

# Coulter A<sup>c</sup>T 10 Automated Cell Counter

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The purpose of this Operating Instruction (OI) is to outline the general operation of the Coulter A<sup>c</sup>T 10 hematology analyzer. Refer to the Coulter A<sup>c</sup>T Series Analyzer reference manuals for more in-depth information.

1. PRINCIPLE: Coulter Method - The Coulter method accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture.

1.1. The A<sup>c</sup>T 10 system uses triplicate counting, internal voting criteria and proprietary flagging algorithms to confirm parameter results prior to reporting. After the computer corrects for coincidence, it compares the three counts each for WBC, RBC, and PLT. If the unit finds disagreement among all count periods or does not meet internal criteria, the instrument displays a total vote out.

1.2. Computed Parameters - The computer computes HCT, MCH, MCHC, and LY%.

1.3. Hemoglobin Concentration - The system uses the lysed WBC dilution to measure the hemoglobin. The absorbance of light from an incandescent light is measured at 525 nm through the optical path length of the bath. A beam of light from the lamp passes through the sample, through the 525 nm filter, and is measured by a photodiode. The signal is amplified and the voltage is measured and compared to the blank reference reading.

2. SPECIMEN:

2.1. Specimen of choice - EDTA anticoagulated whole blood or fresh whole blood that is prediluted directly into diluent. Visually examine samples for clots or use a wooden applicator stick to check for fibrin strands or clots.

2.2. Mix samples at least 8-10 times by hand inversion before analysis. Run whole blood samples within 24 hours of collection. Whole blood cell counts that include PLT must be performed within four (4) hours after collection of specimen. Run pre-diluted samples within 4 hours of collection. The prediluted sample must be prepared at least 2 minutes before running. Run at room temperature.

2.3. Specimens that are clotted, hemolyzed, grossly lipemic, contain chromatogenic dyes or substances, or do not meet the above stated criteria are not acceptable for analysis.

3. REAGENTS: Coulter A<sup>c</sup>T Tainer - The pack contains Reagent 1, diluent; Reagent 2, lytic agent; and Reagent 3, rinse shutdown diluent. The reagent pack can be stored at room temperature and can be used until the expiration date stated on the pack.

3.1. Reagent 1 is an isotonic electrolyte solution that:

- Dilutes the whole blood samples.
- Stabilizes cell membranes for accurate counting and sizing.
- Conducts aperture current.
- Rinses instrument components between analyses.
- Prevents duplicate cell counts by using the sweep-flow process

3.2. Reagent 2 is a lytic reagent that:

- Lyses red blood cells (RBCs) for WBC count and hemoglobin measurement.
- Causes a differential shrinkage of leukocytes into predictable volume components.

3.3. Reagent 3 shutdown diluent prevents protein buildup that occurs in and around the apertures.

4. CALIBRATION: Perform calibration utilizing a Coulter S-Cal calibrator initially upon EMEDS set up, semiannually, when you replace any A<sup>c</sup>T major component that involves the primary measurement characteristics (such as an aperture), or when experiencing any problems with the quality control materials.

4.1. Preliminary Procedures:

4.1.1. Ensure that all required maintenance (including replacement of parts) has been performed on the instrument. See \_\_\_\_ below for maintenance schedule.

4.1.2. Clean the baths according to the Special Procedures and Troubleshooting Manual, Heading 1.3, Clean the Baths.

4.1.3. Calibrate only within the ambient temperature of 20-25C.

4.1.4. Before you begin these calibration procedures, shut down your instrument in Coulter AC.T rinse shutdown diluent.

4.1.5. Check that you have a sufficient supply of reagents to complete this procedure.

4.1.6. Perform Startup.

4.2. Reproducibility/Carryover Check: Reproducibility is a check to ensure that the instrument measures blood parameters consistently.

4.2.1. Set analyzing mode to whole blood.

4.2.2. Cycle one fresh, normal, properly mixed, whole-blood sample to prime the instrument.

4.2.3. Analyze 1 fresh, normal, properly mixed whole-blood sample ten times.

4.2.4. Record results (attachment 1) for WBC, RBC, HGB, MCV, and platelet from the ten cycles.

4.2.5. With a scientific calculator, calculate the mean, standard deviation, and CV% for the constituents listed above. The CV% should be less than or equal to those listed.

$$\text{Note: CV\%} = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Precision Limits for 10 Replicate Samples Coefficient of Variation

Parameters	Coefficient of Variation
WBC at 6.0 - 15.0	</= 3.0%
RBC at 3.00 - 6.00	</= 3.0%
HGB at 12.0 - 18.0	</= 2.0%
MCV at 80.0 - 100.0	</= 2.0%
PLT at 200 - 500	</= 7.0%

If CV% of any parameter is greater than those listed, you might have an instrument problem. Call your Coulter Representative.

