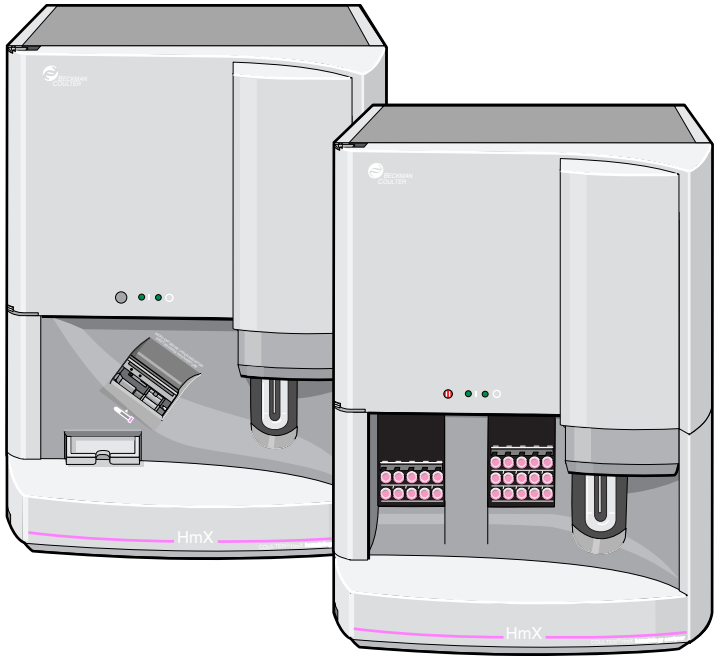
COULTER HmX Hematology Analyzer
COULTER HmX Hematology Analyzer with Autoloader

Operator's Training Guide



PN 4237530BB (March 2011)

Beckman Coulter, Inc.
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Brea, CA 92821

BECKMAN COULTER

WARNINGS AND PRECAUTIONS

READ ALL PRODUCT MANUALS AND CONSULT WITH BECKMAN COULTER-TRAINED PERSONNEL BEFORE ATTEMPTING TO OPERATE INSTRUMENT. DO NOT ATTEMPT TO PERFORM ANY PROCEDURE BEFORE CAREFULLY READING ALL INSTRUCTIONS. ALWAYS FOLLOW PRODUCT LABELING AND MANUFACTURER'S RECOMMENDATIONS. IF IN DOUBT AS TO HOW TO PROCEED IN ANY SITUATION, CONTACT YOUR BECKMAN COULTER REPRESENTATIVE.

HAZARDS AND OPERATIONAL PRECAUTIONS AND LIMITATIONS

WARNINGS, CAUTIONS, and IMPORTANTS alert you as follows:

WARNING - Can cause injury.
CAUTION - Can cause damage to the instrument.
IMPORTANT - Can cause misleading results.

BECKMAN COULTER, INC. URGES ITS CUSTOMERS TO COMPLY WITH ALL NATIONAL HEALTH AND SAFETY STANDARDS SUCH AS THE USE OF BARRIER PROTECTION. THIS MAY INCLUDE, BUT IT IS NOT LIMITED TO, PROTECTIVE EYEWEAR, GLOVES, AND SUITABLE LABORATORY ATTIRE WHEN OPERATING OR MAINTAINING THIS OR ANY OTHER AUTOMATED LABORATORY ANALYZER.

WARNING Risk of operator injury if:

- All doors, covers and panels are not closed and secured in place prior to and during instrument operation.
- The integrity of safety interlocks and sensors is compromised.
- Instrument alarms and error messages are not acknowledged and acted upon.
- You contact moving parts.
- You mishandle broken parts.
- Doors, covers and panels are not opened, closed, removed and/or replaced with care.
- Improper tools are used for troubleshooting.

To avoid injury:

- Keep doors, covers and panels closed and secured in place while the instrument is in use.
- Take full advantage of the safety features of the instrument. Do not defeat safety interlocks and sensors.
- Acknowledge and act upon instrument alarms and error messages.
- Keep away from moving parts.
- Report any broken parts to your Beckman Coulter Representative.
- Open/remove and close/replace doors, covers and panels with care.
- Use the proper tools when troubleshooting.

CAUTION System integrity might be compromised and operational failures might occur if:

- This equipment is used in a manner other than specified. Operate the instrument as instructed in the Product Manuals.
- You introduce software that is not authorized by Beckman Coulter into your computer. Only operate your system's computer with software authorized by Beckman Coulter.
- You install software that is not an original copyrighted version. Only use software that is an original copyrighted version to prevent virus contamination.

IMPORTANT If you purchased this product from anyone other than Beckman Coulter or an authorized Beckman Coulter distributor, and, if it is not presently under a Beckman Coulter service maintenance agreement, Beckman Coulter cannot guarantee that the product is fitted with the most current mandatory engineering revisions or that you will receive the most current information bulletins concerning the product. If you purchased this product from a third party and would like further information concerning this topic, call your Beckman Coulter Representative.

Initial Issue, A 6/99
Software version 1.0

Revision B, 3/01
Software version 1.3
Information concerning the IVD parameters, MRV and IRF, was added to this training guide.
Changed pages: Cover, iii, ix, 3-5, 3-6, 3-7, 4-10, 4-14, 4-22, 6-26, 7-39, Running 5C® Cell Control summary page 2 of 2, and Control File Management summary page 3 of 3.

Note: Changes that are part of the most recent revision are indicated in text by a bar in the margin of the amended page.

Revision BA, 5/10
Software Version 1.3.

Updates were made to the company corporate address.

Note: Changes that are part of the most recent revision are indicated in text by a bar in the margin of the amended page.

Revision BB, 03/11
Software Version 1.3.

Changes were made to pages, 6-3, 6-22.

Note: Changes that are part of the most recent revision are indicated in text by a bar in the margin of the amended page.

This document applies to the latest software listed and higher versions. When a subsequent software version changes the information in this document, a new issue will be released to the Beckman Coulter website. For labeling updates, go to www.beckmancoulter.com and download the most recent manual or system help for your instrument.

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PURPOSE OF THIS DOCUMENT

To provide an authorized trainer with corporately accepted training materials for instructing a new operator in the proper use and operation of a COULTER HmX Hematology Analyzer with Autoloader or a HmX Hematology Analyzer.

DOCUMENTATION

Several manuals and related documents are shipped with a new instrument. It is important that you familiarize yourself with these materials as soon as possible. Since these materials may be used during your training, please make sure the following manuals and related documents are readily available before the training session begins.

HmX Hematology Analyzer with Autoloader

- **Reference** Manual, PN 4237523, provides in-depth information about what the instrument does, the methods it uses, its specifications, and information on installation, safety, and software options.
- **Operator's Guide**, PN 4237521, provides step-by-step instructions for the day-to-day running of your instrument.
- **Special Procedures and Troubleshooting** Manual, PN 4237522, provides in-depth information about how to run a calibration, how to clean, replace, or adjust a component of the instrument, and provides troubleshooting tables as diagnostic tools.
- **Host Specifications**, PN 4237518, defines the contents of records transmitted from a HmX Hematology Analyzer with Autoloader to a host computer.
- **Master Index**, PN 4237525, is a combined index for the **Operator's Guide**, **Reference**, and **Special Procedures and Troubleshooting** manuals. Each manual also has an individual index.

HmX Hematology Analyzer

- **Reference** Manual, PN 4237523, provides in-depth information about what the instrument does, the methods it uses, its specifications, and information on installation, safety, and software options.
- **Operator's Guide**, PN 4237519, provides step-by-step instructions for the day-to-day running of your instrument.
- **Special Procedures and Troubleshooting** Manual, PN 4237520, provides in-depth information about how to run a calibration, how to clean, replace, or adjust a component of the instrument, and provides troubleshooting tables as diagnostic tools.
- **Host Specifications**, PN 4237518, defines the contents of records transmitted from a HmX Hematology Analyzer to a host computer.
- **Master Index**, PN 4237524, is a combined index for the **Operator's Guide**, **Reference**, and **Special Procedures and Troubleshooting** manuals. Each manual also has an individual index.

ABOUT THIS TRAINING GUIDE

Scope

This training guide provides the trainer with the opportunity to customize the training experience to best fit the individual trainee and laboratory. Since trainee experience levels and laboratory needs differ, this training outline is designed with flexibility in mind.

