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| **SOP Number:** | **CH010** | **Effective Date** |  |
| **Department & Section:** | Hematology | **Revision Date(s):** |  |
| **Author:** | K.Clark MT.(ASCP)K. Miller MT.(ASCP)R. Bernshausen MT (ASCP) | **Version:** |  |

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| Applicable Standards |  | Version History |
| Standard | Organization  |  | Version | Effective Date | Retired Date |
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| Related Documents |  |  |  |  |
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| Review History (Up to the Last 15 Occurrences) |
| Date | Version | Revision Type | Review By/Initials & Date |
|  |  | Major Revision | System Laboratory Medical Director, Joe A. Lewis, M.D., F.C.A.P. |
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**Clinical Significance:**

The CBC and differential provides in-depth quantitative analysis of the three cellular/particulate components of peripheral blood. This provides valuable information regarding a wide variety of disease states, and is an essential component of the evaluation of hematologic diseases (e.g. anemia, leukemia, myeloproliferative disorders).

**Principle:**

The ADVIA ®2120*i* / ADVIA® 120 Hematology System is a fully automated diagnostic instrument that uses cytochemical reactions to differentiate and count white blood cells, red blood cells, and platelets. There are two main components of the system: the analyzer and the personal computer. In the analyzer, blood samples are aspirated and divided into aliquots for the different types of tests. Reagents and segmented samples are delivered to reaction chambers where they are mixed and a cytochemical reaction takes place. Once the reactions are complete, the sample and reagent mixtures from the perox, RBC, and baso reaction chambers are sent to the flow cells for analysis. The hemoglobin measurement is read in the Hgb reaction chamber that serves as an optical cuvette. After analysis, the sample and reagent mixture is evacuated into the waste container and the appropriate pathways and reaction chambers are rinsed. Test results are sent to the computer to be reviewed and edited.

The ADVIA ®2120*i* / ADVIA® 120 Hematology System can run five selectivity’s: CBC, CBC/DIFF, retic, CBC/retic, and CBC/DIFF/retic. The system has a throughput of 120 samples per hour when running CBC or CBC/DIFF and a throughput of 74 samples per hour when running the other selectivity’s. Up to 150 sample tubes can be loaded onto the bar-coded racks of the autosampler. Single or STAT samples can be loaded on the manual samplers.

**Complete Blood Count**

The ADVIA ®2120*i* / ADVIA® 120 Hematology System Complete Blood Count (CBC) method is intended to quantitatively measure the following hematological parameters:

* White Blood Cell count (WBC)
* Red Blood Cell count (RBC)
* Hemoglobin concentration (HGB)
* Hematocrit (HCT)
* Mean Corpuscular Volume (MCV)
* Mean Corpuscular Hemoglobin (MCH)
* Mean Corpuscular Hemoglobin Concentration (MCHC)
* Corpuscular Hemoglobin Concentration Mean (CHCM)
* Cellular Hemoglobin Content (CH)
* Red Cell Volume Distribution Width (RDW)
* Hemoglobin Concentration Distribution Width (HDW)
* Platelet Count (PLT)
* Mean Platelet Volume (MPV)

White Blood Cell Differential

The ADVIA ®2120*i* / ADVIA® 120 Hematology System White Blood Cell Differential (WBC DIFF) methods, consisting of both the Peroxidase method and the Basophil/Lobularity method, are intended to quantitatively measure the following WBC hematological parameters:

* Neutrophils: percentage of WBC (%NEUT) and absolute count (#NEUT)
* Lymphocytes: percentage of WBC (%LYMPH) and absolute count (#LYMPH)
* Monocytes: percentage of WBC (%MONO) and absolute count (#MONO)
* Eosinophils: percentage of WBC (%EOS) and absolute count (#EOS)
* Large Unstained Cells: percentage of WBC (%LUC) and absolute count (#LUC)
* Basophils: percentage of WBC (%BASO) and absolute count (#BASO)

**Chemical Principles**

**WBC Count**

The whole blood sample is mixed with ADVIA 2120i/ADVIA 120 BASO reagent that contains acid and surfactant. The red cells are hemolyzed, and the white blood cells are then analyzed using 2 angle laser light scatter signals.

**RBC / Platelet Count**

Both red blood cells and platelets are analyzed by a single optical cytometer after appropriate dilution of the blood sample with ADVIA 2120i/ADVIA 120 RBC/PLT reagent. The red blood cells are isobolumetrically sphered and lightly fixed with

glutaraldehyde to preserve the spherical shape. Red cells and platelets are counted from the signals from a common detector with 2 different gain settings.

On the ADVIA 2120i/ADVIA 120 system the platelet signals are amplified considerably more than the RBC signals. Coincidence correction is made to each of the counts so that accurate counts are made over a wide range of each cell type.

**RBC / Platelet Size**

The method of sizing red cells and platelets uses the simultaneous measurement of laser light scattered at 2 different angular intervals, which eliminates the adverse effect of variation in cellular hemoglobin concentration on the determination of cell volume.

**Hemoglobin Concentration**

The hemoglobin method is a modification of the manual cyanmethemoglobin method developed by the International Committee for Standardization in Hematology (ICSH).

**Indices**

The red cell indices MCH and MCHC are derived from the mathematical calculation of the RBC count the total hemoglobin, and the MCV determination.

The HCT value is calculated from the RBC count and the MCV.

The RDW and HDW values are calculated from the cell-by-cell measurement of cell volume and hemoglobin concentration.

The CH represents the mean of the cell hemoglobin content histogram.

The CHCM is calculated as the mean of the RBC hemoglobin concentration histogram.

**Peroxidase Method**

The peroxidase method was developed by Cremin, Kim, Malin, and Sclafani, based on the principles of differential cellular staining outlined by Ansley and Ornstein. According to these principles, leukocytes are classified by the characteristic properties exhibited by cell-specific constituents when the cells are treated with cytochemical stains. The enzyme peroxidase is present and active in several leukocyte types. In the presence of hydrogen peroxide and an appropriate electron acceptor chromogen, peroxidase develops a darkly colored material which precipitates in the cells. Normal neutrophils and eosinophils possess significant levels of peroxidase activity, with enzyme activity corresponding to cell maturation.

The monocytes were demonstrated to contain lower amounts of peroxidase, which made it possible to define them as a cell population with relatively large light-scatter signals and absorption signals that extend from the unstained cells up to, and partly overlapping, the most weakly-stained neutrophils.

The lymphocyte population analyzed with the Peroxidase Method contains both lymphocytes and basophiles. The basophil count (obtained from the Basophil/Lobularity method) is subtracted from the lymphocyte population to obtain the lymphocyte count.

The peroxidase cytochemical reaction consists of 2 steps. In the first step, EDTA anticoagulated whole-blood sample is diluted with ADVIA 2120i/ADVIA 120 PEROX 1 reagent. Surfactants and thermal stress cause lysis of the red blood cells. Formaldehyde in ADVIA 2120i/ADVIA 120 PEROX 1 reagent fixes the white blood cells. During the second step, ADVIA 2120i/ADVIA 120 PEROX 2 reagent and ADVIA 2120i/ADVIA 120 PEROX 3 reagent are added to the peroxidase reaction chamber. The 4-chloro-1-napthol in ADVIA 2120i/ADVIA 120 PEROX 2 reagent and the hydrogen peroxide in ADVIA 2120i/ADVIA 120 PEROX 3 reagent stain the sites of peroxidase activity in the granules of neutrophils, eosinophils, and monocytes. Lymphocytes, basophiles, and large unstained cells contain no granules with peroxidase enzyme activity.