4.2.6. Review each parameter for trending. If a trend exists, you might have an instrument problem. Call your Coulter representative.

4.2.7. Record the results in attachment 1.

4.2.8. Perform the Carryover check. Carryover is a check to ensure that no part of the sample is carried over to the next sample, thus affecting the next sample's results.

4.2.8.1. Press the aspirate switch three times, recording the results from each of the three cycles (attachment 2).

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4.2.8.2. Using the following formula, compute the carryover for WBC, RBC, HGB, and PLT.

$$\text{Carryover} = \frac{\text{first cycle} - \text{third cycle}}{\text{result \#10 reproducibility check}} \times 100$$

4.2.8.3. The results must not exceed the following values:

Parameter	Carryover (%)
WBC	</= 2.0
RBC	</= 2.0
HGB	</= 2.0
PLT	</= 2.0

4.2.8.4. Record the results on attachment 2.

#### 4.3. Calibration Procedure:

4.3.1. S-CAL Calibrator Kit. The S-CAL calibration kit helps you determine whether the calibration factors of the instrument need to be changed. Assigned values are provided in the kit package insert. The calibration procedure requires the use of attachment 3, calibration worksheet. Prepare the S-CAL calibrator according to the instructions in the S-CAL calibrator package insert.

4.3.2. Refer to attachment 4, Coulter calibration procedures, pages 1-10 through 1-13 in the Coulter A<sup>c</sup>T Series "Special Procedures and Troubleshooting manual for the entire calibration procedure.

#### 5. QUALITY CONTROL:

5.1. Coulter 4C Plus cell controls are available for quality control use. Follow the manufacturer's insert for proposed expiration dates and open-vial stability information. Run all levels of controls at the beginning of each shift.

##### 5.2. Running Coulter 4C Plus Cell Controls.

Important: Risk of misleading results. Only run 4C Plus in the whole blood mode. Running 4C Plus in the incorrect analyzing mode results in misleading results.

5.2.1. Ensure that the controls are not past their expiration date. Bring the controls to room temperature and mix each control by gently rolling the tubes in the palms of your hands and inverting at least 8 times. Inspect the tube contents to ensure that all cells are uniformly distributed; if not, repeat this step.

5.2.2. Set the analyzing mode to Whole Blood. Set the sample ID to the lot number on the 4C Plus tube. Invert the tube once or twice prior to cycling. Cover the top of the control tube with lint-free tissue and remove the cap.

5.2.3. Hold the control tube up to the probe so tip is into the control and press the aspirate switch. When you hear the beep, remove the control tube and replace the cap. Return the control vial to the refrigerator.

5.2.4. Document the control results on the form provided with the 4C Controls. Compare the instrument values with those stated on the 4C Plus Table of Expected Results.

5.2.5. If the results are within the expected range, begin patient testing. If the results are not within the expected range, repeat the control. If the control is still out of range, perform and document the appropriate corrective action.

## 6. PROCEDURE:

6.1. At the beginning of the day, press "Startup" icon. The Startup checks the instrument's pneumatics, cleans the apertures, and performs a background check. Once the startup cycle is complete, review the screen for any error messages. If none are present, go on to the next step below. If any errors are present, refer to the Coulter Special Procedures and Troubleshooting Guide.

### Background Counts

<u>Parameter</u>	<u>Count</u>
WBC	</= 0.4
RBC	</= 0.04
HGB	</= 0.2
PLT	</= 7.0

6.2. Perform and document the 4C Plus controls as stated in 5 above.

### 6.3. Running Samples - Whole Blood:

6.3.1. Set the analyzing mode to Whole Blood. Set the sample identification to the correct number or let the A<sup>c</sup>T increment the current number by 1.

6.3.2. Mix the sample 8-12 times by gentle inversion. Present the sample to the probe and press the aspirate switch. When you hear the beep, remove the sample.

