LH780 Series Procedure **Technical Procedure #1510.t**

PURPOSE

The BeckmanCoulter LH780 series system, with attached Slide Maker and Slide Stainer, are quantitative, automated hematology analyzers for in vitro diagnostic use in clinical laboratories. They provide automated complete blood counts and reticulocyte counts, leukocyte differentials and make and stain blood smears according to pre-determined criteria. Integrated algorithms and user-defined criteria allow separation of normal specimens from those that need additional studies such as interference checks or manual leukocyte (WBC) differentials. The following table provides an overview of procedures involved in completing CBC and Reticulocyte testing.

Procedure	Procedure Overview
LH780 Procedure, #1510.t	Operation, Maintenance and QC
LH780 Result Interpretation and Rechecks, #1515.t	Result interpretation, critical values, rechecks, automated diff and reticulocyte technologist review criteria, pathology review criteria. Appendix with recheck methods.
LH780 Automated Differential Review Procedure, #1516.t	Procedure for technologist review of slides and data entry of differentials via keyboard.
Reporting Peripheral Blood Morphology, #1140.t	Procedure and glossary for identification and quantitation of hematopoietic cells and other structures found on peripheral blood smears.
Pathology Review of Abnormal Hematology Results, #1077.t	Procedure for submission of abnormal results to the pathologist for review.
Reticulocytes by Manual Method, #1241.t	Procedure for performing manual retics on specimens unsuitable for automated retics.
LH Calibration - S-CAL Method, #1518.t	Calibration procedure for “Reference” LH analyzer.
LH780 Calibration - Patient Sample Method, #1518.t	Calibration procedure for non-reference LH780 analyzers.
LH 1500 Hematology Automated System #1500.t	Procedure for LH1500 Hematology Automated Line

Beckman Coulter Remisol Middleware #1503.t — Procedure for releasing results using Middleware.

The following Analyzer Parameters are available on the LH780:

WBC	White Blood Cell (leukocyte) count	
RBC	Red Blood Cell (erythrocyte) count	
HGB	Hemoglobin concentration	
HCT	Hematocrit (relative volume of erythrocytes)	
MCV	Mean Corpuscular (erythrocyte) Volume	
MCH	Mean Corpuscular (erythrocyte) Hemoglobin	
MCHC	Mean Corpuscular (erythrocyte) Hemoglobin Concentration	
RDW	Red Cell (erythrocyte volume) Distribution Width	
PLT	Platelet (thrombocyte) count	
MPV	Mean Platelet Volume	
LY%	Lymphocyte percent	
MO%	Monocyte percent	
NE%	Neutrophil percent	
EO%	Eosinophil percent	
BA%	Basophil percent	
LY#	Lymphocyte number (absolute)	
MO#	Monocyte number (absolute)	
NE#	Neutrophil number (absolute)	
EO#	Eosinophil number (absolute)	
BA#	Basophil number (absolute)	
RET%	Reticulocyte percent	
RET#	Reticulocyte number (absolute)	
NRBC%	Nucleated RBCs/100 WBC	
NRBC#	Nucleated RBCs (absolute)	
*HLR%	High Light Scatter Reticulocytes %	*For Research Use Only.
*HLR#	High Light Scatter Reticulocytes #	*For Research Use Only.
*IRF	Immature Reticulocyte Fraction	*For Research Use Only.
*MRV	Mean Reticulocyte Volume	*For Research Use Only.
*MSCV	Mean Sphered Cell Volume	*For Research Use Only.
*Pct	Plateletcrit	*For Research Use Only.
*PDW	Platelet Distribution Width	*For Research Use Only.

PRINCIPLE

The Coulter method 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.

Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path

between two submerged electrodes, one located on each side of the aperture. This causes an electrical pulse that can be counted and sized. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

White blood cell (WBC) differentials and reticulocyte counts are done in the flow cell, where:

Low-frequency current measures volume

High-frequency current senses cellular internal content by measuring changes in conductivity, and

Laser light scatter characterizes cellular surface, shape and reflectivity.

The LH780 operates in both Automatic and Manual modes of sampling. In the Automatic aspiration mode the system automatically transports, mixes, aspirates and processes specimens. The Manual mode of operation uses the same sampling system as the Automated mode with a different sample needle. The sample identification number for the Manual mode must be entered manually or by handheld scanner, and the open vial must be introduced directly to the aspirator tip to begin the cycle.

SPECIMENS

- Freshly drawn whole blood collected in dipotassium or tripotassium EDTA anticoagulant is the specimen of choice.

- Tubes must be at least half full and must not contain clots.

- Tubes must not be overfilled. Misleading results could occur if you fail to leave space at the top of the tube between the sample and the stopper (overfilled tubes), thereby inhibiting specimen mixing. Ensure you leave space at the top of the tube between the sample and the stopper to facilitate mixing and that the sample is properly mixed before analysis.

- Citrated specimens may be used for platelet counts for patients with platelet clumping due to EDTA antibodies provided that the PLT count (or WBC, RBC, HGB, and HCT, if reported) is multiplied by 1.1 to correct for dilutional error. The indices (MCV, MCH, MCHC, and RDW) do not require correction. The effect of citrate on the automated differential has not been investigated and so should not be reported.