A constant volume of the cell suspension from the Perox reaction chamber passes through the flow cell. The two fluids flow as independent, concentric streams (no mixing), with the PEROX SHEATH stream encasing the sample stream. The absorbance and the forward light-scattering signatures of each blood cell are measured. The optical signals are converted to electrical pulses by photodiodes. After processing, the information is displayed in two histograms. The Perox Y histogram contains the forwared-scattering data (cell size). The Perox X histogram contains the absorption data (peroxidase staining). The two histograms are combined to form the Perox cytogram from which cells are identified and counted.

**Basophil / Lobularity Method**

The Basophil/Lobularity method was developed by Cremins and Orlik to provide both accurate basophil counts and a measure of cellular lobularity.

This method provides precise, accurate, and rapid recognition of basophiles. Cremins and Orlik discovered that basophiles are particularly resistant to lysis by a combination of acid and surfactant.

When the EDTA anticoagulated whole blood sample is mixed with ADVIA 2120i/ADVIA 120 BASO reagent, the red blood cells are hemolyzed and the cytoplasm is stripped from all white cells except basophiles. The sample is then analyzed by two-angle laser light scattering detection using a laser diode. The white cells are classified into three categories: basophiles, mononuclear (MN) cells, and polymorphonuclear (PMN) cells.

**EQUIPMENT AND REAGENTS:**

Equipment

**ADVIA 2120I / ADVIA 120 Hematology System**

* Storage Conditions: -45°C to 70°C (-49°F to 158°F)
* Operating Conditions: 18°C to 35°C (64°F to 95°F) with 15% - 80% (noncondensing) relative humidity
* Clean the analyzer daily as described in the ADVIA 2120I / ADVIA 120 Operator’s Guide in the Daily Routine section.

Reagents

**ADVIA 2120i/ADVIA 120 Reagents**

* Store ADVIA 120 reagents at room temperature 15°C - 30°C (59°F - 86°F).
* All unopened reagents are stable until the expiration date printed on the product label.
* Open containers are stable as follows:

**Table: CH10-01**

|  |  |  |  |
| --- | --- | --- | --- |
| Reagent | Uses | Stability | Handling |
| CBC TIMEPAC CN Free | Use for CBC method. Results include WBC, RBC, and HGB.Use for WBC DIFF method for BASO count. | 45 days | BASO: CAUTION! Causes Burns. Do not get in eyes, on skin, or on clothing. Avoid breathing vapor. Use with adequate ventilation. Wash thoroughly after handling. Avoid ingestionHGB: CAUTION! Avoid contact with eyes or prolonged contact with skin. Wash thoroughly after handling. Avoid ingestionRBC/PLT and DEFOAMER: CAUTION! Avoid contact with eyes, skin, or clothing. Wash thoroughly after handling. Avoid ingestion. |
| DIFF TIMEPAC | Use for WBC DIFF method. Results include NEUT, EOS, and LUC.  | 90 days | PEROX 1 and PEROX 2: POISON! CALL A PHYSICIAN. Danger! May be fatal if swallowed. Avoid breathing vapor. Use with adequate ventilation. Wash thoroughly after handling.PEROX 3 and PEROX SHEATH: CAUTION! Avoid contact with eyes, skin, or clothing. Wash thoroughly after handling. Avoid ingestion. |
| autoRETIC | Use for Reticulocyte Method. Results include #RETIC and MCVr | 120 days | CAUTION! Avoid contact with eyes, skin, or clothing. Wash thoroughly after handling. Avoid ingestion. |
| DEFOAMER  | Use for CBC, Reticulocyte, and WBC DIFF methods | 90 days | CAUTION! Avoid contact with eyes, skin, or clothing. Wash thoroughly after handling. Avoid ingestion.  |
| EZ KLEEN | Use for CBC, Reticulocyte, and WBC DIFF methods | 90 days | CAUTION! Avoid contact with eyes, skin, or clothing. Wash thoroughly after handling. Avoid ingestion. |
| SHEATH / RINSE | Use for CBC, Reticulocyte, and WBC DIFF methods | 45 days | CAUTION! Avoid contact with eyes, skin, or clothing. Wash thoroughly after handling. Avoid ingestion.  |

Reagent Ingredients

**Table: CH10-02**

Ingredients for ADVIA 120 reagents are as follows:

| Reagent | Ingredients |
| --- | --- |
| CBC TIMEPAC(CN-Free Hgb) | BASO (2 x 1100 mL): Hydrochloric acid, 9.00 mmol/L; Phthalic acid, 21.49 mmol/L; Preservative; Surfactant CN-FREE HGB (2 x 1100 mL): Dimethyllaurylamine oxide, 2.0%RBC/PLT (2 x 2775 mL): Sodium dodecyl sµLfate, 0.035 mmol/L; Disodium EDTA dihydrate, 4.03 mmol/L; Tetrasodium EDTA dihydrate, 3.36 mmol/L; Sodium chloride, 109.3 mmol/L; Glutaraldehyde, 0.11%; BufferDEFOAMER (1 x 75 mL): Silicone emulsion, 100% |
| CBC TIMEPAC | BASO (2 x 1100 mL): Hydrochloric acid, 9.00 mmol/L; Phthalic acid, 21.49 mmol/L; Preservative; Surfactant HGB (2 x 1100 mL): Potassium cyanide, Dimethyllaurylamine oxide, 2.0%RBC/PLT (2 x 2775 mL): Sodium dodecyl sµLfate, 0.035 mmol/L; Disodium EDTA dihydrate, 4.03 mmol/L; Tetrasodium EDTA dihydrate, 3.36 mmol/L; Sodium chloride, 109.3 mmol/L; Glutaraldehyde, 0.11%; BufferDEFOAMER (1 x 75 mL): Silicone emulsion, 100% |
| DIFF TIMEPAC | PEROX 1 (2 x 650 mL): Sodium dodecyl sµLfate, 0.36 mmol/L; Sorbitol, 620 mmol/L; Sodium chloride, 8.35 mmol/L; Formaldehyde, 5.5%; BRIJ-35, 0.100 mmol/L; Buffer PEROX 2 (2 x 305 mL): 4-Chloro-1-naphthol, 44.8 mmol/L; Diethylene glycol, 99.2%PEROX 3 (2 x 585 mL): Stabilizer; Hydrogen peroxide, 0.3%PEROX SHEATH (2 x 2800 mL): Propylene glycol, 4.06 M; Surfactant |
| autoRETIC(4 x 820 mL) | Oxazine 750, 11.4 mg/L; Buffer; N-Tetradecyl-N, N-dimethyl-3-ammonio-1-propane sulfonate, 0.023 mmol/L |
| DEFOAMER (1 x 75 mL) | Silicone emulsion, 100% |
| EZ KLEEN(4 x 838 mL) | Sodium hydroxide, 50 mmol; 2-(2-Ethoxyethoxy)ethanol, 894 mmol; Surfactant |
| SHEATH / RINSE(1 x 10 L or 1 x 20 L) | Preservatives; Buffers; Surfactant |

# **Reagents Special Preparation**

No special preparation of the reagents is required.

# **Specimen:**

**Sample Collection and Preparation**

**⬛ WARNING! POTENTIALLY BIOHAZARDOUS MATERIAL**

Any samples of human blood should be handled cautiously as a biohazardous material, according to good laboratory practices. Follow body substance isolation precautions as outlined by safety policy in the laboratory. Recommended: WEAR GLOVES AND LAB COAT.