This document is designed to support both HmX Hematology Analyzer with Autoloader and HmX Hematology Analyzer training. Material that is unique to the autoloader or the single-specimen, rotary cap-pierce analyzer is clearly identified, usually with the analyzer's name at the top of the page.

- HmX Hematology Analyzer with Autoloader headers indicate the autoloader version of the analyzer.
- HmX Hematology Analyzer headers indicate the single-specimen, rotary cap-pierce version of the analyzer.
- Using the term instrument or HmX instrument indicates the material applies to both.

Topics

The materials in this guide support the training of eight different topics:

- Topic 1 GETTING TO KNOW YOUR INSTRUMENT
- Topic 2 STARTUP / SHUTDOWN
- Topic 3 SET UP OPTIONS
- Topic 4 QUALITY CONTROL
- Topic 5 CALIBRATION
- Topic 6 SAMPLE ANALYSIS
- Topic 7 DATA REVIEW
- Topic 8 BASIC TROUBLESHOOTING

Each topic consists of several related subjects that may be used, as needed, in your training.

Organization

The eight individual topics contained in the Operator's Training Guide are part of a larger organization that includes four basic types of information:

Introduction	Explains how to use this training guide
Topics	Heart of this document, each topic consists of multiple subjects and operational summaries relating to the overall topic
Summary Pages	Master copy for use by the Key Operator in their laboratory once training is complete
Training Checklist	Method for documenting the specific training an operator receives

Summary Pages

Routine operational tasks detailed in the various instrument manuals are abbreviated in this training document. These abbreviated instructions are referred to as either summary pages or operational summaries. Each summary page is an individual, stand-alone document that is designed for use in your laboratory as a training aid and/or as a reference tool for a trained operator. A master copy of each summary page is located behind the Summary Masters tab.

These summary pages contain essential operational instructions. As a result, it is important that you become proficient in the use of this tool during your training. To eliminate the need to flip back and forth between the topic and the summary masters, the appropriate operational summaries are inserted within each topic. When an operational summary is inserted in the training material, a rotated chevron (➤) appears to the left of the Summary Page title. For example, at the point of insertion, the Startup summary would begin with

➤ Startup Summary

Since this is a stand-alone summary for use after training is complete, some of the instructions discussed in the training topic may be repeated in the summary. Two rotated chevrons (➤ ➤) mark the end of the inserted summary.

Training Checklist

This checklist provides the trainer with an official method to document the completion of training on the COULTER HmX Hematology Analyzer with Autoloader or the HmX Hematology Analyzer.

When the training process is complete, we recommend that both the trainer and trainee sign the Training Checklist to show agreement that the trainee is sufficiently trained and able to meet the objectives listed for each topic.

RESPONSIBILITIES

Beckman Coulter's Responsibility

Beckman Coulter is responsible for instructing a Key Operator in the proper use and operation of a COULTER HmX Hematology Analyzer with Autoloader or a HmX Hematology Analyzer.

Our goal is to provide your laboratory with high quality training that best fits your laboratory's needs. This means the emphasis given to each topic may differ, with some topics needing to be covered more thoroughly than others.

Key Operator's Responsibility

It is the Key Operator's responsibility to train all other operators in their laboratory. To assist you in this process, a master copy of each operational summary is located at the end of the Operator's Training Guide. Once trained by an authorized Beckman Coulter trainer, the Key Operator is authorized to copy these masters for use in training other laboratory personnel.

CONVENTIONS

This training guide uses the following conventions:

- *Italics font* indicates screen text and/or messages displayed by the instrument such as *RESET THE SYSTEM* or *Press any key.*
- **Note** (in bold font) prefaces information that is important to remember or helpful in performing a procedure.
- **Bold** indicates
  - a menu item such as **Run Samples**
  - or a function such as **F3 Run**.
- ▢ indicates a key such as Enter.
- F4 indicates the operator needs to press and release the F4 key.
- ▢ ▢ indicates to press and release the first key listed, then press and release the next key listed.
- ▢ + ▢ indicates to press and hold the first key listed, then press the next key.
- Software navigation to access a needed function or screen appears as a sequence of menu items separated by double arrow heads. For example, the path to the screen for setting up reagents is **Special Functions ▸▸ Set Up ▸▸ System set up ▸▸ Reagents**. Each menu item contains one letter displayed in black.
- To select a particular menu item,
  - Use the ↑ or ↓ keys to highlight the menu item then press Enter

    or
  - Note the black letter within the desired menu item and press the corresponding alphabetic key. (It is not necessary to press the Enter key.)
- A rotated chevron (➤) placed to the left of a Summary Page title identifies an operational summary inserted within the training materials. For example,

  ➤ Changing Reagents Summary
- Two rotated chevrons (➤ ➤) designate the end of an operational summary.
- HmX Hematology Analyzer with Autoloader or HmX Analyzer with Autoloader indicates an autoloader unit.
- HmX Hematology Analyzer or HmX Analyzer indicates a single-specimen, rotary cap-pierce unit.
- Instrument or HmX instrument indicates information concerning both the autoloader and the single-specimen, rotary cap-pierce versions of the analyzer.

GRAPHICS

All graphics, including screens and printouts, are for illustration purposes only and must not be used for any other purposes.

OBJECTIVES

When the subject is complete, you will be able to . . .

Data Management System (DMS)

- Locate and name the three major components of the peripheral computer system.
- Locate and explain the function of specific keys on the keyboard.
- Quickly access a commonly used area of software using the Access screen.
- Select a desired option from the DMS Main Menu or submenu.
- On the Status line, explain the meaning of the
  - Symbols
  - Background colors
  - Up arrows versus down arrows.
- Replace the ribbon or ink cartridge and paper in the printer.

Main Unit

- Locate and name the hardware controls and indicators.
- Given an instrument that appears to be without power,
  - Tell whether it is powered down or in Standby.
  - Bring the instrument back into operation.
- Reset the system.
- Locate, name, and explain the major function of each module.

Modes of Operation

- State the four modes of operation available on HmX instruments.
- Briefly overview the Primary mode of operation versus the Secondary mode of operation.
- List four situations when the Secondary mode can be used.
- Demonstrate how to silence an alarm.