- Perform whole blood analysis as soon as possible for maximum accuracy.

- CBC and differential are valid for 24 hours at room temperature and 48 hours when stored at 2 – 8°C. Use 24 hour limit unless the tube has been under constant refrigeration since the time of collection.

- Specimens for reticulocyte testing should be analyzed within 24 hours after collection when stored at room temperature and 72 hours when stored at 2 to 8 oC.

- Aspiration volume is 300 µL in the Automated aspiration mode and 200 µL in the Manual aspiration mode. Approximately 200 µL additional volume is required when the Slide Maker is in use (Automated aspiration mode only).

- Tubes of blood may be used for sample analysis and various quality control procedures, but caps may only be pierced a maximum of five times. This is important as excessive piercing may result in cap deterioration that could introduce latex pieces into the system.

REAGENTS and SUPPLIES

Listed below are the Coulter Reagents, Controls, Calibrator and Consumables used.

REAGENTS/SUPPLIES			
Name	Volume	Part No.	Open Stability
LH 700 Series Diluent	20 Liter	PN 8547194	Mfg Exp Date
LH 700 Series Pak	1900/500 mL	PN 8547195	60 days
LH 700 Series Retic Pak	380/1900	PN 8547196	60 days
LYSE S III	10 Liter	PN 8546796	60 days
CLEANER	10 Liter	PN 8546931	3 months
5C-ES Cell Control	12-Pak	PN 7547192	13 days
RETIC C	6-Pak	PN 7547125	30 days

REAGENTS/SUPPLIES			
Name	Volume	Part No.	Open Stability
LATRON Primer	6-Pak	PN 7546915	30 days
LATRON Control	6-Pak	PN 7546914	30 days
S-CAL Kit	1 use	PN 7546808	1 hour
Slide Maker Slides	Gross	PN 6605505	N/A
Slide Maker Ribbon	5000/roll	PN 2016732	N/A
Slide Maker Labels	1500/roll	PN 2016733	N/A
Slide Baskets	N/A	6806894	N/A
Bath	N/A	1024888	N/A
Cubitainer	20 L empty	7547021	N/A
Filters:			
Liquid with tubing and connectors	N/A	6806976	N/A
Liquid without tubing and connectors	N/A	6233013	N/A
Trays:			
Basket holding position	N/A	1024891	N/A
Basket 13 position, input and output queue	N/A	1024913	N/A

REAGENTS/SUPPLIES

Name	Volume	Part No.	Open Stability
Bath tray holds 5 baths	N/A	1024889	N/A
Tray divider	N/A	1024915	N/A
Tubing:			
Pickup assembly (Methanol and Sedona Stain)	10-20 L	6706295	N/A
Pickup assembly (containers)	5 L	6706296	N/A
Pickup assembly (DI H20)	With float	5120249	N/A
Waste and level sensor	N/A	5120248	N/A
Methanol	4 L	EMD MX0485-3	N/A
Sedona Hemastain Stain	2 L	21-105	Mfg Exp Date
Hemastain Buffer	2 L	SVHB-024	Mfg Exp Date

SYSTEM DESCRIPTION

- Power Supply

  This unit consists of two assemblies. The Electronic Power Supply assembly provides the regulated and unregulated voltages required by the circuitry of the system. The Pneumatic Power Supply assembly is the source of pressure and vacuum.

- Diluter

  This unit is the primary operating unit of the system. It performs the mixing, transporting, pipetting, diluting, lysing, and sensing functions. The majority of all controls and indicators needed for normal daily operation are on the front of the Diluter.

- Analyzer

  This unit controls the electronic sequence of each operating cycle, and calculates and analyzes the results. It receives count and size information directly from the Diluter while the sample is being cycled; then it counts, measures, and computes the parameters. The Analyzer then sends this information to the Workstation.

- Reset Button (located on right side of analyzer)

  Press when directed by specific procedures and to reset the analyzer when data transmission to the Workstation is suspended.

- Analyzer CRT
  - Keypad available to select/deselect many functions such as:
    - Number of aspirations/sample
    - Enable/Disable alarm functions
  - Voting matrix display
    - Displays when results for any parameter are not available from an aperture.
    - Used to monitor persistent vote-outs.
  - System Status Indicators
  - Digitized Oscilloscope Display

- Graphic interface displays real-time cell count patterns.
- Useful in monitoring aperture and flow cell counting.

- Workstation
  - Receives information from the Analyzer
  - Displays, stores, and transmits it to the Laser Printer and the Host Computer
  - Provides storage for results, including scatterplots and histograms
- Handheld Scanner
  - Used to manually read barcodes on samples and reagents
- Laser Printer
  - This printer prints the data displayed on the Workstation screen, including parameter data and graphics.
- Slide Maker
  - Makes and labels wedge slides aspirated in the primary mode of the LH780 series analyzer. Labelled with Medical Record Number,Name, Bar Code, bar code number, and date and time.
- Slide Stainer
  - Stains slides from the slide maker and slides made elsewhere and loaded onto stainer.