* The specimen of choice is whole blood anticoagulated with EDTA.
* Specimen volumes required:
1. Optimal draw is a tube drawn to capacity. The collecting tube should be filled to a minimum of one-half full for optimum results.
2. A minimum of 1 mL whole blood is required for running specimens.
3. A 2.5 mL tube filled less than one-half is NOT acceptable.
4. An EDTA microtainer filled above the 250 μL line is adequate for testing.
5. Specimens must NOT be clotted (clots, fibrin strands, or platelet clumps), grossly hemolyzed, or drawn above an IV. All specimens will be checked visually for obvious clots prior to sampling by the analyzer. Microhematocrit tubes (bullets) will be physically checked for clots.
6. Optimal time to run the specimen is within 8 hours from draw time. If samples cannot be run within 8 hours of collection, they may be refrigerated and run within 24 hours without loss of accuracy. Warm samples to room temperature before running.
7. Sodium citrate samples may be used for hemoglobin, hematocrit and rechecking low platelet counts for clumping. A dilution correction is required for sodium citrate samples. Multiply result by 1.11.
8. Specimens that do not meet the above requirements must be redrawn. Please refer to the Specimen Rejection Policy (LAB-103).
9. Allow the specimen to equilibrate to room temperature before mixing. Do not warm specimen by placing it into a 370C water bath or incubator.
10. ADVIA 2120i/ADVIA 120 analyzer requires 175 μL to complete analysis.

**✯ IMPORTANT!**

If the whole blood specimen is brought to room temperature quickly, there is a strong tendency for the platelets to clump upon mixing.

**Do not use previously frozen whole blood samples.** Freezing the blood can disrupt the cellular structure, thus resulting in aberrant cell counts.

Unless otherwise stated, no special treatment of the whole blood specimen is required. However, samples must be collected in the specified collection tube and gently, but thoroughly mixed at the time of collection and again before sampling.

**Sample Stability**

The effect of the aging of blood was studied over a 72-hour period on the ADVIA 2120i.

Two whole-blood specimens drawn from 15 normal, apparently healthy donors were assayed shortly after phlebotomy and then again at intervals of 8,24,36,48,56, and 72 hours.

 One of the whole-blood specimens from each pair was stored at room temperature while the corresponding specimen was stored at 20C to 80C in capped, blood collection tubes that contained EDTA as the anticoagulant.

The results indicate that CBC parameters are stable within 2 standard deviations (within run precision) of the initial recovery for the specified timer interval. The stability of the calculated parameters is limited to the stability of the least stable primary parameter.

**Table: CH10-03**

**Specimen Stability**

**Parameter Room Temperature Refrigerated Temperature**

 **Stability (hours) Stability (hours)**

%NEUT 36 72

%LYMPH 36 72

%MONO 72 72

%EOS 8 72

%BASO 72 56

%LUC 72 72

**SPECIMEN VOLUME(Minimum/Optimum)**

**Sample mode volumes**

Automatic Closed-Tube 175 μL

Manual Closed-Tube 175 μL

Manual Open-Tube 175 μL

**Patient Preparation:** No special patient preparation is required.

**Handling Conditions:**

1. Samples must be properly labeled with two patient identifiers.
2. All specimens will be checked visually for clots.
3. Microtainer specimens will be physically checked for clots, using applicator sticks.
4. All specimens should be mixed thoroughly before testing.
* Tubes should be left on the rocker for 30 seconds-1 minute before manual aspiration.
* The ADVIA 2120i and the ADVIA 120 rock the specimen a minimum of 10+ times before aspirating the first specimen on the autosampler rack.
* Manually fed specimens should be inverted a minimum of 10 times before aspiration
1. Specimens must be checked for possible interfering substances before resulting. All analyzer flags must be resolved before releasing a patient result
* Refer to the Spurious Result Procedure located elsewhere in this manual.
1. All specimens should be mixed thoroughly before testing.
* Tubes should be left on the rocker for at least 1 minute before manual aspiration.
* The ADVIA 2120i and the ADVIA 120 rock the specimen a minimum of 10+ times before aspirating the first specimen on the autosampler rack.

**Sample Handling:**

Running Patient Samples from the Autosampler

1. Load samples in the following order:
* Whole blood primer (primer label)
* Optional: Controls (control label)
* Patient samples (sample ID label)
1. Insert tube into rack with the barcode label visible above the rack barcode label that indicates the rack number and sample position. Do not twist tube within rack.
2. Load rack onto input queue with labels facing front of analyzer.
3. If the Standby indicator is lit, press **Standby**.
4. On the touchpad, press **Start/Stop Sampler**. The Start and Rack in Sampler indicators are lit.
5. Validate results when available.

Running Patient Samples from the Manual Closed-Tube Sampler

1. If the Standby indicator is lit, press **Standby**.
2. Run samples in the following order:
* Whole blood primer (primer label)
* Optional: Controls (control label)
* Patient samples (sample ID label)
1. Scan the tube label or enter the sample information in the Manual Sample ID tab.

**IMPORTANT:** Make sure the correct sample ID appears on the status line before aspirating a sample using either the manual open-tube sampler or the manual closed-tube sampler. Waiting appears on the status line while the system searches for a matching work order.

1. Aspirate each sample.
	1. Insert and push down tube containing the well-mixed sample into the manual closed-tube sampler. Hold tube parallel to the sampler well wall.
	2. Sample is automatically aspirated – the sampling light flashes.
	3. When the sampling light stops flashing, remove the tube.
2. Validate results when available.

Running Patient Samples from the Manual Open-Tube Sampler

1. If the Standby indicator is lit, press **Standby**.
2. Run samples in the following order:
* Whole blood primer (primer label)
* Optional: Controls (control label)
* Patient samples (sample ID label)
1. Scan the tube label or enter the sample information in the Manual Sample ID tab.

**IMPORTANT:** Make sure the correct sample ID appears on the status line before aspirating a sample using either the manual open-tube sampler or the manual closed-tube sampler. Waiting appears on the status line while the system searches for a matching work order.

1. Aspirate sample.
2. Position tube so that the sampler probe is immersed into the well-mixed sample. You should only immerse the sampler probe deep enough (approximately 0.25-in.) to ensure aspiration.
3. Press the aspirate plate.
4. Sampling light flashes during aspiration.
5. When the sampling light stops flashing, remove the tube.
6. Validate results when available.

# **Reporting Results**

1. Reference Ranges are reported with each result.
2. All critical values will be reported to the appropriate patient care personnel. Please review the Critical Value Policy for proper notification/documentation.
3. All STAT results are to be reported within 40 minutes.
4. All routine results are to be reported within 4 hours.
5. All technologists must be familiar with the criteria stating when to perform an RBC morphology scan, platelet estimate, manual differential or to leave the peripheral blood smear for the pathologist to review. These criteria can be found elsewhere in this manual.
6. All CBC orders with **manual differential** (CBCMD) will require that a differential be performed on the specimen.
7. All CBC orders with **no differential** (CBCND) will have no manual differential or RBC morphology generated. Please Note: **All CBC’s with no differential will have a platelet estimate performed if the platelet count from the analyzer is >100.000 X10~3/µL.** This result can be entered under the Enter Result function.
8. All CBC orders with **automated differential** (CBCAD) will have to have their criteria evaluated before results are reported. If the specimen does not need to have a manual differential or a RBC morphology scan performed, the technologist must answer the Morphology and Platelet queries on the Process Analyzer Batch screen. The results would be a Normal Morphology and Adequate Platelet.