Reagents

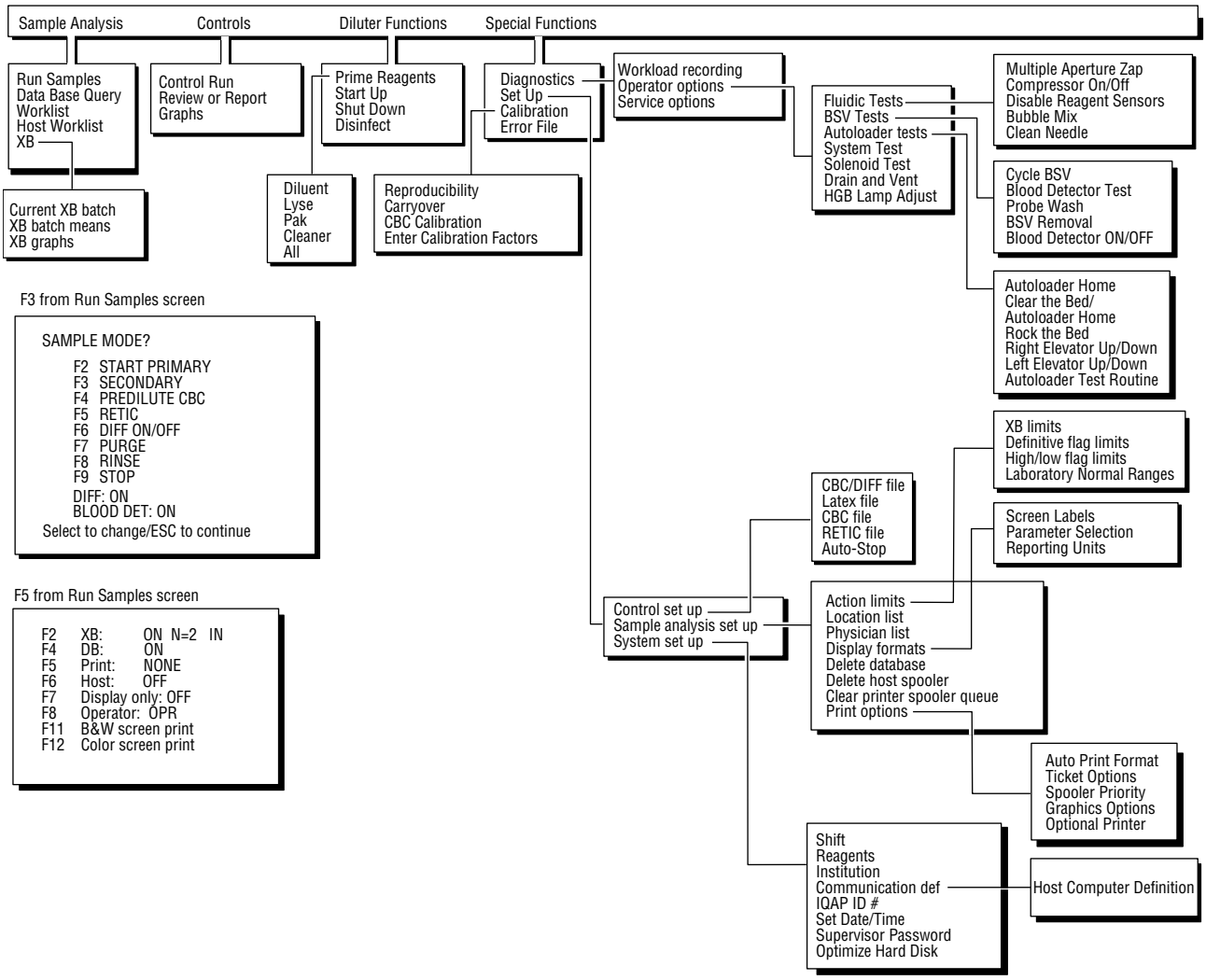
- Name the reagents used on a HmX instrument.
- State if the reagent is used for CBC analysis, DIFF analysis, or both.
- State if the reagent is used for Retic analysis.
- State the open container stability of each reagent used on a HmX instrument.
- Explain how to change reagents.
- Explain how to replace a waste container, if applicable.

ACCESS SCREEN

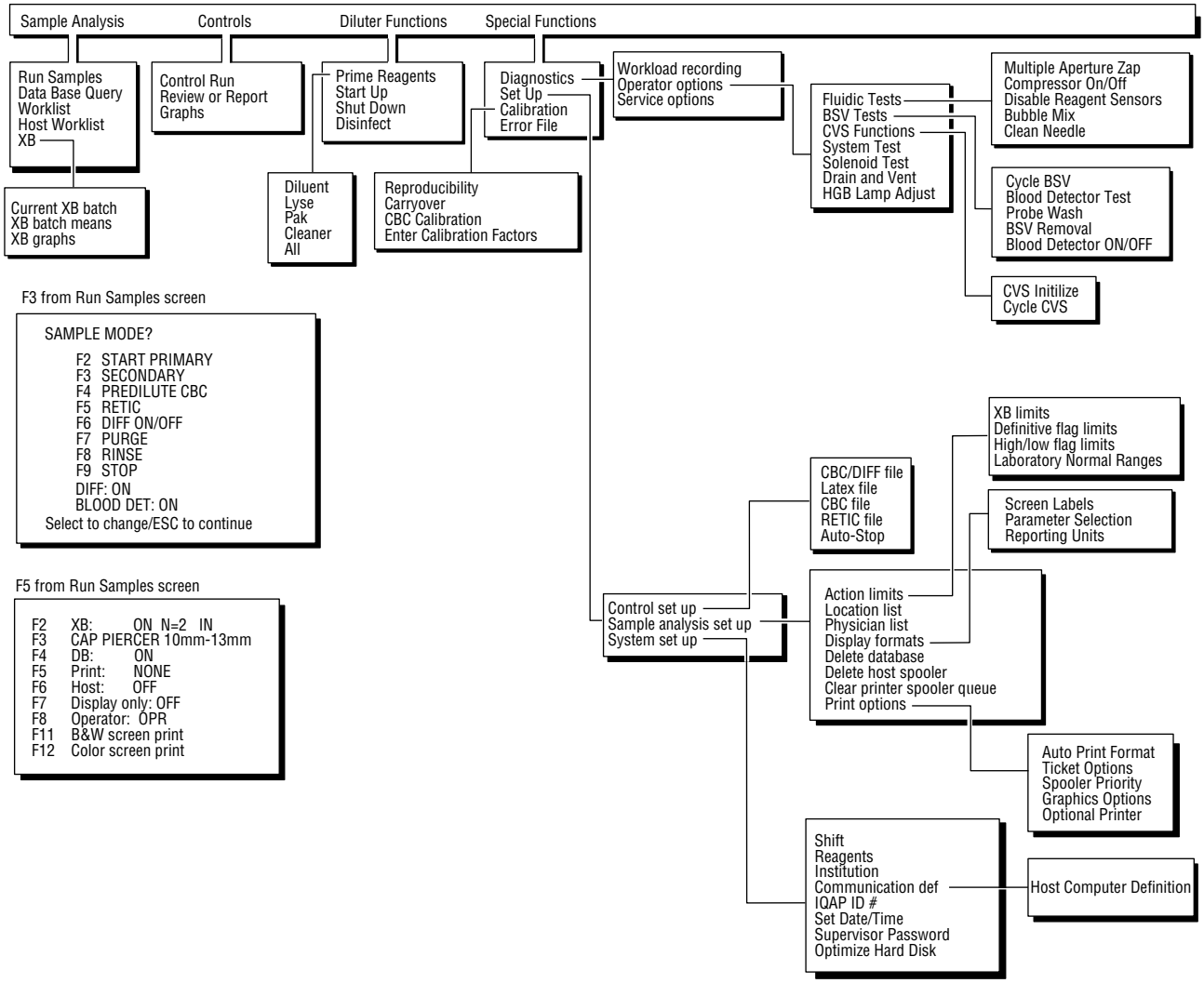


- First screen to appear after a system reset or power up
- Screen provides operator with quick access to the most commonly used areas of the software
- Display automatically returns to the Access screen upon exit when this screen is used to access any of the designated software areas
- May also display this screen from the Main Menu by pressing F1

SOFTWARE MENU TREE - HmX Hematology Analyzer with Autoloader



SOFTWARE MENU TREE - HmX Hematology Analyzer



STATUS LINE TABLE



Symbol	Refers to	↑	↓	Red	Yellow	White
DMS	Data Management System	Connected to Analyzer.	Not connected to Analyzer.	Not communicating with Analyzer.	DMS busy or receiving data.	DMS is OK.
PR*	Graphics Printer	Autoprint is set to ALL, ABNORMALS or NORMALS.	Autoprint is set to NONE.	Printer is off-line OR Printer is out of paper.	Printer is printing.	Printer and DMS are connected.
HC	Host Computer	Auto transmission ON.	Auto transmission OFF.	Not connected to host.	Sending to host computer.	Host and DMS are connected.
DB	Data Base	Store is ON.	Store is OFF.	Data base is not functional. System stops. Reset the system and rerun last 2 specimens.	Data base is storing data.	Data base is OK.
XB	$\bar{X}_B$ Analysis	XB is ON.	XB is OFF.	Last completed batch was OUT (outside the XB Action Limits).	N/A	Last completed batch was IN (within the XB Action Limits).
WL	Worklist	Preassigned entries pending on Worklist.	No preassigned entries on the Worklist.	3 consecutive or 10 total error messages are in the status field.	The Worklist is full (300 preassigned samples).	Worklist is OK.
HWL	Host Worklist	Preassigned entries are on the Host Worklist.	No preassigned entries on the Host Worklist.	Host Worklist is full.	DMS is receiving preassigned samples from the host computer.	Host Worklist is OK.
QC	Quality Control	Auto-Stop is ON.	Auto-Stop is OFF.	Last control run had an error message.	Receiving a control run.	Results of last control run are OK.
P2	Additional Graphics Printer	Autoprint is ON.	Autoprint is OFF.	Printer is off-line OR printer is out of paper.	Printer is printing.	Printer and DMS are connected.

* Changes to MA for manual printing, BA for batch printing, and AU for autoprinting.