CALIBRATION

For CBC parameters, the reference LH is calibrated with Coulter S-Cal, a commercial calibrator, using the method recommended by the manufacturer. All other instruments are calibrated using patient blood, matched to the reference instrument. Refer to separate calibration procedures (Coulter Calibration-S-CAL Method, 1518.t, and Coulter Calibration - Patient Sample Method, 1519.t) for details. The differential and reticulocyte measurement devices are set for optimum performance by service personnel and do not require calibration.

QUALITY CONTROL

The quality control program integrates several processes to ensure that the system is operating correctly and that reliable results are being generated. These include instrument checks, maintenance, running of commercial controls, and delta checks.

The following instrument checks and procedures are incorporated into the Coulter LH780 Quality Control program:

- Instrument Checks
  - Workstation
  - Analyzer
    - Enable the blood detector
    - Verify that the number of aspirations per tube is set to 1.
  - Default Mode on Workstation
    - C, CD, CDR, R. Select CD.
    - LH Random Access overrides this setting on bar-coded specimens
  - Process Type
    - Set Process Type to AUTO ANALYSIS
  - Select Run Configuration Icon (Lightening Bolt icon)
    - Slide Maker Tab
      - Enable the Slide Maker with check mark
    - Slide Stainer Tab
      - Enabel the Slide Stainer with check mark
    - Printer Tab
      - Select “SpecificFlags”
        - Yes Action Limits, Critical Limitis, Definitive Messages, Partial Aspiration, No read, Suspect Messages, Reflex Manager, Non-Numeric Flags
      - LIS Tab
        - Select “All Samples”
      - Enable System Functions for Slide Maker and Slide Stainer

- Maintenance and System Checks

  - Place initial in appropriate space on Preventative Maintenance Log as each procedure is completed.

  - Daily Maintenance

    - Analyzer

      - Each LH780 and Slide Maker should be in SHUT DOWN for a minimum of 2 hours once every 24 hours of operation. The Slide Maker is dependent on the attached LH780 for vacuum and pressure so it **must** be shut down first.

        - On the Slide Maker Keypad, press:

          - Routine Functions

          - Routine Fluidics

          - Shut-down

        - Slide Maker Shutdown takes about 2-3 minutes

        - On the Diluter Keypad on Analyzer, press "Shut-down

        - Allow the instrument to remain in Cleaner for at least 60 minutes.

    - Optimize the Workstation during shutdown (Power off)

      - Using the Log Off icon (Door) on the command center, select shut down the Workstation.

      - Allow the Workstation to process. When it is safe to shut down the computer, power off by depressing the round power button.

      - Allow the computer to remain powered off for at least 1 - 2 minutes.

      - Power the computer on by depressing the round power button.

      - Enter the logon name (labadmin) and press OK. There is no password.

      - At this point, leave the Workstation alone. In the background, the computer is working to optimize the hard drive. DO NOT DO ANYTHING AT THE WORKSTATION UNTIL AFTER THE

PROCESS INDICATOR ICONS HAVE STOPPED FLASHING COLORS.

- Clean BSV probe and rinse block with a cotton swab moistened with DIH_2O.

- Slide Maker Daily Maintenance
  - Turn off the power.
  - Unlatch the front cover and raise it up completely.
  - Clean the dispense probe and rinse cup with a cotton swab moistened with DIH_2O. If necessary, manually move the smear truck and shuttle so they do not obstruct your view of the dispense probe.
  - Close the cover.
  - Turn on the Slide Maker.
- Slide Stainer Daily Maintenance. Only one Slide Stainer is in use each day. Maintenance is done on rotating schedule based on usage.
  - Disable the Slide Stainer in System Configuration
  - Put Slide Stainer in Standby Mode
  - Drain baths
  - Home the arm with Reinitialize Arm command
  - Power off the Slide Stainer
  - Clean agitator assembly (basket holders, liquid level sensors, overflow sensors), baths, bath tray, and dryer with Methanol by lifting and pulling tray lever forward.
  - Replace baths and bath tray with clean baths and tray.
  - Power on the Slide Stainer
  - Fill Baths according to staining protocol
  - Enable Slide Stainer in System configuration. Put Slide Stainer in Auto Mode in the Workstation
  - Stain slides and check quality of stain
- Start Up Analyzer after 2 hours.
- Press Start-up on the Analyzer Diluter Keypad and allow it to process
  - Check the startup test results.

- Select QA on the Command Center to display the Quality Assurance application.
- Select the Day 31 icon to display the daily startup test results.
- Check the reagent status, background status and subsystem status for any items that failed (will appear in red).
- Select plus/plus icon to see startup test details. You can also select 0.00 icon to see background test results on the QA Results & Graphics window.
- Repeat any failed components of the Startup on the Analyzer module. It is not necessary or desirable to repeat Startup.
  - To Repeat Start Up test on the Analyzer screen, press:
    - ★ System Run
    - ★ Analyzer Functions
    - ★ Startup Tests
    - ★ Select RAMP, PRECISION or BACKGROUND, as needed.
    - ★ Refer to the Workstation's Help System for the appropriate action to resolve any failed items.
    - ★ Document corrective action in the written instrument log book.