**Table: CH10-04**

**Reference Ranges**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **ANALYTE** | **AGE** | **NORMAL RANGE BOTH SEXS** | **MALE** | **FEMALE** | **UNITS** |
|  |  |  |  |  |  |
| ***WBC*** | < 1 DAY | 9.0-30.0 |   |   | X10~3 uL |
|   | 1D-6D | 9.0-34.0 |   |   | X10~3 uL |
|   | 7D-13D | 5.0-21.0 |   |   | X10~3 uL |
|   | 14D-29D | 5.0-20.0 |   |   | X10~3 uL |
|   | 1M-11M | 5.0-19.5 |   |   | X10~3 uL |
|   | 1YR-1YR 11M | 6.0-17.5 |   |   | X10~3 uL |
|   | 2YR-3YR 11M | 6.0-17.0 |   |   | X10~3 uL |
|   | 4YR-5YR 11M | 5.5-15.5 |   |   | X10~3 uL |
|   | 6YR-7YR 11MO | 5.5-14.5 |   |   | X10~3 uL |
|   | 8YR-15YR 11MO | 4.5-13.5 |   |   | X10~3 uL |
|   | 16YR-17YR 11MO | 4.5-13.0 |   |   | X10~3 uL |
|   | ADULT | 4.8-10.8 |   |   | X10~3 uL |
|   |   |   |   |   |   |
| ***RBC*** | 0-2 DAYS | 3.90-5.50 |   |   | X10~6/uL |
|  | 3- 6DAYS | 4.00-6.60 |   |   | X10~6/uL |
|   | 7-13 DAYS | 3.90-6.30 |   |   | X10~6/uL |
|   | 14-29 DAYS | 3.60-6.20 |   |   | X10~6/uL |
|   | 1MO -59 DAYS | 3.00-5.40 |   |   | X10~6/uL |
|   | 2MO-5MO | 2.70-4.90 |   |   | X10~6/uL |
|   | 6MO-23MO | 3.70-5.30 |   |   | X10~6/uL |
|   | 2YR-5YR | 3.90-5.30 |   |   | X10~6/uL |
|   | 6YR-11YR | 4.00-5.20 |   |   | X10~6/uL |
|   | 12YR-17YR |   | 4.50-5.30 | 4.10-5.00 | X10~6/uL |
|   | 18-20 |   | 4.50-5.20 | 4.00-5.20 | X10~6/uL |
|   | ADULT |   | 4.70-6.10 | 4.20-5.40 | X10~6/uL |

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| ***HGB*** | 0-2 DAYS | 13.5-19.5 |   |   | G/dL |
|   | 3- 6DAYS | 14.5-22.5 |   |   | G/dL |
|   | 7-13 DAYS | 13.5-21.5 |   |   | G/dL |
|   | 14-29 DAYS | 12.5-20.5 |   |   | G/dL |
|   | 1MO -59 DAYS | 10.0-18.0 |   |   | G/dL |
|   | 2MO-5MO | 9.0-14.0 |   |   | G/dL |
|   | 6MO-23MO | 10.5-14.5 |   |   | G/dL |
|   | 2YR-5YR | 11.5-13.5 |   |   | G/dL |
|   | 6YR-11YR | 11.5-15.5 |   |   | G/dL |
|   | 12YR-17YR |   | 13.0-16.0 | 12.0-16.0 | G/dL |
|   | 18-20 |   | 13.5-17.5 | 12.0-16.0 | G/dL |
|   | ADULT |   | 14.0-18.0 | 12.0-16.0 | G/dL |
|   |   |   |   |   |   |
|  |  |  |  |  |  |
| ***HCT*** | 0-2 DAYS | 42-60 |   |   | % |
|   | 3- 6DAYS | 45-67 |   |   | % |
|   | 7-13 DAYS | 42-66 |   |   | % |
|   | 14-29 DAYS | 39-63 |   |   | % |
|   | 1MO -59 DAYS | 31-55 |   |   | % |
|   | 2MO-5MO | 28-42 |   |   | % |
|   | 6MO-23MO | 33-39 |   |   | % |
|   | 2YR-5YR | 34-40 |   |   | % |
|   | 6YR-11YR | 35-45 |   |   | % |
|   | 12YR-17YR |   | 37-49 | 36-46 | % |
|   | 18-20 |   | 41-53 | 36-46 | % |
|   | ADULT |   | 42-52 | 37-47 | % |

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| ***MCV*** | 0-2 DAYS | 98-118 |   |   | fL |
|   | 3- 6DAYS | 95-121 |   |   | fL |
|   | 7-13 DAYS | 88-126 |   |   | fL |
|   | 14-29 DAYS | 86-124 |   |   | fL |
|   | 1MO -59 DAYS | 85-123 |   |   | fL |
|   | 2MO-5MO | 77-115 |   |   | fL |
|   | 6MO-23MO | 70-86 |   |   | fL |
|   | 2YR-5YR | 75-87 |   |   | fL |
|   | 6YR-11YR | 77-95 |   |   | fL |
|   | 12YR-17YR |   | 78.98 | 78-102 | fL |
|   | 18-20 |   | 80-100 | 80-100 | fL |
|   | ADULT |   | 80-94 | 81-99 |   |
|   |   |   |   |   |   |
| ***MCH*** | 0-6 DAYS | 31-37 |   |   | pcG |
|   | 7-59 DAYS | 28-40 |   |   | pcG |
|   | 2MO-5MO | 26-34 |   |   | pcG |
|   | 6MO-23MO | 23-31 |   |   | pcG |
|   | 2YR-5YR | 24-30 |   |   | pcG |
|   | 6YR-11YR | 25-33 |   |   | pcG |
|   | 12YR-17YR | 25-35 |   |   | pcG |
|   | ADULT | 26-34 |   |   | pcG |
|   |   |   |   |   |   |
| ***MCHC*** | 0-2 DAYS | 30-36 |   |   | G/dL |
|  | 3- 6DAYS | 29-37 |   |   | G/dL |
|  | 7-29 DAYS | 28-38 |   |   | G/dL |
|  | 1MO-5M0 | 29-37 |   |   | G/dL |
|  | 6MO-23MO | 30-36 |   |   | G/dL |
|  | 2YR-ADULT | 31-37 |   |   | G/dL |
|  |  |  |  |  |  |
| ***RDW*** | ALL | 11.5-14.5 |   |   | % |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   |   |   |   |   |   |
| ***PLT*** | ALL | 130-400 |   |   | X10~3 uL |
|   |   |   |   |   |   |
| ***MPV*** | ALL | 7.4-10.4 |   |   | fL |
|  |  |  |  |  |  |

**Criteria for ordering a Manual WBC Differential or RBC Morphology Smear Review from the ADVIA 2120i and ADVIA 120 Automated Hematology Analyzers**

Please review the Table of Sample System Flags (Table: CH10-06)

List of flags that can occur to a specimen, and the action that needs to be taken with that flag.

**Criteria for performing a Manual Differential**

**Table: CH10-05**

|  |  |
| --- | --- |
| **WBC** | **<3.0 mm³ or >15.0 mm³** |
| **Neutrophil %** | **>85%** |
| **Lymphocyte Absolute #** | **>4.5 (4500)** |
| **Lymphocyte %** | **> Neutrophils %** |
| **Monocyte Absolute #** | **>1.5 (1000)** |
| **Eosinophils Absolute #** | **>1.5 (1000)** |
| **Basophils Absolute #** | **>1.0 (1000)** |
| **Luc% (large unstained cells)** | **>4.8 %** |

If any of the above criteria are met, a manual white blood cell differential must be performed on a specimen that is ordered as a CBC with automated diff. (CBCAD)

* All LS flags (Left Shift) of 2+,3+ perform a manual differential
* All IG flags (Immature Granulocytes) of 2+,3+ perform a manual differential
* All MPO (Myeloperoxidase deficiency: granulocytes will be 0 or very low; lymphocytes and monocytes will be very high) perform manual differential. The automated differential from the analyzer should **NOT** be reported. (NP the results). Answer “NO” to report differential in the analyzer screen.
* All Blast flags need a manual differential
* All Atypical Lymphocytes need a manual differential

**\*Please note**: A manual differential is performed on all specimens of children less than 6 years of age. The automated differential result is NOT reported.

**Criteria for review of RBC Morphology:**

1. First specimen received by laboratory on an admission that must have a RBC morphology performed:
	* Hct: <25%
	* MCV <75 or >105
	* RDW >22
	* Hypo,Aniso,Micro, Macro -2+,3+ analyzer flags.
	* **Disregard any 1+** **flags.**
	* **Disregard any NRBC flag** (if this is the only flag)
2. Successive samples on the same admission must have a RBC morphology performed, only if there are any deltas to the RBC parameters.