DATA MANAGEMENT SYSTEM (DMS)

Functions of the Data Management System (DMS)

- Controls and monitors instrument operation
- Displays, stores, outputs, and allows recall of sample results
- Stores and graphs control results for the QC program
- Automatically calculates new calibration factors
- Allows bidirectional communication with a host computer
- Consists of three major components: the computer, the monitor, and the printer

Major Components

Computer

- **Must not use this system as a personal computer**
- Consists of hardware components and software
- Hardware is the term used to describe the physical components of a computer system
  - Locate the following hardware components:
    - Power ON/OFF button
    - Power ON/OFF indicator light
    - Hard drive indicator and symbol
    - Diskette drive
- Software refers to a sequence of detailed instructions (called a program) that directs the computer to perform certain actions
  - Data Management System or DMS refers to the software used in the computer

Monitor

- Computer uses the monitor to visually display information
- Locate the Power ON/OFF switch and the indicator light that lights when power is on
- Locate screen adjustment controls

Printer

- Used to either print a copy of the screen displayed on the monitor or of data stored in the computer's data base
- Up to two printers can be used with your system
- Locate the Power ON/OFF switch
- Explain use of the ONLINE/OFFLINE button
- Demonstrate how to load the paper
- Demonstrate how to change the ribbon or ink cartridge

Keyboard

- Direct line of communication between the operator and the peripheral computer containing the DMS software program

Layout

- Alphanumeric keys
  - Configured in a standard typewriter keyboard layout
  - Consists of all standard alphanumeric keys plus a handful of special computer keys and symbols
- Caps Lock
  - Works like the Shift Lock key on a typewriter
  - With Caps Lock on,
    - Pressing any alphabet key on the keyboard produces an uppercase letter
    - Pressing the Shift key plus a letter produces a lowercase letter
    - Only shifts the letter keys, all other keys on the keyboard remain the same
- Numeric keypad
  - Keypad contains the numbers 0 through 9, plus the period, an Enter key, and various mathematical symbols
  - Can expedite manual entry of patient ID numbers and certain quality control entries
- Num Lock
  - Controls operation of the numeric keypad
  - Locate light that illuminates when Num Lock is turned on
  - With Num Lock on, the numeric keypad produces numbers
  - DMS keyboard is designed to be used with Num Lock on
  - With Num Lock off, the numeric keypad on a typical keyboard doubles as a cursor keypad; however, the DMS program is designed for use with the cursor control keys
- Cursor control keys
  - ← ↑ ↓ →
    - Also called arrow keys
    - Directional keys for moving the cursor on the screen
    - Used to highlight menu items, scroll up and down screens, or move to a field on a screen to enter or edit data
  - Page Up and Page Down
    - Commonly used to page through stored sample and control data
  - Home and End
    - Generally used to locate the beginning or the end of stored data
  - Insert and Delete keys are not used with the DMS program
    - When appropriate, a function key is designated as a delete key
- F1 through F12 Function Keys
  - Pressing a Function Key activates the function assigned to that key
  - For example, if a screen displays **F10-Save/Esc** as an option, pressing F10 saves the displayed data and exits the screen display at the same time
- Print Screen key is not used with the DMS program
  - When appropriate, a function key is designated as a print key

- Enter
  - Mainly used to confirm a menu or submenu selection which then activates a change in the screen display
  - Do not use Enter to bring up the DMS display as pressing this key may activate a command
  - Keyboard has two Enter keys
    - Both work identically
    - Enter key was placed by the numeric keypad to facilitate rapid entry of numbers
- Esc
  - Used to back out of an option one screen at a time
  - Use F10 **Save/Esc** instead of Esc to store data you just entered or edited
- Spacebar
  - Used to toggle among options when a choice is required
  - Use the Spacebar to bring up the DMS display when the screen is dark
- Ctrl and Alt
  - Modifier keys that have no action by themselves but can be used in conjunction with other keys to generate special characters or operations not readily available at the keyboard

Hot Keys

- Also called shortcut keys because they allow the operator to do certain functions quickly
- Includes the following:

F1	Displays the Access screen (only available when the Main Menu is displayed)
F4	Prints
F9	Exits the current screen and returns to either the Main Menu or the Access screen depending on point of origination Exception → if the F3 Run window is displayed, then the function of F9 is Stop
F10	Saves and/or returns to the previous screen
Alt + End	Stops instrument beeping and removes the error message at the bottom of the screen
Ctrl + F2	Moves from the current screen to the Error file and back to the original screen
Ctrl + W	Moves from Sample Analysis screen to Worklist and back when a submenu or window is not displayed
Ctrl + F9	Stops the cycle (HmX Hematology Analyzer with Autoloader only)
Ctrl + C	Clears the bed (HmX Hematology Analyzer with Autoloader only)

Screen Conventions

- DMS uses several conventions that either reduce keystrokes or aid the operator in obtaining the proper screen needed to complete a desired task

Menu Item Conventions

- Are displayed in blue text
- Each menu item contains one black letter
  - Pressing the corresponding alphabetic key is one way of selecting that particular menu item
  - Pressing Enter is not necessary

- Main Menu items have designated colors
  - **Sample Analysis** blue
  - **Controls** green
  - **Diluter Functions** magenta
  - **Special Functions** orange

- Designated color coding appears on
  - Submenu borders
  - Screen titles
  - Titles that are part of a bordered display box

Display Screen Conventions

- As a general rule, black text indicates a screen label or a field that cannot be edited; blue text indicates a field that can be edited
- Numeric data stored in a table generally has a turquoise background

Instructional and Informational Message Convention

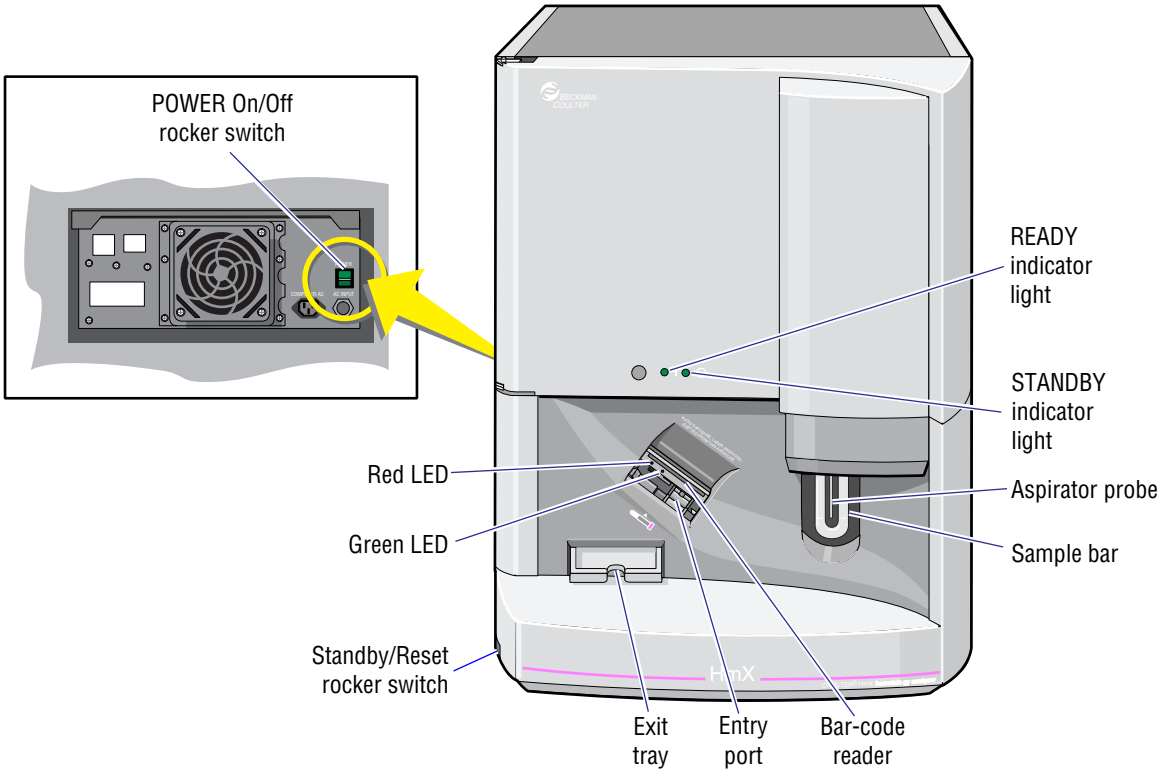
- Most, but not all, instructional or informational messages appear as black text with a black border

To continue HmX Hematology Analyzer training, proceed to page 1-10.