- To Start Up Slide Maker on the Slide Maker keypad, press:
- Note: Slide Maker should start up automatically when properly shut down
  - Routine Functions
  - Routine Fluidics
  - Start-up
- Print the Start-up log following the Slide Maker start-up and place it in the instrument maintenance book

- Weekly Maintenance
  - Exercise BSV using function 06.

- Clean Sheer Valves by dropping DIH_2O on top and on moving sides while exercising solenoids 85,87, and 93 using function 85, 87, and 93.
- Clean Air Filters. Wash in water and dry completely, or vacuum, return to original location
    - Analyzer has 1 air filter.
        - Analyzer air filter on back of the analyzer
            - ★ Loosen the screws on the back of the Analyzer
            - ★ Pull the air filter down over the disk-shaped retainers on the screws to remove the air filter.
    - Power Supply has 3 air filters
        - One is located underneath the front of the Power Supply. Push downward on the front edge of the filter cover and slide the filter out.
        - Two are located on either side of Power Supply.
            - ♣ Lossen screws to remove.
    - Slide Maker has 2 air filters
        - One is located on the left rear side of unit. ***Must remove Slide Stainer cover to access.***
        - One is located inside Slide Maker behind the slide bracket area, in front of the slide dryer fan. Power Slide Maker off when cleaning this filter.
    - Slide Stainer has 1 air filter on left rear of unit. Lossen screws to remove.

- Monthly Maintenance is only needed for Slide Stainer
    - Slide Stainer Monthly Maintenance
        - Put Slide Stainer in Standby Mode
        - Home the arm by choosing Reinitialize Arm

- Drain all baths and reagent lines using Drain Reagent Line icon (icon with dripping drain)
- Place reagent pickup tubes from stain 2 and stain buffer 3 into a container of methanol
- Fill all baths. Baths 1-3 will fill with methanol. Bath 5 will fill with Di H_20
- Place stainer in Auto Mode and let agitate for 15 minutes
- Drain all baths using Drain Reagent Line icon
- Replace reagent pickup tubes into the correct containers
- Fill baths as needed

- As Needed Maintenance
  - Replace Reagents
    - Remove the plastic collar, if any, which secures the pick-up tube assembly.
    - Lift the assembly straight up and out, being careful not to touch or contaminate it.
    - Place the assembly into the new container, sliding the cap over the plastic collar and screwing it securely onto the container.
    - Use Hand Held Bar Code reader to enter data into workstation
      - Click on System Sep Up
      - Click on QA
      - Choose Reagent Tab
      - Click on Setup
      - Read both bar codes on reagent box
      - Click on green check mark
    - When changing Lyse, Diff Pak, or Retic Pak:

- Run Start Up. Print and file report
- Run one level of 5C control

- Replace Slides on Slide Maker
  - Place the cassette on the slide-loading stand and move cover back.
  - Place the slides in the cassette. Discard top slide if it is contaminated from handling.
  - Load the cassette into the Slide Maker.
- Replace Slide Maker Label Roll
  - Press the Slide Maker power button to turn it off.
  - Unlatch the front cover and raise it up completely.
  - Open the clip on the label supply.
  - Remove the empty supply roll and full take-up roll.
  - Place the new supply and the take-up rolls on their retainer spools.
  - Close the clip on the label supply.
  - Thread the label tape through the form sensor, through the printer head and around the two label tension bars.

NOTE:	The Slide Maker contains an arrow path to show the proper direction to thread the label tape.

  - Close the cover.
  - Press the power supply button to turn on the Slide Maker.
- Replace Slide Maker Printer Ribbon

NOTE:	Replacing the printer ribbon is necessary after using approximately four rolls of labels.

  - Lift the cover and press the button to turn the Slide Maker off.
  - Unlatch the front cover and raise it up completely.
  - Remove the old printer ribbon.

Important: Risk of breakage. The ribbon connecting the supply roll and take-up roll can break if you pull it too hard. Pull gently to separate. If the connecting ribbon breaks, tape it to the take-up roll.

- While letting the take-up roll hang, thread the ribbon through the printer head.

NOTE: The Slide Maker contains an arrow path to show the proper direction to thread the printer ribbon.

- Place the supply roll on its retainer spool.
- Place the take-up roll on its retainer.
- Thread the ribbon around the tension bar.
- Wind the ribbon until you see the ribbon reach the tension bar.
- Close the cover.
- Press the Slide Maker power button to turn it on.

- XB Analysis

  XB continuously monitors instrument performance utilizing patient samples. It is based on the theory that, in a given population, the red cell indices tend to remain stable and any variance from established target values indicates either a shift in the patient population or instrument malfunction.

  - XB analysis is used as needed in troubleshooting and in making calibration decisions.
  - XB analysis is not used at the Cancer Center lab due to the low number and type of specimens tested.

- Reproducibility and Carryover

  - These studies are performed on CBC/Diff as needed for troubleshooting purposes.

