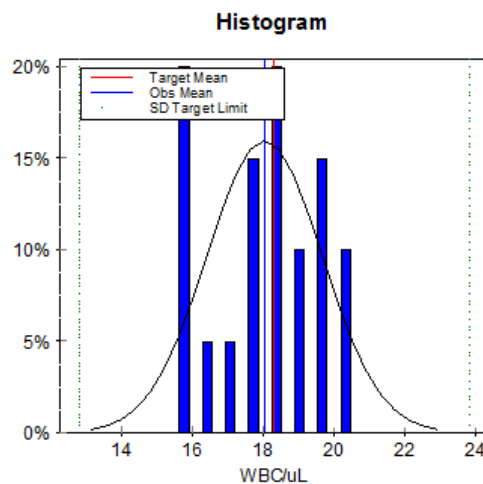
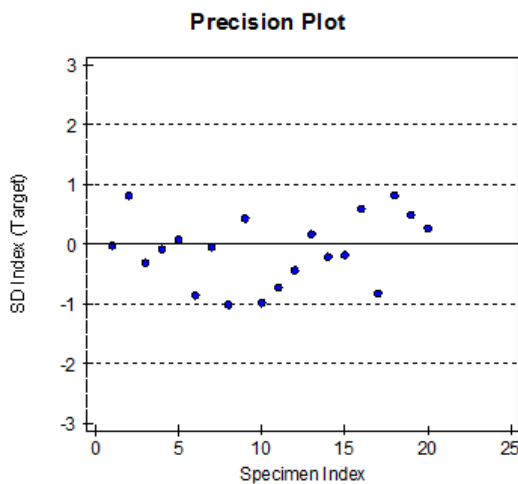
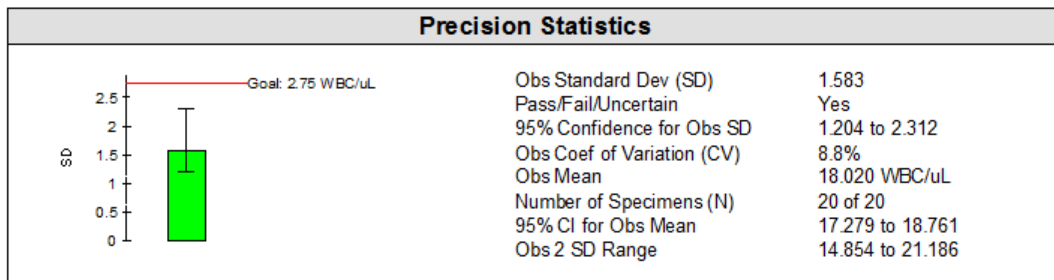
Donor Test	Replicate	WBC/uL	Mean	SD	Acceptable Range	Acceptable?
PLT Donor 1	Rep 1	0.26	0.267	0.07	0.127 - 0.407	Yes
	Rep 2	0.34				
	Rep 3	0.2				
PLT Donor 2	Rep 1	0.35	0.42	0.075	0.27 - 0.57	Yes
	Rep 2	0.41				
	Rep3	0.5				
PLT Donor 3	Rep 1	0.09	0.173	0.085	0.003 - 0.343	Yes
	Rep 2	0.17				
	Rep 3	0.26				
PLT Donor 4	Rep 1	0.97	1	0.052	0.896 - 1.104	Yes
	Rep 2	0.97				
	Rep 3	1.06				
PLT Donor 5	Rep 1	1.35	1.477	0.121	1.235 - 1.719	Yes
	Rep 2	1.59				
	Rep 3	1.49				