To continue HmX Hematology Analyzer with Autoloader training, go to page 1-18.

MAIN UNIT - HmX Hematology Analyzer

Front View of the Main Unit



IMPORTANT Risk of misleading results. Electrical interference can produce erratic parameter results. Operate this instrument with all the doors closed to minimize external electrical interference.

Controls and Indicators

POWER On/Off Rocker Switch

- Main POWER On/Off switch located on the back of the Main Unit
- Recommend the Main Power be left on at all times
  - Prolongs the life of the lasers
  - Increases stability of electronic components

Standby/Reset Rocker Switch

- Located on the left side of the lower front door, near the bottom
- Used to place instrument in a Ready or Standby state
  - Symbol for indicating the Ready state is "I"
  - Symbol for indicating the Standby state is "O"

- Instrument state is determined by the position of this rocker switch
  - In the Ready state, the back portion of the rocker switch is flush with the instrument's base; this is position I
  - In the Standby state, the front portion of the rocker switch is flush with the instrument's base; this is position O
- Also used to reset the system or reestablish communication between components
  - To reset the system, place the Standby/Reset rocker switch in Standby (position O) for 15 seconds then flip the switch back to Ready (position I)

Ready Indicator Light

- Used to quickly identify instrument status
- When the light is ON, the Main Power is ON and the instrument is ready for operation
- When the Standby (O) **and** Ready (I) indicator lights are OFF (not lighted), Main Power is OFF

Standby Indicator Light

- Used to quickly identify instrument status
- When the light is ON, the Main Power is ON and the instrument is in the Standby state
- To return to the Ready state, the
- Standby/Reset rocker switch must be moved to Ready, position I
- In the Standby state, voltages are still being applied to a memory location in the Analyzer but everything else is powered down
- When the Standby (O) **and** Ready (I) indicator lights are OFF (not lighted), Main Power is OFF

Entry Port

- Port for inserting closed-vial specimen tubes

Exit Tray

- Output tray for specimen tubes processed using the carousel in the Rotary Cap-Pierce module in the Closed-Vial mode

Aspirator Probe

- Used to aspirate from an open vial containing a whole-blood specimen, prediluted specimen, or a retic preparation
- Also used to aspirate from an open bottle of LATRON Primer or Control

Sample Bar

- Press to start aspiration from an open vial

Bar-code Reader

- Scanner for reading the bar-code label on a specimen tube before either closed-vial or open-vial sampling

Green LED

- When scanning a bar-code label, a glowing green LED indicates the barcode was read successfully - the operator can process the specimen

Red LED

- Glowing red LED means wait
- When scanning a bar-code label, a glowing red LED indicates the barcode was not read successfully

MODES OF OPERATION

HmX Hematology Analyzer . . . a Single-Specimen, Rotary Cap-Pierce System

Primary Mode

- The lower door of the Main Unit must remain closed while the instrument is cycling
- Also referred to as the Closed-Vial or Automatic mode
- Carousel rotates the specimen tube from the entry port to the cap-piercing station then when sampling is complete, carousel rotates again to deposit the tube onto the exit tray for removal by the operator
- Aspirates 185 µL of sample from each tube
- Specimen tubes must contain at least 1.0 mL of whole blood (minimum volume with the proper proportion of blood to anticoagulant)
- Tube and tube devices can be used if they fall within the following ranges:
  diameter of 10 to 16 mm / length of 47 to 77 mm / capacity of 2 to 7 mL
  - Beckman Coulter control and calibration tubes can also be used
  - Before inserting a specimen tube through the entry port, it may be necessary to change the size of the carousel tube slot from the 10mm-13mm tube slot to the 16mm tube slot, or vice versa
  - See Appendix A of the Reference Manual for a listing of tested collection devices
- May run specimen tubes with or without bar-code labels

Secondary Mode

- Also referred to as the Open-Vial or Manual mode
- Aspirates 125 µL of sample from each tube via the aspirator probe
- Opening for aspiration is located on the back side of the aspirator probe's tip
- Mode used for processing
  - Microsamples
  - Retics
  - LATRON Primer and Control
  - Any specimen tube that does not fit into the carousel
  - Any specimen tube with insufficient sample volume for processing in the Primary (Closed-Vial) mode of operation (that is, tubes containing <1.0 mL of whole blood)
  - Any samples requiring dilution

Predilute Mode

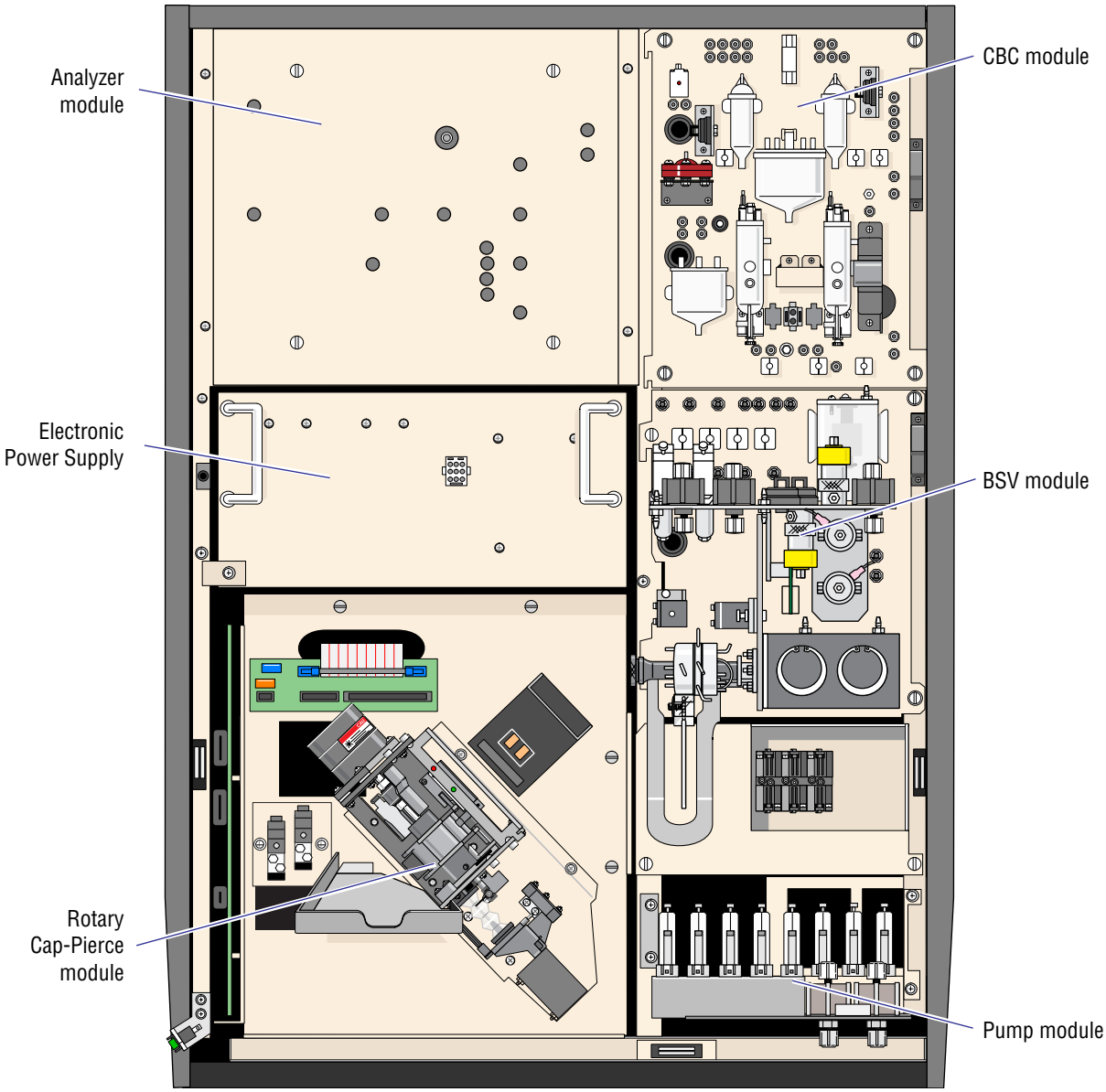
- Aspirator probe also used for running samples in the Predilute mode
- CBC mode only
- Requires specimen to be diluted 1:3 (minimum 50 µL of blood and 100 µL of diluent)
- Aspirates 125 µL of the prediluted sample via the aspirator probe
- Final results are calculated automatically