- Controls

  - Set up Control Files (when needed)
    - Set Up 5C and Retic controls

- Select System Set-Up (yellow diamond) icon on the Command Center.
- Select the Quality Assurance (QA) icon.
- Select Controls to display the control information already set up for the instrument.
- Select the Setup New Controls (New page) icon and the Setup New Controls Folder window appears.
- Select 5C-ES or Retic C for the type of control.
- Select All Levels for the level of the control
- Select the yellow diamond to set up reference values
- Import control data by reading the barcode on the control package insert for
- When controls are downloaded, change each file from active to accumulating data file.

- Run Controls using Assayed values. Run new lots of controls in parallel with existing lot of controls, accumulating 12 control values.

- Set Up LATRON control if needed.
  - The Levels field is blank as there is only one level.
  - Select the yellow diamond to set up reference values.
  - The LATRON Control Folder Setup Window appears.
  - Fill in the lot number, expiration date and reference values manually from the assay sheet
  - Check the box to select this lot as the default lot. All Latron results will be transmitted to this control file.
- Latron primer and Latron are run once per day
  - Ensure the latex primer and control are within the correct temperature range, 18-30°C or 64-86°F (room temperature).
  - Select QA on the Command Center to display the Quality Assurance application.

- Select QC to display the Controls.
- Verify the lot number of the latex control. If you must use a new lot number, ensure that it has been set up properly.

° Run Latron Primer and Latron Control

- Run LATRON Primer first
  - ➢ On the Diluter keypad, press F57 - ENTER to aspirate LATEX primer and LATEX control for combined Diff and Retic test modes. The Diluter Keypad displays PRESS MANUAL OR CLEAR APERTURE
  - ➢ Press CLEAR APERT.
  - ➢ Remove the cap of the latex primer vial. DO NOT MIX.
  - ➢ Immerse the aspirator tip in the latex primer vial. The instrument automatically aspirates the primer. The Diluter Keypad displays DIFF + RETIC PRIMER, while the instrument performs this function. When complete, the Diluter Keypad displays FUNCTION = 57.
  - ➢ Remove the vial from the aspirator tip when you hear a beep and the Analyzer Status line displays PREPARING SAMPLE. The probe cleaner retracts the aspirator and automatically cleans it.
  - ➢ Review LATRON primer results.

    ♣ *Results >500 are unacceptible*

    ★ Repeat the primer.

    ★ If you do not get a result below 500, cycle a new vial of primer.

    ★ If you still do not get a result below 500, run Start Up.

    ★ Results <500, run the Latron Control.

- Run LATRON Control
  - ➢ On the Diluter Keypad, press ENTER to reactivate the function 57 for the latex control.

- The Diluter Keypad displays PRESS MANUAL OR CLEAR APERTURE. There is no need to press anything, as presenting the specimen to the aspiration tip activates aspiration.

- Gently mix the latex control according to the directions in the package insert.

- Immerse the aspirator tip in the latex control vial. The instrument automatically aspirates the control. The Diluter Keypad displays LATEX - DIFF + RETIC while the instrument performs this function. When complete, the Diluter Keypad displays FUNCTION = 57.

- Review LATRON Control Results: When a Latron Control is outside its expected ranges:

  - *Ensure the control setup information (assigned values and expected ranges) match those on the package insert. If they do not, change the control information to match the package insert, and rerun the control.*

  - *Ensure no bubbles exist in the flow cell by rerunning the primer and the control. If the control is still outside the expected ranges:*
    - Go the Diluter Keypad.
    - Use F13 to purge the flow cell.
    - Run primer and control again.

  - *Check the control:*
    - Ensure the control is not contaminated, is properly mixed, and is not expired.
    - Ensure the aspirator tip is clean.

  - If necessary, use a new vial of latex control. Be sure to mix according to the directions on the package insert.

  - Ensure the flow cell is clear by performing the procedure for clearing a clogged flow cell, found in the Workstation's Help System.

- ♣ Rerun the control. If the control is still outside the expected ranges, initiate troubleshooting, notify supervisor and document in Corrective action section of the written instrument logbook.

➢ When the Latron is acceptable, press STOP on the analyzer to exit this function. The Diluter Keypad displays READY.

° Commercial Controls run each 8 hour shift

- COULTER 5C-ES

Controls are run once per shift; two levels are rotated throughout each 24 hours as follows:

Night shift - Normal and Abnormal 2

Day shift - Normal and Abnormal 1

Evening shift - Abnormal 1 and Abnormal 2

All three levels of controls are run at the Cancer Center Lab.

- COULTER RETIC-C Controls

Controls are run once per shift; two levels are rotated throughout each 24 hours as follows:

Night shift - Normal and Abnormal 2

Day shift - Normal and Abnormal 1

Evening shift - Abnormal 1 and Abnormal 2

- Prepare controls.

Remove control vials from refrigerator and bring to room temperature, about 15 minutes. Avoiding the barcode, record open container expiration date on vial when new ones are opened ((5C Control-13 days, Retic C Control-30 days). Mix controls thoroughly by rolling between hands and by inversion. DO NOT PLACE ON ROCKER.