Between Run Reproducibility

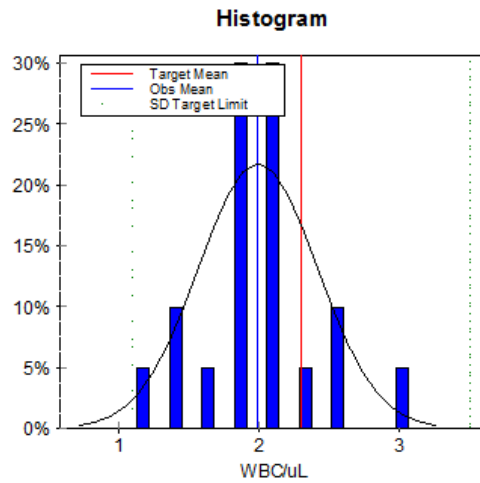
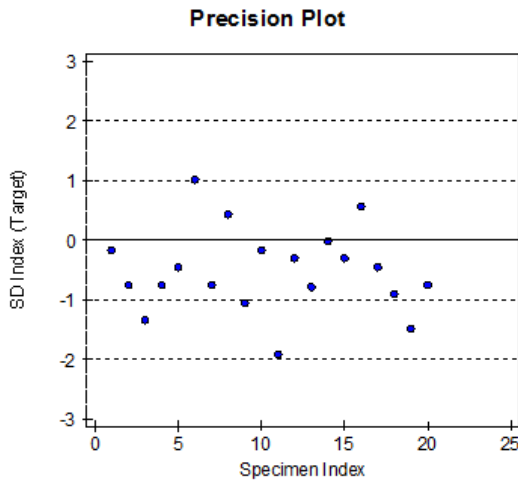
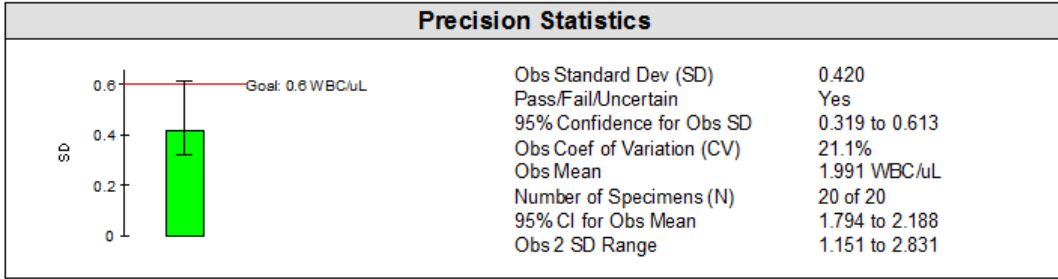
High-level and low-level control material for each analyte (leukoreduced red blood cell and leukoreduced platelet) were tested twice per day at two separate time intervals (AM and PM) across 20 days. One level of control for each product was tested in the morning and the other level of control for each product was tested in the afternoon.

	Analyte	Sample	N	Mean	SD	CV
●	LR-PLT QC High	Between Run	20 of 20	23.175 / 22.5	1.459 / 2.75	6.3% / 11.9
●	LR-PLT QC Low	Between Run	20 of 20	2.544 / 2.2	0.463 / 0.81	18.2% / 31.8
●	LR-RBC QC High	Between Run	20 of 20	18.020 / 18.3	1.583 / 2.75	8.8% / 15.3
▶	LR-RBC QC Low	Between Run	20 of 20	1.991 / 2.3	0.420 / 0.6	21.1% / 30.1