**Delta Checks**: When a patient’s current result differs significantly from the immediate previous result. It may be due to a number of factors:

* Clerical error
* Analytical error
* Specimen mislabel
* Change in the patient’s physiological state.

 Delta Checks are utilized in the following way:

* HGB: ±2.0 gm/dL
* HCT: ± 10%
* MCV: ± 5.0 fl

If this is not the first specimen for a patient on this admission and there are no delta checks, another scan of the RBC morphology is not necessary. RBC morphology does not need to be ordered. This applies whether the RBC’s were reviewed during a manual differential or a RBC morphology scan. An answer of PR (previously reviewed) can be placed under the morphology result in the analyzer

**Criteria for review of Platelet Counts:**

1. Platelet counts <100,000 mm³. These must have a manual estimate from the peripheral blood smear even if the test ordered is a CBC No Diff.
2. Platelet counts >700,000 mm³
3. Platelet asterisk LP, SR, or NF

**Manual platelet estimates that are performed for a CBC No Differential specimen are entered under the result entry screen**

**Reporting format for Abnormal Results**

Through the use of flagging algorithms, laboratory personnel are alerted to suspected abnormal conditions. These conditions are indicated by the appropriate flag (such as \*, +, and/or color highlighting). Whenever a flag is triggered, the user should **review** the results and take appropriate action. Please refer to the **Spurious Results Procedure** located in elsewhere this manual.

**Table: CH10-06**

**System Flags**

|  |  |
| --- | --- |
|  | **Sample System Flags** |
| **ADVIA 120Code** | **ADVIA 2120i Code** | **Description of Flag** | **Action** |
| **BTO** | **BTO** | BASO Chamber Temp Out | Troubleshoot problem then Verify Differential if needed |
| **CC** | **CHCMCE** | MCHC-CHCM Comparison Error | Analyze sample for lipemia, cold aggl or high WBC. Correct HGB |
| **TX** | **PXTO** | PEROX Chamber Temp Out | Troubleshoot problem then Verify Differential if needed |
| **RR** | **RBCIFR** | RBC Irregular Flow Rate | Rerun sample, Accept if No CC |
| **HR** | **HGBIFR** | HGB Irregular Flow Rate | Rerun sample, Accept if No CC |
| **PH** | **HGB-PL** | HGB Lamp Low | Accept if No CC |
| **NW** | **PLT-CLM** | Platelet Clumps | Verify PLT Count if platelets decreased |
| **NT** | **PLT-NO** | PLT Noise | Verify PLT Count if platelets decreased |
| **OT** | **PLTORN** | PLT Origin Noise | Verify PLT Count if platelets decreased |
| **VX** | **PX-NV** | PEROX No Valley | Verify Differential |
| **CT** | **RTCint** | Platelet threshold questionable | Manual Retic |
| **CR** | **RTCIFR** | Retic Irregular Flow Rate | Manual Retic |
| **FC** | **RTC-FS** | Retic Abnormal Distribution | Manual Retic |
| **NO** | **RTC-NO** | Noise at retic Origin | Manual Retic |
| **CL** | **RTC-L** | Cells analyzed <10,000 | Manual Retic |
| **RF** | **RTC-FL** | Retic Irregular Flow Rate | Manual Retic |
| **CS** | **RTCSAT** | Cells in saturation >10% | Manual Retic |
| **SE** | **RTC-SE** | Abnormal Distribution of Retic RBCs | Manual Retic |
| **PX** | **PX-PL** | PEROX Light Intensity Low | Change perox lamp |
| **XS** | **PX-SAT** | PEROX Saturation | Rerun sample |
| **PL** | **LAS-PL** | Laser Light Intensity Low | Manual Differential |
| **MPO-D** | **MPO** | Myeloperoxidase deficiency | Manual Differential |
| **WC** | **WBC-CE** | WBCB and WBCP do not match | Rerun sample, Verify Differential |
| **WS** | **WBCSUB** | WBC Substitution Rule was used. | Rerun sample, Verify Differential |
| **BC** | **B-SUSP** | BASO Count Suspect | Verify Differential |
| **BR** | **B-IRF** | BASO Irregular Flow Rate | Rerun sample |
| **NB** | **B-NO** | BASO Noise | Rerun sample |
| **VB** | **B-NV** | BASO No Valley | Ignore this flag |
| **BS** | **B-SAT** | BASO Saturation | Rerun sample |
|  | **NRCELL** | Suspect Cellular Interference - Unlysed RBCs | Manual Differential |
|   | **NR-LPD** | Suspect Lipid Interference | Verify Differential |
|  | **NRLPLT** | Suspect Large Plt Interference | Verify Differential |
|  | **NRPXNV** | No Perox NRBC/Lymph Valley | Verify Differential |
|  |   |   |   |
|  |  |  **Morphology Flags** |  |
|  | **Code** | **Description of Flag** | **Action** |
|  | **ANISO 2+** | RDW Increased  | Scan RBC Morphology |
|  | **MICRO 2+** | MICRO Increased | Scan RBC Morphology |
|  | **MACRO 2+** | MACRO Increased | Scan RBC Morphology |
|  | **HYPO 2+** | HYPO Increased | Scan RBC Morphology |
|  | **HYPER 2+** | HYPER Increased | Scan RBC Morphology |
|  | **RBCF** | RBC Fragments present | Scan RBC Morphology |
|  | **RBCG** | RBC Ghosts present | Scan RBC Morphology |
|  | **ATYP 1+** | LUC Increased | Verify Differential |
|  | **BLASTS 1+** | % Blasts Increased | Manual Differential |
|  | **IG 1+** | Immature Granulocytes | Manual Differential |
|  | **LS 1+** | Bands  | Manual Differential |
|  | **MO 1+** | Myeloperoxidase deficiency | Manual Differential |
|  | **LPLT 2+** | **Large Platelet** | **Verify PLT Count if platelets decreased** |

**Criteria for Rechecking Results:**

Delta checks are implemented to alert the technologist of a significant change in a patient’s results. It is the technologist’s responsibility to determine whether or not there is a need to repeat the results/recollect the specimen/or accept the results for that specimen.

This is the investigation process for a delta check”

1. Retest Hematology specimens on another instrument, if possible. If results are similar then proceed.
2. WBC and Platelets can change rapidly due to a stimulus, even in a healthy patient. If all other parameters are consistent with previous results, accept the results. Footnote in the LIS that the specimen has been reran and verified.
3. Review cumulative results on the patient for comparison. Use the Previous Test Results Tab on the analyzer screen.
4. Check in the Electronic Medical Record (EMR) for information and/or call nursing staff or the doctor for information. Current conditions or treatments may provide clues that explain significant changes (e.g. surgery, blood loss or transfusion, and dehydration).
5. If no reason for the delta change for Hgb (∆>2) and/or Hct (∆>6) and/or MCV (∆>5) can be determined, it is logical to suspect improper collection or labeling of the specimen (e.g., collection near an IV line) and request a new specimen. Confirm specimen identification by comparing the specimen label to the laboratory request. If an improperly labeled specimen is identified, notify other areas of the laboratory that may have also received an improperly labeled specimen (e.g.,chemistry) and arrange for recollection. If the specimen appears to be labeled properly, continue.
6. If there is no evidence of a specimen error, footnote in the LIS to document that the unusual result has been reran and verified.

All delta checks must be footnoted in the LIS with the action taken for that decision. Established Hematology footnotes will be used for this process.

**Reporting Results outside of the Analytical Measuring Range of the Analyzers:**

Results that fall above the measuring range of the analyzers, (as determined by the manufacture), will be reported as greater than this range. Please see that attached table. No manual dilution will be performed on these specimens.