- Run Controls

Put the analyzer into Stat Cassette Mode. Place the controls in a cassette, centering the barcode labels. Place cassette in the right-hand loading bay of the diluter. The Analyzer will premix and run cassette.

NOTE:	If Coulter controls are run without setting them up, the Workstation will automatically create control setup information, however control ranges are not determined and results will appear to be outside limits. Performing Control Setup will add ranges and apply limits to controls.

- Review Control Files:
  - Access Control file by selecting QA on the Command Center to display the Quality Assurance application.
  - Select QC to display the Controls window.
  - Select the specific control for which you want to review results. The control results table, statistics and graphs appear on the window. Use the scroll bars on the window to view other parameter results and graph.
  - If controls are within limits place a check in the Commercial Controls section of the Daily Coulter Check-Off Sheet to indicate that the controls are acceptable.

IMPORTANT:	DO NOT REPORT PATIENT RESULTS UNTIL CONTROLS ARE ACCEPTABLE.

- If any control is outside limits: Check for the following:
  - Material was mixed properly. If not, mix it according to the package insert. DO NOT USE MECHANICAL ROCKER.
  - Identification information was entered correctly. If using a bar-code reader, ensure the bar-code labels are clean and positioned correctly. If using the Diluter Keypad, ensure you typed the correct information.
  - Setup information (assigned values and expected ranges) matches the control package insert. If they do not, change the control's information to match the package insert.
  - If any of the above problems existed, rerun the control; otherwise, proceed to the next step.
  - Rerun the control ONCE to ensure it was not a statistical outlier.

- Run another vial or level of control ONCE to rule out contamination.
- Watch for normal sample flow as part of troubleshooting the instrument. Control codes and flags are explained in detail in the Workstation's Help System. If problem persists, institute troubleshooting, notify supervisor.
- Document all corrective action in the CMNT dialog box in the online Coulter QC log (click on the box to open it). Document in LH Maintenance book under Corrective Action log

- Mode-to-Mode Comparison
  - Mode-to-mode comparison is run once daily to document that the Manual Aspiration Mode agrees with the Automated Sampling Mode (which is controlled by commercial controls).
  - Obtain the results of a patient sample run in the Automated Sampling Mode. Always put analyzer into Stat Cassette Mode when running in primary mode.
  - Run the same sample in the Manual Aspiration Mode.
  - Compare results of the directly measured parameters, recording them on the Mode-to-Mode Comparison worksheet.
  - They must agree within the following limits.

MODE-TO-MODE COMPARISON LIMITS	
WBC	±0.4 or 5%
RBC	±0.2 or 2%
HGB	±0.3 or 2%
PLT	± 20 or 7%

- If they do not agree, repeat the control. If the out-of-control situation persists, initiate troubleshooting, notify supervisor and document in the Corrective Action section of logbook.

DO NOT REPORT RESULTS FROM THE MANUAL ASPIRATION MODE UNTIL THE PROBLEM IS RESOLVED.

- Interinstrument Comparison (ABC Control)
  - Select a patient
  - Print barcode labels, if needed
    - QC Labels
    - QC Menu
    - STD/CTL = ABC
    - LOT = ABC
    - FORMAT = QC
    - CODESET = I25
    - CNT = the number of labels to be printed
  - Place a barcode label on the selected specimen
  - The same tube of blood is run on each instrument in operation. Verify each run before running on next analyzer.
  - Results obtained on all instruments are compared to the reference instrument and must agree within established limits to ensure that instruments match each other. The supervisor will evaluate the results daily.
    - Do not repeat samples that do not match. The results will be evaluated and used to troubleshoot instrument problems.
- Quarterly Proficiency Testing by College of American Pathologists (CAP)
  - Randomly assign to CLS working bench
  - Include in regular workload

- There can be NO inter-laboratory communications about the Survey test results until after the reporting date of that Survey. Specimens cannot be referred to another laboratory.
- Enter the survey results into the form and have it rechecked for clerical errors by a CLS not involved in testing.
- Return the completed survey to CAP where results are compiled, analyzed statistically, and returned to participants.
- Close correlation with mean results is required for continued accreditation.

SAMPLE ANALYSIS

- Cycling Samples:
- Automated Aspiration Mode (Primary Mode or Closed Mode)
  - Load samples into the cassettes
  - Hemogard tubes 4 ml are placed in the cassette with the lip.
  - Non-Hemogard tubes 5 ml are placed in the cassette without the lip.
  - Slide each sample firmly into the cassette with bar codes facing up.

WARNING:	Do not force a tube into a cassette. Forcing a tube into the cassette improperly could cause the tube to break. If a tube should break, follow the laboratory's safety procedure for cleaning broken glass and biological spills.

  - Place analyzer into Stat Cassette Mode. Place the cassettes firmly and securely into the loading bay on the right side of the Diluter. The instrument automatically begins cycling the cassettes.

NOTE:	Samples that have been sitting may not mix adequately on the rocker bed. Premix them by inversion.