Retic Mode

- Aspirator probe also used for running samples in the Retic mode
- Sample preparation is required before processing
- Aspirates approximately 2 mL of the sample dilution via the aspirator probe

MAIN UNIT - HmX Hematology Analyzer

Modules on the Front of the Main Unit



INSTRUMENT MODULES

Modules on the Front of the Main Unit

Analyzer Module

- Also referred to as the Analyzer/Systems Control module
- Contains the various circuit boards needed to operate the instrument
- Controls the timing and sequencing of the operating cycles
- Receives pulses and raw data from the CBC and VCS modules
- Counts, measures, and computes CBC parameter results
- Sends final CBC parameter results and VCS data to the DMS

Electronic Power Supply

- Supplies the necessary power for all instrument operations

Rotary Cap-Pierce Module

- Single specimen tube transport and cap-piercing system
- When the specimen tube is inserted through the entry port, the carousel rotates the tube to the cap-piercing station where the needle pierces the tube's stopper so that 185 µL of whole blood can be aspirated from the specimen tube into the instrument for analysis
- When the aspiration is complete, the carousel rotates the specimen tube away from the needle and deposits it onto the exit tray for removal by the operator

CBC Module

- Contains all the components necessary for CBC analysis

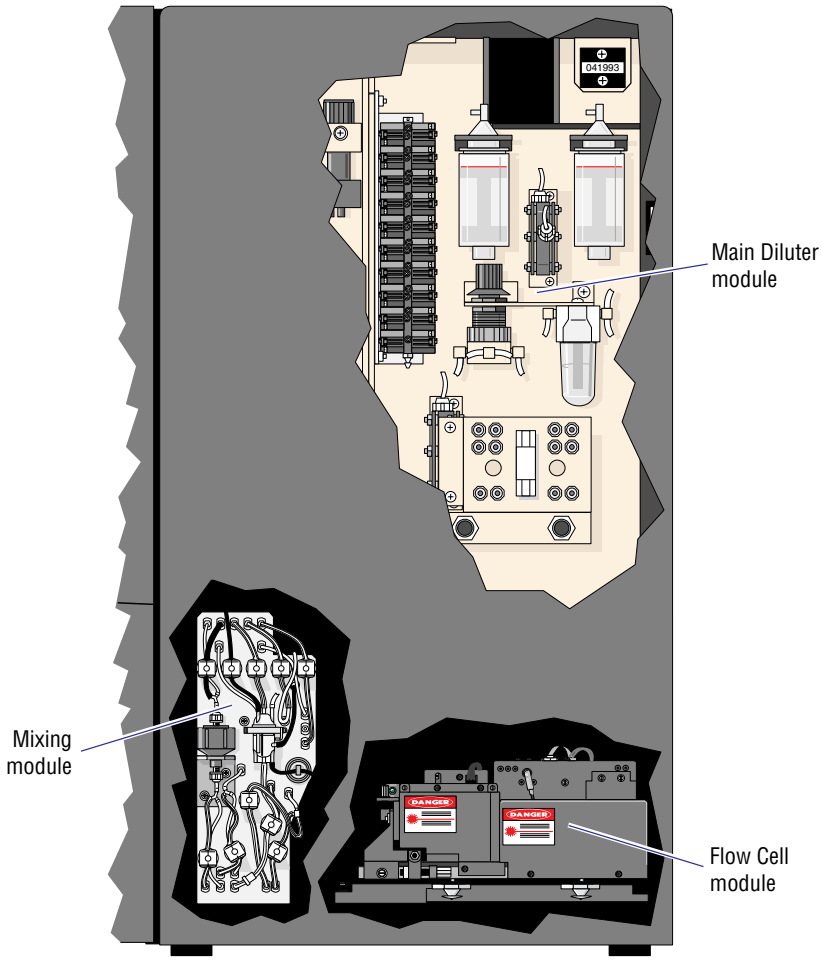
BSV (Blood Sampling Valve) Module

- Contains components associated with the sampling of blood including the aspiration pumps for pulling sample from the specimen tube into the instrument
- Also contains the sheath tank that supplies the diluent needed for proper sample flow for differential analysis using VCS technology

Pump Module

- Contains the two Erythrolyse II reagent pumps needed for differential analysis using VCS technology

Right Side of the Main Unit



Modules on the Right Side of the Main Unit

Main Diluter Module

- Contains various components relating to sample dilution and cycling as well as vacuum and pressure regulators

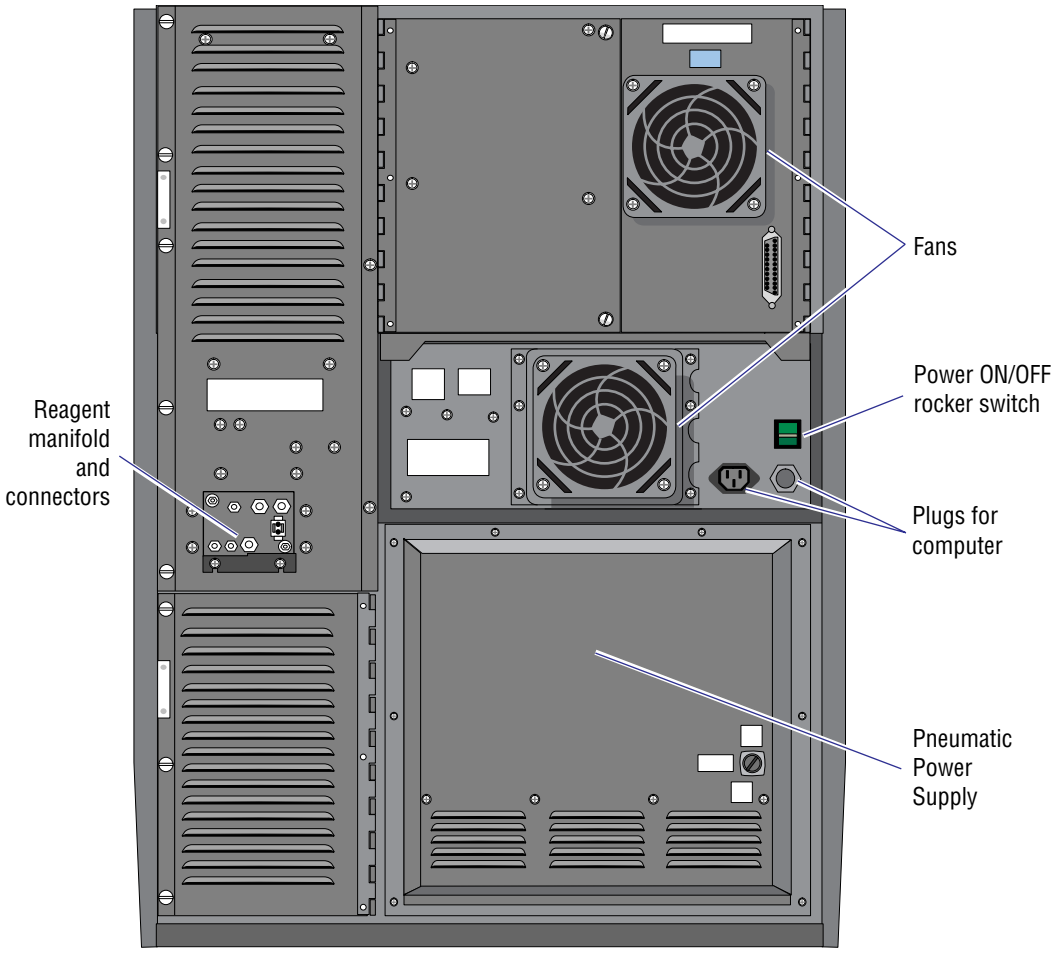
Flow Cell Module

- Contains the components necessary for analyzing the WBC Diff and Retics using VCS technology
- Also referred to as the Triple Transducer Module (TTM)
- Flow cell and laser are inside a protective housing