6.3.3. The A<sup>c</sup>T displays the sample results on the screen. Record and review the test results. If there are no flags on results, the A<sup>c</sup>T is ready to run the next sample.

### 6.4. Running Samples - Prediluted Blood:

6.4.1. Set the analyzing mode to prediluted blood. Set the sample identification to the correct number or let the A<sup>c</sup>T increment the current number by 1.

6.4.2. Touch the "Dispense Diluent" icon on the screen. Present an empty tube to the probe and press the aspirate switch to dispense 1580 ul of diluent into the empty tube. If you have no more samples to prepare, press the "Exit" icon to return to the "Sample Results" screen.

6.4.3. Add 20 ul of blood specimen to the diluent in the tube. Mix the sample according to your laboratory's protocol. Wait at least 2 minutes before running the sample.

6.4.4. Present the mixed, prediluted sample to the probe and press the aspirate switch. When you hear the beep, remove the sample.

6.4.5. The A<sup>c</sup>T displays the sample results on the screen. Record and review the test results. If there are no flags on results, the A<sup>c</sup>T is ready to run the next sample.

7. CALCULATIONS: Perform manual differential/smear review if:

7.1. Any vote-out on repeat.

7.2. Platelet count is <130; perform platelet estimate only.

7.3. "R" Code is printed next to any analyte.

7.4. MCV >105.0 (RBC morphology only).

7.5. WBC <2.0 or >20.0.

8. RESULTS:

8.1. Reporting units are printed on the printout.

8.2. The hemoglobin and hematocrit should reflect a 1:3 ratio (1:3 ratio +/- 3).

8.3 All panic values are reported in accordance with SGSAL OI 44-MA.4, Critical Values.

8.4. Linearity Limits.

Parameter	Linearity Range
WBC	0 - 99.9
RBC	0 - 7.0
HGB	0 - 25
PLT	0 - 999

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8.5. Reference ranges for the A<sup>c</sup>T 10:

PARAMETERS	NORMAL VALUES		PANIC VALUES	
	Male	Female	Male	Female
WBC	4.8 - 10.8	4.8 - 10.8	<2.0	>20.0
RBC	4.7 - 6.1	4.2 - 5.4		NONE
Hgb	14.0 - 18.0	12.0 - 16.0	<7.0	>20.0
HCT	42.0 - 52.0	37.0 - 47.0	<20.0	>60.0
MCV	80.0 - 94.0	81.0 - 99.0		NONE
PLT	130 - 500	130 - 500	<50	>999
LY%	20.5 - 51.1	20.5 - 51.1		
LY#	1.2 - 3.4	1.2 - 3.4		

9. PROCEDURAL NOTES:

9.1. CBC specimens will be checked for in vitro hemolysis and possible interfering lipemia before reporting results by examining the numeric data for anomalous results (especially indices) as well as particle histogram inspection.

9.2. Known interfering substances:

9.2.1. WBC: Unusual RBC abnormalities that resist lysing, nucleated RBCs, fragmented WBCs, and large platelets.

9.2.2. RBC: Very high WBC count, high concentration of very large platelets, microcytic RBCs, and autoagglutination.

9.2.3. Hgb: Very high WBC counts, severe lipemia, certain unusual RBC abnormalities that resist lysing.

9.2.4. MCV: Very high WBC count, high concentration of very large platelets, autoagglutination.

9.2.5. PLT: Very small erythrocytes or leukocytes or cell fragments may cause no-fit conditions in some cases.

9.2.6. Run 4C controls whenever the reagent Pak is changed.

9.3. Refer to Coulter A<sup>c</sup>T 10 Reference Manual and Operator's Guide for cleaning, replacement, adjustment, and basic troubleshooting.

9.4. Data Review:

9.4.1. All panic values will be verified by repeat analysis.

9.4.2. If a cold agglutinin is suspected, pre-warm the specimen in a 37°C water bath for 15 minutes and rerun the specimen.

9.4.3. If "R" is printed next to any analyte, review smear.

9.4.4. Platelet estimates will be performed from smear on all platelet counts <130.

9.4.5. MCV > 105.0 must be examined on a smear.

10. REFERENCES:

10.1. Coulter A<sup>c</sup>T Series Analyzer Operator's Guide, PN 4237287D, March 1997.

10.2. Coulter A<sup>c</sup>T Series Analyzer Reference Manual, PN 4237288B, March 1997.