**Analytical Measuring Range**

**Table: CH10-07**

|  |  |
| --- | --- |
|  | Advia 2120i/ 120 |
| WBC | 0.02-400.0 x10³μL |
| RBC | 0.0-7.0x10↑6μL |
| HGB | 0-22.5 g/dL |
| PLT | 5-3500 x10³μL |
| Reticulocyte | 0.2-24.5% |

**Within run Precision**

**Table: CH10-08**

|  |  |  |  |
| --- | --- | --- | --- |
|  | Mean | SD | CV |
| WBC | 7.5 | 0.2 | 2.7 |
| RBC | 5.0 | 0.06 | 1.2 |
| HGB | 15.0 | 0.14 | 0.93 |
| MCV | 90. | 0.7 | 0.78 |
| PLT | 300 | 8.8 | 2.93 |
| Retic% | 2.0 | 0.25 | 12.5 |

Carryover: < or = to 1% for all parameters

**Critical Results**

Please refer to the Critical Value Procedure LAB-106-P.

* Protocol to follow if a critical value is obtained, is to repeat the testing of the original specimen (preferably on the other analyzer). After determining that the result is correct, the critical value will be called to the appropriate nursing unit or patient caregiver. This result must be repeated back to laboratory testing personnel. This is documented in the Laboratory LIS system.

**Table: CH10-08**

**Critical Values**

|  |  |  |  |
| --- | --- | --- | --- |
| **Test**  | **Units of Measure**  | **Critical Low**  | **Critical High**  |
| WBC\* | x103/μL | Less than 2.0 | Greater than 35 |
| ***Neonatal WBC (0-7 days)*** | ***x103/μL*** | ***Less than 2.0*** | ***Greater than 50*** |
| Hemoglobin | g/dL | Less than 7.0 | N/A |
| ***Neonatal Hemoglobin*** | ***g/dL*** | ***Less than 9.5*** | ***Greater than 25*** |
| Hematocrit | % | Less than 20.0 | N/A |
| ***Neonatal Hematocrit*** | **%** | ***Less than 29.0*** | ***Greater than 75*** |
| Platelets\* | x103/μL | Less than 50 | Greater than 1,000 |

Calculations

The system automatically performs all calculations necessary for obtaining final results. The calculations for patient reporting parameters are summarized in the following table.

**Calculations: Table: CH10-09**

| Method | Parameter | Calculation |
| --- | --- | --- |
| CBC | WBCB | Raw WBC x (Baso Cal Factor / [1-FracDT]) |
| RBC | Number of Red Cells x RBC Cal Factor x Dilution Factor x Coincidence correction Factor |
| HGB | Log (Sample Mean / Baseline Mean) x Hgb Cal Factor x 50.0 |
| HCT | (RBC x MCV) / 10 |
| MCV | Mean of RBC Volume histogram |
| MCH | (HGB / RBC) x 10 |
| MCHC | (HGB / [RBC x MCV]) x 1000 |
| CH | Mean of RBC CH histogram |
| CHCM | Mean of RBC HC histogram |
| RDW | 100 x (CV of RBC Volume histogram / MCV) |
| HDW | SD of RBC HC histogram |
| PLT | Corrected PLT Count x RBC Cal Factor x PLT Cal Factor x Dilution Factor |
| MPV | Mean of Platelet VOL histogram |
| WBC DIFF | WBCP |  Raw WBC x (Perox Cal Factor / [1-FracDT ] ) |
| %NEUT | ([100 x Neutrophil Count] + %HPX) / PHA Cells |
| #NEUT | (%NEUT / 100) x WBC |
| %LYMPH | ([100 x Lymphocyte Count] / PHA Cells) - %BASO |
| #LYMPH | (%LYMPH / 100) x WBC |
| %MONO | (100 x Monocyte Count) / PHA Cells |
| #MONO | (%MONO / 100) x WBC |
| %EOS | (100 x Eosinophil Count) / PHA Cells |
| #EOS | (%EOS / 100) x WBC |
| %LUC | (100 x LUC Count) / PHA Cells |
| #LUC | (%LUC / 100) x WBC |
| %BASO | 100 x (BASO Count / BASO PHA Cells ) |
| #BASO | (%BASO / 100) x WBCB |

The calculations for additional research or laboratory use parameters are available in the ADVIA 2120I / ADVIA 120 Operator’s Guide in the Methods section.

**Calibrators and Controls:**

**ADVIA OPTIpoint** : is used to adjust the gains of the ADVIA 120/2120i Hematology Systems. This product is processed from human whole blood and is formulated for use as a test material for the electro-optical channels of the ADVIA 120/2120i Hematology systems.

* Store at 2°C - 8°C (36°F - 46°F).
* Unopened bottles are stable until the last day of the month of the expiration date printed on the product label, or for one year.
* Open bottles are stable for 7 days.
* Refer to the product insert for complete details concerning description and handling.
* Before use allow ADVIA OPTIpoint to reach room temperature, then vortex vigorously for 30 to 60 seconds

**ADVIA SET point Calibrator**: is a hematology calibration material for calibrating ADVIA 120/2120i Hematology Systems.

|  |  |  |
| --- | --- | --- |
| med_biohazard alert 1 |  | BIOHAZARD All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing. |

* Store at 2°C - 8°C (36°F - 46°F).
* Unopened bottles are stable until the last day of the month of the expiration date printed on the product label.
* Open bottles are stable for 5 days.
* Refer to the product insert for complete details concerning description and handling.

**ADVIA 3·in·1 TEST point Hematology Controls**: are hematology reference materials for monitoring precision and accuracy of ADVIA 120/2120i Hematology Systems.

|  |  |  |
| --- | --- | --- |
| med_biohazard alert 1 |  | BIOHAZARD All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing. |

* Store at 2°C - 8°C (36°F - 46°F).
* Unopened bottles are stable until the last day of the month of the expiration date printed on the product label.
* Open bottles are stable for 10 days.
* Refer to the product insert for complete details concerning description and handling.

**ADVIATEST point Hematology Controls**: are hematology reference materials for monitoring precision and accuracy of ADVIA 120/2120i Hematology Systems.

**Note: These controls are used at the CSHCC-Cancer Center only**

|  |  |  |
| --- | --- | --- |
| med_biohazard alert 1 |  | BIOHAZARD All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing |

* Store at 2°C - 8°C (36°F - 46°F).
* Unopened bottles are stable until the last day of the month of the expiration date printed on the product label.
* Open bottles are stable for 10 days.
* Refer to the product insert for complete details concerning description and handling

# **Calibration**

Preparation

ADVIA SETpoint Calibrators

Follow the directions in the package insert.

* Allow the calibrator product to equilibrate to room temperature for approximately 15 minutes.
* Manually mix vials by inversion until the RBCs are completely resuspended.
* Return the calibrator material to the refrigerator immediately after use.

Whole Blood Calibrators

All parameters except the %RETIC use ADVIA SETpoint Calibrator and 1 whole blood sample. The %RETIC parameter uses ADVIA SETpoint and 5 whole blood samples. Avoid calibrating the %RETIC parameter unless it is necessary for correlation with another method.

Whole blood calibrators must meet the following requirements:

* Specimens must be less than 8 hours old.
* Specimens should produce values within the expected clinical ranges. While some variation from these ranges is allowed, specimens that produce test results that exceed the reportable test ranges or that produce morphology flags are not acceptable.

Calibration Frequency

Calibrate on an as needed basis and in each of the following cases:

* at installation
* when there is a significant shift in control values after replacing a critical hydraulic or optical component
* when installing reagent with a different lot number and determining a shift in controls or patient results.
* any time commercial control products and/or moving averages are out of range and you have verified that the out-of-control condition is not instrument related
* at 6 month interval

Calibration Procedure

Before calibrating, perform the following:

1. Verify that there is sufficient reagent for the calibration. Do not change the reagent lot number during the calibration.
2. Visually inspect the hydraulics during operation to ensure that they function properly.
3. Check pressure and vacuum readings on the Analyzer Status tab of the Utilities menu.
4. Verify that the optical system is functioning properly. Do not calibrate unless the gain and alignment of all the optics channels have been verified.
5. Clean the shear valve pathways.