Mixing Module

- Associated with the VCS portion of the system
- Used to mix the whole blood sample with the reagents needed to prepare the sample for WBC differential analysis using VCS technology

Back View of the Main Unit



Module on the Back of the Main Unit

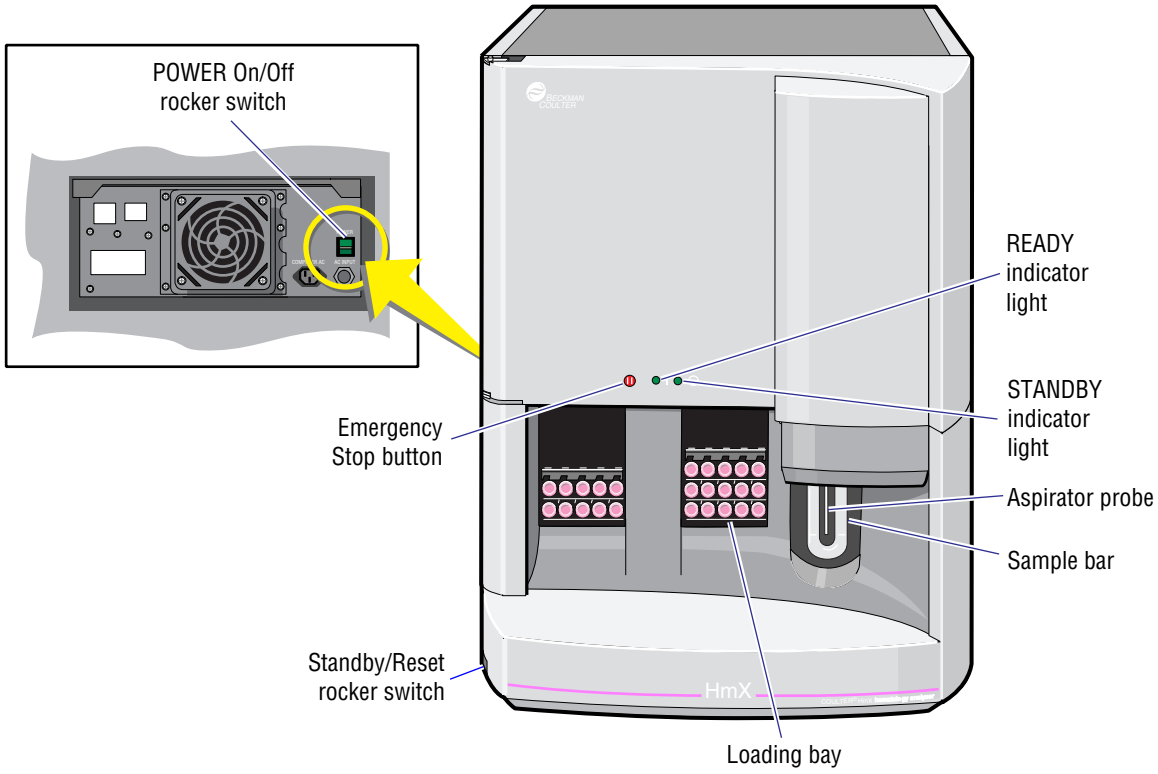
Pneumatic Power Supply

- Source of the vacuums and pressures used throughout the instrument
- The compressor/vacuum pump produces one service adjustable level of air pressure, 60 psi and one nonadjustable level of vacuum, >22" Hg

To continue HmX Hematology Analyzer training, go to page 1-26.

MAIN UNIT - HmX Hematology Analyzer with Autoloader

Front View of the Main Unit



IMPORTANT Risk of misleading results. Electrical interference can produce erratic parameter results. Operate this instrument with all the doors closed to minimize external electrical interference.

Controls and Indicators

POWER On/Off Rocker Switch

- Main POWER On/Off switch located on the back of the Main Unit
- Recommend the Main Power be left on at all times
  - Prolongs the life of the lasers
  - Increases stability of electronic components

Standby / Reset Rocker Switch

- Located on the left side of the lower front door, near the bottom
- Used to place instrument in a Ready or Standby state
  - Symbol for indicating the Ready state is "I"
  - Symbol for indicating the Standby state is "O"
- Instrument state is determined by the position of this rocker switch
  - In the Ready state, the back portion of the rocker switch is flush with the instrument's base; this is position I
  - In the Standby state, the front portion of the rocker switch is flush with the instrument's base; this is position O
- Also used to reset the system or reestablish communication between components
  - To reset the system, place the Standby/Reset rocker switch in Standby (position O) for 15 seconds then flip the switch back to Ready (position I)

Ready Indicator Light

- Used to quickly identify instrument status
- When the light is ON, the Main Power is ON and the instrument is ready for operation
- When the Standby (O) **and** Ready (I) indicator lights are OFF (not lighted), Main Power is OFF

Standby Indicator Light

- Used to quickly identify instrument status
- When the light is ON, the Main Power is ON and the instrument is in the Standby state
- To return to the Ready state, the Standby/Reset rocker switch must be moved to Ready, position I
- In the Standby state, voltages are still being applied to a memory location in the Analyzer but everything else is powered down
- When the Standby (O) **and** Ready (I) indicator lights are OFF (not lighted), Main Power is OFF

Loading Bay

- Cassettes are loaded here for processing in the Primary (Closed-Vial) mode

Aspirator Probe

- Used to aspirate from an open vial containing a whole-blood specimen, prediluted specimen, or a retic preparation
- Also used to aspirate from an open bottle of LATRON Primer or Control

Sample Bar

- Press to start aspiration from an open vial

Emergency Stop Button

- Press this button to immediately stop the autoloader mechanism

MODES OF OPERATION

HmX Hematology Analyzer with Autoloader

Primary Mode

- The lower door of the Main Unit must remain closed while the instrument is cycling
- Also referred to as the Closed-Vial or Automatic mode
- Autoloader uses the rocker bed and sensors to move specimen tube holders called cassettes from the loading bay to a cap-piercing station, then to the unloading bay
- Aspirates 185 µL of sample from each tube
- Specimen tubes must contain at least 1.0 mL of whole blood (minimum volume with the proper proportion of blood to anticoagulant)
- Up to five specimen tubes of various types and sizes can be loaded into a cassette
- Tube and tube devices can be used if they fall within the following ranges:
  diameter of 10 to 16 mm / length of 47 to 77 mm / capacity of 2 to 7 mL
  - Beckman Coulter control and calibration tubes can also be used
  - See Appendix A of the Reference Manual for a listing of tested collection devices
- Proper size cassette must be used
  - Use only 10 to 13 mm o.d. tubes in the universal cassette with gray inserts
  - Use only 16 mm o.d. tubes in the cassette with black inserts
  - When loading tubes into a cassette, make sure each specimen tube fits securely inside the cassette to prevent blood spills that may occur if a tube falls or a cap is pierced off center
- May run specimen tubes with or without bar-code labels
- Stop button is for emergency use only

Secondary Mode

- Also referred to as Open-Vial or Manual mode
- Aspirates 125 µL of sample from each specimen tube via the aspirator probe
- Opening for aspiration is located on the back side of the aspirator probe's tip
- Mode used for processing
  - Microsamples
  - Retics
  - LATRON Primer and Control
  - Any specimen tube that does not fit into the cassette
  - Any specimen tube with insufficient sample volume for processing in the Primary (Closed-Vial) mode of operation (that is, tubes containing <1.0 mL of whole blood)
  - Any samples requiring dilution