WARNING:	If a problem occurs while the system is cycling, press STOP and wait for the system to stop before you do anything to correct the problem. Attempting to correct an instrument problem while the instrument continues to process samples could cause injuries.

- Manual Aspiration Mode (Secondary Mode or Open Mode):

- Mix specimens thoroughly.
    - Tubes may be mixed for 5 minutes on rocker or by 20 complete inversions.
    - Microtainers may be mixed by gently aspirating up and down with a transfer pipette, or by inversion. MIXING ON THE ROCKER IS INADEQUATE. DO NOT VORTEX. The only execption is when the patient has known platelet clumping problems. See procedure 1515.t
    - Check each microtainer for clots using applicator sticks.
- Enter ID
    - Enter the sample ID on bar-coded specimens using the handheld scanner
        - Put the cursor in the barcode field at the Workstation
        - Scan the sample ID using the handheld scanner.
        - Press ID (wait for ID XXXXXXX to display on the Keypad)
        - Type a period (Confirm that the correct ID number appears on the Analyzer CRT)
        - Press ENTER to accept the bar-code ID or STOP to reject the bar-code ID
    - Entering the sample ID manually on non-bar-coded specimens
        - Press ID and key in the sample ID on the Diluter Keypad.
        - Press ENTER.
- Remove the stopper from the specimen tube, using gauze to prevent aerosolization.
- Introduce well-mixed whole-blood specimen to manual mode aspiration tip for automatic aspiration.
- Remove the tube from the aspirator tip at the beep
- The probe cleaner retracts and cleans the aspirator.

- Display Sample Results and Graphics:

- Select the Patient Bed Icon on the Command Center to access Patient Results database.
- Current results are displayed on the Results & Graphics window.

NOTE:	If the results on the screen are not changing, check to see if the Screen Lock is activated (Specific Toolbar on the right side of the screen)

- Previous results may be recalled from the database.
  - Select Database Explorer (binoculars) icon.
  - Enter search criteria when the pop-up window opens (can use scanner to enter Sample ID)
  - Click OK or press Enter.
  - Screen will display list of specimens.
  - Select specimen by clicking on the gray box to the left of the spec. ID.
  - Select Show Results and Graphics (Scatterplot with red arrow) icon, located on the Specific Toolbar (right side of screen) to display results.

- To print (or re-transmit) results, select the appropriate icon (printer or computer) on the Common Toolbar (top of the screen).
- To edit the ID number (automated aspiration mode):
  - Change the ID
  - Tab to the next field (floppy disk icon should change from gray to blue)
  - Click on the Save (floppy disk) icon
  - Results may now be re-transmitted or re-printed.
- Review, Interpret and Report CBC/Diff and Reticulocyte results using:

LH780 Result Interpretation and Rechecks Technical Procedure #1515.t

Reference Values

ADULT NORMAL VALUES (UCDMC)		
PARAMETER	MALE	FEMALE
WBC (X 103/mm3)	4.5-11.0	same
RBC (X 106/mm3)	4.5 - 5.9	4.0-5.2
HGB (gm/dL)	14 - 18	12 -16
HCT (%)	40 - 52	34 - 46
MCV (fl.)	80 - 100	same
MCH (ug)	27 - 33	same
MCHC (%)	32 - 36	same
RDW (%)	11.9 - 14.7	same
PLT (X 103/mm3)	130 - 400	same
Neutrophil #	1.8 – 7.7	same
Lymphocyte #	1.0 – 4.8	same
Monocyte #	0.1 – 0.8	same
Eosinophil #	0 – 0.5	same
Basophil #	0 – 0.2	same

Pediatric Normal Values

RED BLOOD CELL VALUES AT VARIOUS AGES: MEAN AND LOWER LIMIT OF NORMAL (-2 SD)*

	HGB (g/dL)		HCT (%)		RBC (M/uL)		MCV (fL)		MCH (pg)		MCHC (%)	
Age	Mean	-2sd	Mean	-2sd	Mean	-2sd	Mean	-2sd	Mean	-2sd	Mean	-2sd
Birth (Cord Blood)	16.5	13.5	51	42	4.7	3.9	108	98	34	31	33	30
1 to 3 days	18.5	14.5	56	45	5.3	4.0	108	95	34	31	33	29
1 week	17.5	13.5	54	42	5.1	3.9	107	88	34	28	33	28
2 weeks	16.5	12.5	51	39	4.9	3.6	105	86	34	28	33	28
1 month	14.0	10.0	43	31	4.2	3.0	104	85	34	28	33	29
2 months	11.5	9.0	35	28	3.8	2.7	96	77	30	26	33	29
3 to 6 months	11.5	9.5	35	29	3.8	3.1	91	74	30	25	33	30
0.5 to 2 years	12.0	10.5	36	33	4.5	3.7	78	70	27	23	33	30
2 to 6 years	12.5	11.5	37	34	4.6	3.9	81	75	27	24	34	31
6 to 12 years	13.5	11.5	40	35	4.6	4.0	86	77	29	25	34	31
12 to 18 years												
Female	14.0	12.0	41	36	4.6	4.1	90	78	30	25	34	31
Male	14.5	13.0	43	37	4.9	4.5	88	78	30	25	34	31
18 to 49 years												
Female	14.0	12.0	41	36	4.6	4.0	90	80	33	26	34	31
Male	15.5	13.5	47	41	5.2	4.5	90	80	30	26	34	31

This data has been compiled from several sources. Emphasis is given to recent studies employing electronic counters and to the selection of populations that are likely to exclude individuals with iron deficiency. The mean $\pm$2 SD can be expected to include 95 percent of the observations in a normal population. (From Dallman, PR: In Pediatrics, 16th ed., Rudolph A (ed), New York, Appleton-Century-Crofts, 1977, p. 1111.)