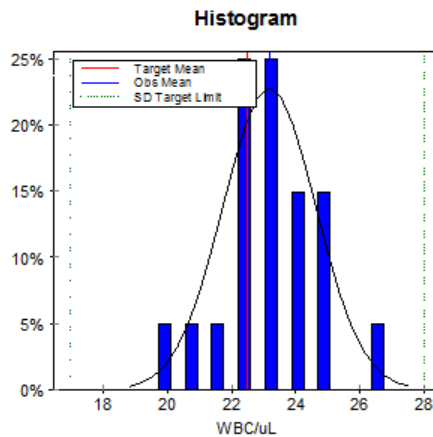
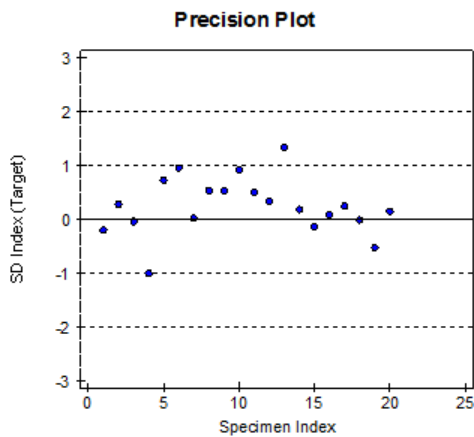
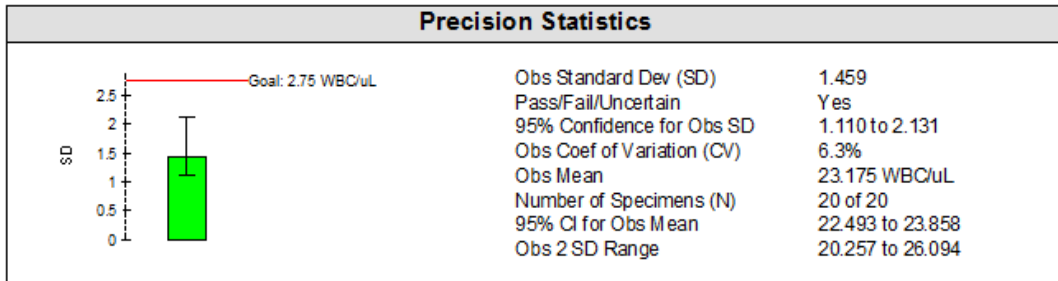
*RBC High Control, Between Run Reproducibility*



*RBC Low Control, Between Run Reproducibility*

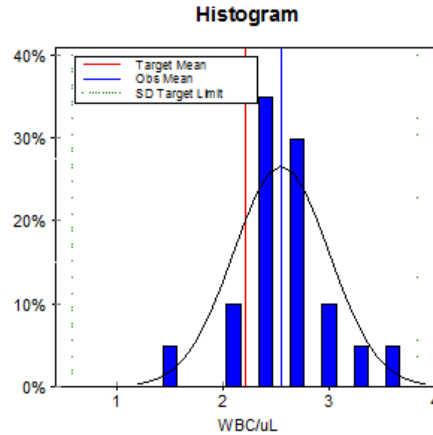
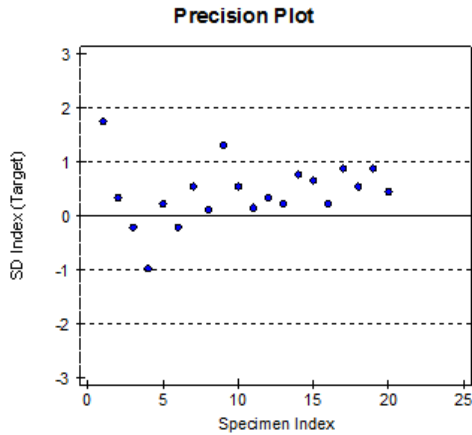
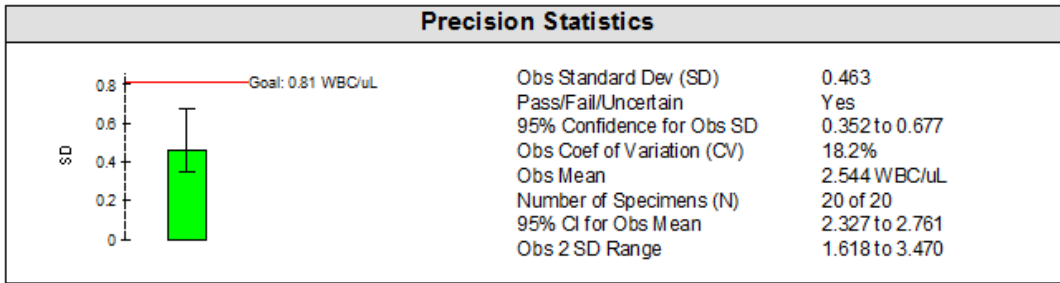


*PLT High Control, Between Run Reproducibility*





PLT Low Control, Between Run Reproducibility



**References**

1. ADAM-rWBC 2 Instruction Manual, NanoEnTek
2. ADAM-rWBC kit Instructions for Use, 09/2018
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.