**IMPORTANT:** Before beginning the calibration procedure, make sure the Aspiration Failures stop criterion is set to 1 in the Alarm / Stop Criteria window.

Using the Calibration Wizard

All CSHCC Laboratory will use the Calibration Wizard to calibrate the analyzers:

1. Click Calibration Wizard on the Calibration tab.
2. Three navigation buttons appear in each wizard window:
* Next—click to advance to the next step in the calibration procedure.
* Back—Click to return to the previous step in the calibration procedure.
* Exit—Click to stop using the Calibration Wizard.
1. Follow the Calibration Wizard instructions.

. **Without the Calibration Wizard**

1. Choose the calibration options using the Calibration Definition window.
	* Select the type of procedure – Whole Blood Calibration or Commercial Calibration.
	* Select the Calibration Parameters.
	* Enter the number of aspirations if using the autosampler.
	* Enter the number of samples when calibrating with whole blood.
2. Enter reference information for the calibration samples in the Calibration Reference window.
3. Run the calibration samples on the ADVIA 2120i system using the primary sampler. When calibrating Baso WBC and Perox WBC, also aspirate samples using the other samplers to calibrate the parameters across all modes of aspiration.

 **NOTE:** Click **Exit** at any time to return to the Calibration Introduction window. You can restart the calibration process or leave the Calibration tab.

**To verify calibration cycle each level of the ADVIA 3·in·1 TESTpoint Hematology Controls (Normal, Abnormal 1, and Abnormal 2) or ADVIA TESTpoint Hematology Control (Lo, Normal, High) through the autosampler mode**.

**QC Materials**

Siemens ADVIA 3-IN-1TESTpoint Hematology Controls (Low, Normal and High) are used as the material of choice.

**QC Frequency**

* Controls tested according to each location/site Quality Control Procedure.
* After a reagent lot number change (patient controls may be used in lieu of TESTpoint controls for checking lot number changes). Refer to the retained patient control log sheet for the defined tolerance limits.
* After replacement of any part or component of the analytical module that may affect analytical performance.
* New lot numbers are overlapped approx. 10 days with the old lot number before the new lot number is placed into service.
* To verify calibration.
* Retained patient samples are run periodically to monitor performance trends.

**Running Control Samples from the Autosampler**

1. Insert control tubes into rack with the barcode label visible above the rack barcode label that indicates the rack number and sample position. Do not twist tube within rack.
2. Load rack onto input queue with labels facing out to front of analyzer.
3. If the Standby indicator is lit, press **Standby.**
4. On the touchpad, press **Start/Stop Sampler**. The Start and Rack in Sampler indicators are lit.
5. In the Sample Control Panel tab, evaluate control results when available.

**Running Control Samples from the Manual Closed-Tube Sampler**

1. If the Standby indicator is lit, press **Standby**.
2. Scan the tube label or enter the sample information in the Manual Sample ID tab.

 **IMPORTANT:** Make sure the correct sample ID appears on the status line before aspirating a sample using either the manual open-tube sampler or the manual closed-tube sampler. Waiting appears on the status line while the system searches for a matching work order.

3. Aspirate each sample.

 a. Insert and push down tube containing the well-mixed sample into the manual closed-tube sampler. Hold tube parallel to the sampler well wall.

 b. Sample is automatically aspirated – the sampling light flashes.

 c. When the sampling light stops flashing, remove the tube.

 In the Sample Control Panel tab, evaluate control results when available.

**Running Control Samples from the Manual Open-tube Sampler**

1. If the Standby indicator is lit, press **Standby**.

 2. Scan the tube label or enter the sample information in the Manual Sample ID tab.

 **IMPORTANT:** Make sure the correct sample ID appears on the status line before aspirating a sample using either the manual open-tube sampler or the manual closed-tube sampler. Waiting appears on the status line while the system searches for a matching work order.

 3. Aspirate sample.

 a. Position tube so that the sampler probe is immersed within the well-mixed sample. You should only immerse the sampler probe deep enough (approx. 0.25 –in.) to ensure aspiration.

 b. Press the aspirate plate.

 c. Sampling light flashes during aspiration.

 d. When the sampling light stops flashing, remove the tube.

 4. In the Sample Control Panel tab, evaluate control results when available.

**Control Results**

The laboratory must evaluate all control results before reporting patient results. If control results fail to meet the laboratory established criteria for acceptability, all patient test results obtained in the unacceptable test run must be evaluated to determine if patient test results were adversely affected. The laboratory should take and document appropriate corrective actions, which may include calibration and re-assaying of patient samples, before reporting patient results.

The Target values are System Specific Values (SSVs) derived from replicate analyses of ADVIA TESTpoint Hematology Controls on properly calibrated instruments. The ranges provided represent estimate of laboratory variation. Inter-laboratory variation is usually accounted for by instrument calibration, maintenance and operating technique. For this reason, the Target values and Ranges are a guide for monitoring instrument performance and are not intended as absolute values for calibration.

For quality control of the instrument each laboratory should establish its own mean values and acceptable ranges for each lot. To verify instrument calibration, the laboratory established mean values should be within the SSV range. While this lot of control is in use, 95% of your recovered values should fall within 2 + SD of your laboratory generated mean value. Occasionally, individual results may fall outside the Range. Each lot of control should be extended to create an overlap period until a mean is established for the new lot of control.

**QC CORRECTIVE ACTION**

 **Indications of instability or deterioration:**

1. Deterioration is suspected when the laboratory mean of several days control results is not within the assay expected range. 2. Examine the tubes for the presence of a darkly colored supernatant indicative of gross hemolysis, and also for the presence of any clots. Do not use this product if it is hemolyzed or if any clots are present.

 a. Review the operating procedure of the instrument.

 b. Assay a new unopened vial of the affected ADVIA TESTpoint control. If values are still outside the expected range, call SIEMENS Technical Support.

**Performance Limits**

Individual laboratories should expect better precision than that shown in the expected range column. Ninety-five (95%) of your recovered values should fall within 2 SD of your individual laboratory mean.

**Troubleshooting Out-of-Range QC Values**

A control must be defined in the Control Dictionary before you run samples of it on the analyzer. The Control Dictionary is available in the Tools tab of the Customize menu.

Evaluate all control results before reporting patient results. Controls will automatically come across the LIS interface and can be evaluated in Meditech.

You can document corrective action in the QC log on the analyzer.

* **Green** — Control results for test are between target and +2 SD or target and -2 SD.
* **Yellow** — At least one control result is between +2 SD and +3 SD or -2 SD and -3 SD.
* **Red** — At least one control result is lower than - 3 SD or higher than + 3 SD.

**Investigational Procedure**

SIEMENS Diagnostics Corp. maintains a Customer Service Department that is available to help you resolve your control recovery problems. Resolution of the problem can be expedited if you will perform preliminary checks and have your data and reagent/control lot number information available when you call.

1. Check the expiration dates of your associated reagents. Note the lot numbers and expiration dates. Check for deterioration by inspecting for gross hemolysis (darkly colored supernatant). However, moderately colored supernatant is normal and should not be confused with deterioration of the product.
2. If this problem occurred with a new lot of control material, note the previous lot number of controls and the values obtained. If you are using more than one level of control, inspect your data to ascertain if the problem exists at all levels.
3. Check the ADVIA 2120i analyzer for obvious malfunctions such as:
4. Reagent leaks and air leaks
5. Prime and rinse to check out reagent flow, paying attention to the RBC and WBC shuttles and sample shear valve.
6. Look at the shuttles to verify proper drainage.
7. If the problem still remains, proceed as follows:
8. Clean the blood shear valve.
9. Do autorinse x 2.
10. Prime instrument with saline and whole blood x 1.
11. Run controls.
12. If a problem still persists:
13. Select a normal patient specimen that is less than 4 hours old. Analyze this specimen 12 times. Discard the first 2 determinations. Inspect the results for trending and unusual scatter. If the data appears to be within a variation that you normally observe, calculate the CV for the parameter that is in question. Compare this CV to the published instrument performance specification in the Product Reference Sheet. If your value exceeds these limits, call Siemens.
14. If error codes are encountered, refer to the error screen or printout and refer to error codes in the on-line Troubleshooting Guide.
15. If your CV is within specification, you have preliminarily qualified the performance of the instrument. Now it is time to call Siemens Customer Service at 1-800-255-3232 or 1-877-229-3711.