NORMAL LEUKOCYTE COUNTS

	Total WBCs		Neutrophils			Lymphocytes			Monocytes		Eosinophils	
Age	Mean	Range	Mean	Range	%	Mean	Range	%	Mean	Range	Mean	Range
Birth	18.1	9.0-30.0	11.0	6.0-26.0	61	5.5	2.0-11.0	31	1.1	6	0.4	2
12 Hours	22.8	13.0-38.0	15.5	6.0-28.0	68	5.5	2.0-11.0	24	1.2	5	0.5	2
24 hours	18.9	9.4-34.0	11.5	5.0-21.0	61	5.8	2.0-11.5	31	1.1	6	0.5	2
1 week	12.2	5.0-21.0	5.5	1.5-10.0	45	5.0	2.0-17.0	41	1.1	9	0.5	4
2 weeks	11.4	5.0-20.0	4.5	1.0-9.5	40	5.5	2.0-17.0	48	1.0	9	0.4	3
1 month	10.8	5.0-19.5	3.8	1.0-9.0	35	6.0	2.5-16.5	56	0.7	7	0.3	3
6 months	11.9	6.0-17.5	3.8	1.0-8.5	32	7.3	4.0-13.5	61	0.6	5	0.3	3
1 year	11.4	6.0-17.5	3.5	1.5-8.5	31	7.0	4.0-10.5	61	0.6	5	0.3	3
2 years	10.6	6.0-17.0	3.5	1.5-8.5	33	6.3	3.0-9.5	59	0.5	5	0.3	3
4 years	9.1	5.5-15.5	3.8	1.5-8.5	42	4.5	2.0-8.0	50	0.5	5	0.3	3
6 years	8.5	5.0-14.5	4.3	1.5-8.0	51	3.5	1.5-7.0	42	0.4	4	0.2	2
8 years	8.3	4.5-13.5	4.4	1.5-8.0	53	3.3	1.5-6.8	39	0.4	4	0.2	2
10 years	8.1	4.5-13.5	4.4	1.8-8.0	54	3.1	1.5-6.5	38	0.4	4	0.2	2
16 years	7.8	4.5-13.0	4.4	1.8-8.0	57	2.8	1.2-5.2	35	0.4	5	0.2	3
21 years	7.4	4.5-11.0	4.4	1.8-7.7	59	2.5	1.0-4.8	34	0.3	4	0.2	3

* Numbers of leukocytes are in thousands per mm^3, ranges are estimates of 95% confidence limits, and percentages refer to differential counts. Neutrophils include band cells at all ages and a small number of metamyelocytes and myelocytes in the first few days of life. (From Dallman, PR: In Pediatrics, 16th ed., Rudolph, A, (ed), New York, Appleton-Century-Crofts, 1977, p 1178).

References

Beckman/Coulter LH 750 Operator's Manual (electronic); Beckman/Coulter, 2002.

Nathan, D.G. and Oski, F.A.; Hematology of Infancy and Childhood, 3rd Ed., pp. 1680 and 1688; W.B. Saunders, 1987.

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LH780 Series Procedure **Technical Procedure #1510.t**

Procedure History

Date	Written/ Revised By	Revision	Approved Date	Approved By
6/30/00	J Cannon	New	6/30/00	E Larkin, MD
10/03/00	J Cannon	Minor	11/03/00	E Larkin, MD
11/21/00	J Cannon	Minor	11/21/00	E Larkin, MD
7/2/02	J Cannon	LH Rev	7/15/02	E Larkin MD
11/02	J Cannon	Del Pat Cont	11/5/2	E Larkin MD
10/03	J Cannon	Annual Review	10/17/03	E Larkin MD
10/26/04	J Cannon	Minor	10/24/04	E Larkin MD
2/24/05	J Cannon	New Director	2/24/05	K Janatpour MD
5/06	J Cannon	Annual Review Minor changes	5/15/06	K Janatpour MD
5/09	L Gandy	Minor changes	06/30/09	D. Dwyre, MD
06/10	L Gandy	Add Slide Stainer	08/18/10	D Dwyre MD
		Biannual Review	8/24/12	D Dwyre MD
9/13	L Gandy	Minor, update ABC info		NA
6/14	L Gandy	Minor, 2 hour shutdown		NA
		Biannual Review	10/1/2014	D Dwyre MD
12/3/14	L Gandy	Add start up and control to reagent change		

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