###### **Calling for Service:**

1. DURING SERVICE HOURS 8 TO 5 PM:
	1. Contact by calling 1-877-229-3711.
	2. If the problem cannot be resolved with Siemens over the phone, request a service call.
	3. Document the problem and the action taken so far.
	4. When service arrives, give an account of the problem. The FSE will email the Lead Tech a copy of the service report. This report will be forwarded to Crothall Clinical Engineering Department for their records.
2. DURING AFTER HOURS:
	1. Use the second analyzer as the backup instrument.
	2. Call Siemens for service and have the field service rep. come in ASAP during normal service hours.
3. THE ADVIA 2120i/ADVIA 120 ARE ON A FIVE DAY SERVICE CONTRACT so you may call ahead and have service come in on Monday morning.

**Moving Average (Performed at Memorial and South only)**

Moving averages are used to verify instrument performance over a period of time. The moving average can help identify a analytical error before this can be determined by running commercial quality control The moving averages are set up to capture MCV, MCH, MCHC,CHCM, NEUTx, NEUTy MNx, and MNy. This will monitor the RBC indices’ and the placement of the WBC’s in the cytogram. The number of patient results included in each calculation is 20. The formula to calculate these parameters is Bull’s Algorithm. All flagged results are excluded. The MVC, MCH, and MCHC can flow across the interface and be evaluated by each technologist like any other control result. The WBC flags can only be monitored on the analyzer control screen.

**Moving Average: Table: CH10-10**

Suggested action if a change in RBC indices is observed in two back –to –back observations.

|  |  |  |  |
| --- | --- | --- | --- |
| **MCV** | **MCH** | **MCHC** | **Suggested parameters to investigate** |
| change | same | change | Hct or MCV |
| same | change | change | Hgb |
| change | change | same | RBC |
| change | change | change | RBC,Hgb,Hct, or MCV |

Please refer to the investigational procedure to assist in troubleshooting these out-of control parameters.

**RETAINED PATIENT CONTROLS:** Retained patient samples are used to verify instrument performance throughout each shift.

1. Select two normal samples. The parameters that will be monitored are: WBC, RBC, Hgb, MCV, and Plt.
2. Retained patient controls (or Precision Controls) will have a defined range of CBC values. Please see the precision control log sheet for these values.
3. Two patient controls are used on each 8 hour shift.
4. Record results on the Precision Control log sheets and verify that they are within tolerance limits.
5. If results are not within the tolerance limits, a different precision control should be analyzed. If the problem persists, ADVIA 3·in·1 TESTpoint Hematology Controls should be analyzed. If there is not an instrument problem, discard this precision control, and start a new one. Patient results are not to be reported until the controls are within tolerance limits.
6. If a problem does exist, consult the Troubleshooting Section in the on-line Operator’s Guide on the ADVIA.
7. The tolerance limits are defined as follows:

 WBC = + 0.6 x 1000/cumm

 RBC = + 0.2 x 1000/cumm

 HGB = + 0.4 gm/dl

 MCV = + 5.0 fl

 PLT = + 30 x 1000/cumm

**Note:** Precision Controls can be tested in either primary, manual closed tube or manual open tube aspiration mode.

**CORRELATION OF NEW LOT NUMBERS:**

* Upon receipt of new lot number of, ADVIA 3·in·1 TESTpoint Hematology Controls the assayed values are entered in the ADVIA ®2120*i* / ADVIA® 120 analyzer QC files.
* New lot numbers are also place into the Laboratory LIS system. It will cross the interface and be evaluated by the technologist performing the controls.
* Laboratory staff will run these controls, in tandem with the current control lot number for at least ten sample aspirations.
* At the end of this period, these controls are evaluated, by the Lead Medical Technologist or designee, and the means are adjusted as indicated.
* The new lot number of ADVIA 3·in·1 TESTpoint Hematology Controls is then placed into use.

**Quality Evaluation:**

* The QC is then sent in monthly to Siemens to be evaluated.
* The QC is reviewed monthly by the Lead Technologist. Please refer to specific quality control procedures for each location.

**Precision statistics are then examined and appropriate action is taken whenever the limits are exceeded.**  This relates to both peer group statistics and/or in-house month to month statistics.  CV and SD are examined and reviewed for significant changes from previous data. If a significant change has occurred, the following actions should be taken:

* Verify the precision by performing a reproducibility study. If precision is off, contact the vendor for troubleshooting suggestions and/or service.
* Verify reagent lots and expiration dates.
* Review any manufactures notifications
* Verify all instrument maintenance.
* Verify the calibration by running the calibrator itself.
* Document all resolutions as specified by each location quality control procedures

# **Method Limitations**

CBC Method

* Some irreversibly sickled cells that occur in cases of sickle cell anemia may not completely sphere in the system, resulting in an elevated RDW and therefore an underestimation of the MCV. Sickled cells may cause erroneous results in other RBC parameters.
* Samples with cold agglutinins may falsely lower the red blood cell count.
* Samples with high WBC counts (>60 x 10³ cells/µL), or platelet clumps, and Samples with extreme lipemia, chylomicrons, or extremely high bilirubin might interfere with and elevate the hemoglobin results obtained from the hemoglobin method, including the MCHC.

Since these interferences do not affect the cell-by-cell hemoglobin concentration values obtained from the RBC method, the CHCM values are unaffected. If the MCHC and CHCM differ by more than 1.9 g/dL, a “Comparison Error” system flag alerts the operator to this condition.

* Patient specimens with nucleated RBCs (particularly neonate specimens) may falsely elevate the WBC count. When correcting the WBC count for the presence of NRBCs, use the WBC count reported from the basophil channel.

 Neonatal samples may interfere with the hemoglobin determination.

WBC DIFF Method

* Certain abnormal specimens such as those from hemodialysis patients, patient specimens with nucleated RBCs (particularly neonates), as well as specimens exhibiting platelet clumping or incomplete lysis of RBCs, may interfere with white cell differential.
* Circulating micromegakaryocytes may be counted as white blood cells
* Incomplete RBC lysis in the Peroxidase channel may be observed in specimens with elevated serum urea nitrogen (BUN > 75 mg/dL).
* Interference with the performance of the Peroxidase channel may be noted in specimens obtained from patients with inherited or acquired deficiency of the myeloperoxidase enzyme. Such deficiency has been observed in approximately 0.5% of laboratory specimens.
* In certain abnormal specimens (specifically lymphoid disorders and leukemias), erroneously elevated Basophil counts may be observed

**Please refer to the Handling of Spurious Results section located in this manual.**

# **References**

1. Siemens HealthCare Diagnostics, ADVIA ®2120*i* / ADVIA® 120 Operator’s Guide, V4.00.00, 2008
2. National Committee for Clinical Laboratory Standards (NCCLS). Clinical Laboratory Procedure Manuals⎯Third Edition (GP2-A3), 1996.
3. Clinical Laboratory Hematology, 1st edition 2003. McKenzie, Shirlyn B.

# **Technical Assistance**

Siemens Diagnostics Technical Care Center: 1-877-229-3711

 1-800-255-3232

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