Predilute Mode

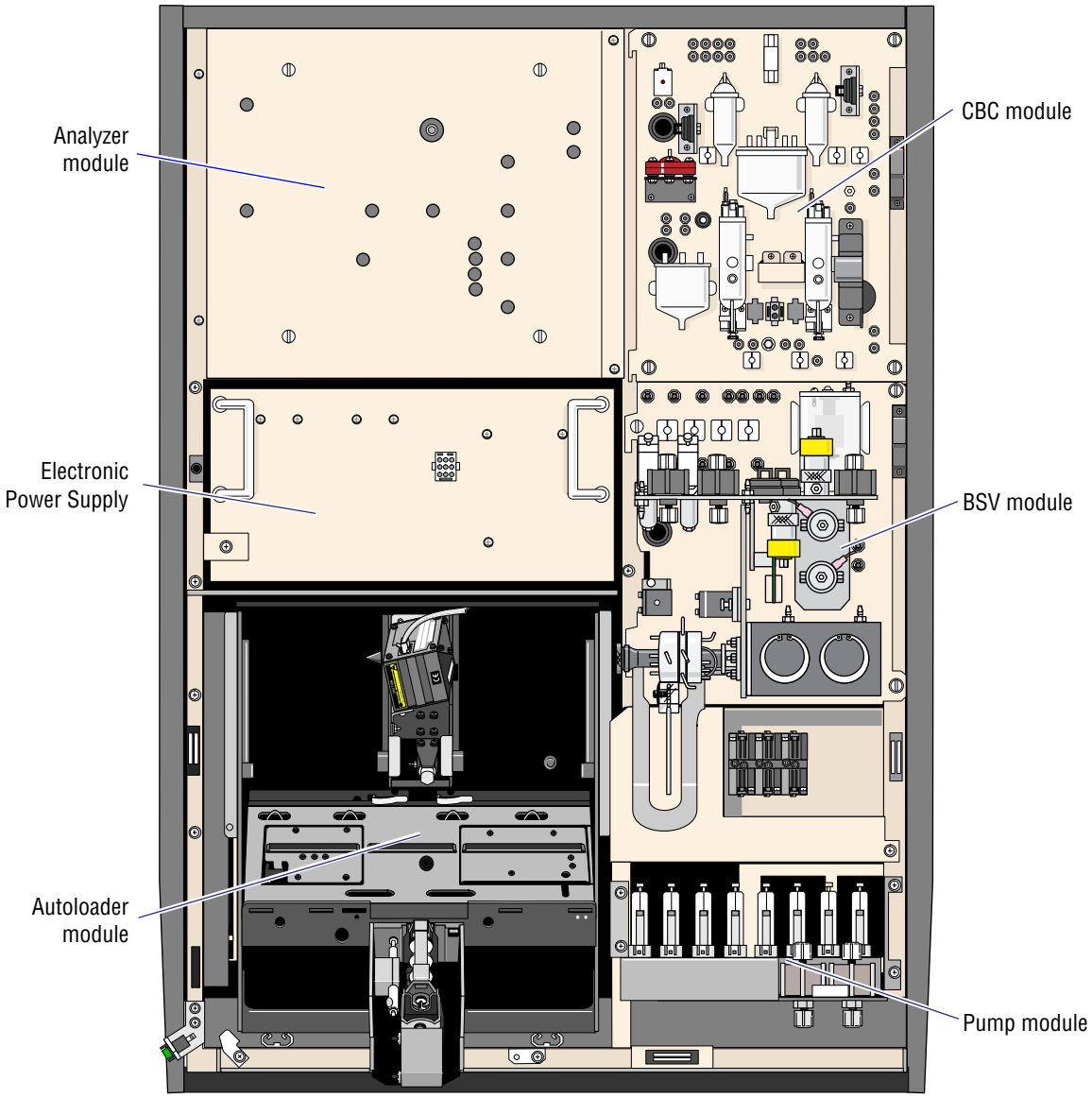
- Aspirator probe also used for running samples in the Predilute mode
- CBC mode only
- Requires specimen to be diluted 1:3 (minimum 50 µL of blood and 100 µL of diluent)
- Aspirates 125 µL of the prediluted sample via the aspirator probe
- Final results are calculated automatically

Retic Mode

- Aspirator probe also used for running samples in the Retic mode
- Sample preparation is required before processing

MAIN UNIT - HmX Hematology Analyzer with Autoloader

Modules on the Front of the Main Unit



INSTRUMENT MODULES

Modules on the Front of the Main Unit

Analyzer Module

- Also referred to as the Analyzer/Systems Control module
- Contains the various circuit boards needed to operate the instrument
- Controls the timing and sequencing of the operating cycles
- Receives pulses and raw data from both the CBC and VCS modules
- Counts, measures, and computes CBC parameter results
- Sends final CBC parameter results and VCS data to the DMS

Electronic Power Supply

- Supplies the necessary power for all instrument operations

Autoloader Module

- Cassette transport and cap-piercing system
- Contains the robotic mechanisms to move the specimen tube holders called cassettes from the loading bay to the cap-piercing station and then to the unloading bay
- Up to five specimen tubes can be loaded into a cassette which is then placed in the loading bay
- Once operation is initiated, the bottom cassette is lowered onto the rocker bed where the cassette is rocked back and forth while it moves along the bed to the cap-pierce station
- Each time a tube reaches the cap-pierce station, the needle pierces its stopper so that 185 µL of whole blood can be aspirated from the specimen tube into the instrument for analysis
- When all the specimen tubes are pierced, the rocker bed moves the cassette to the unloading bay for removal by the operator

CBC Module

- Contains all the components necessary for CBC analysis

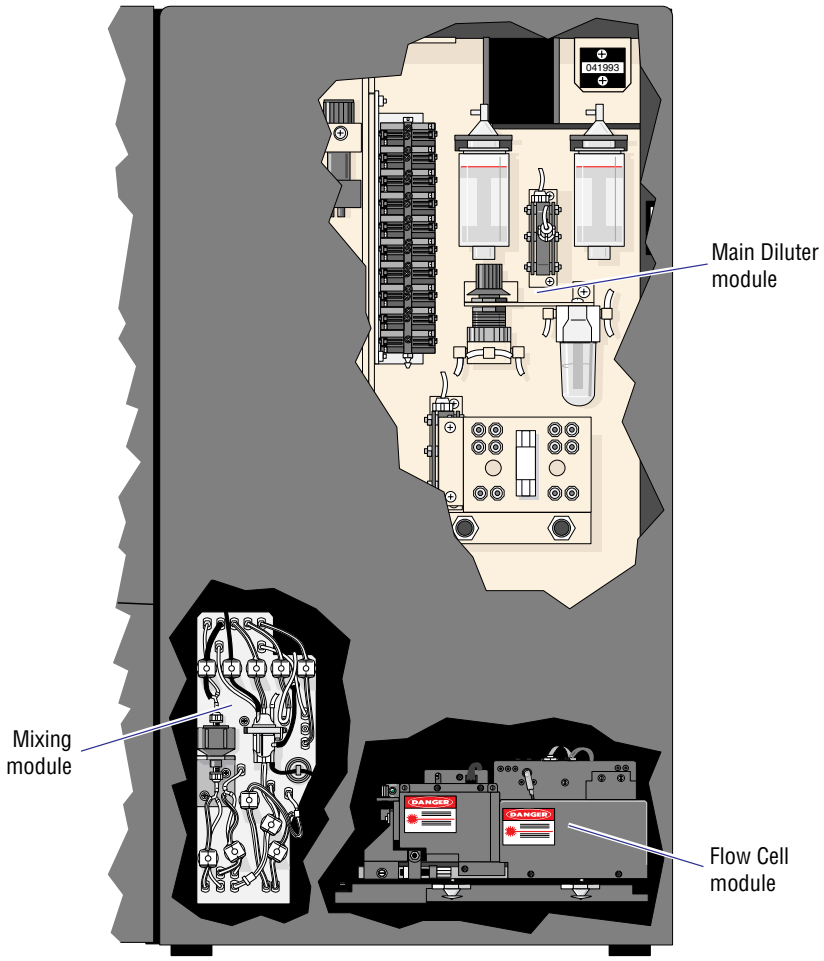
BSV (Blood Sampling Valve) Module

- Contains components associated with the sampling of blood including the aspiration pumps for pulling sample from the specimen tube into the instrument
- Also contains the sheath tank that supplies the diluent needed for proper sample flow for differential analysis using VCS technology

Pump Module

- Contains the two Erythrolyse II reagent pumps needed for differential analysis using VCS technology

Right Side of the Main Unit



Modules on the Right Side of the Main Unit

Main Diluter Module

- Contains various components relating to sample dilution and cycling as well as vacuum and pressure regulators

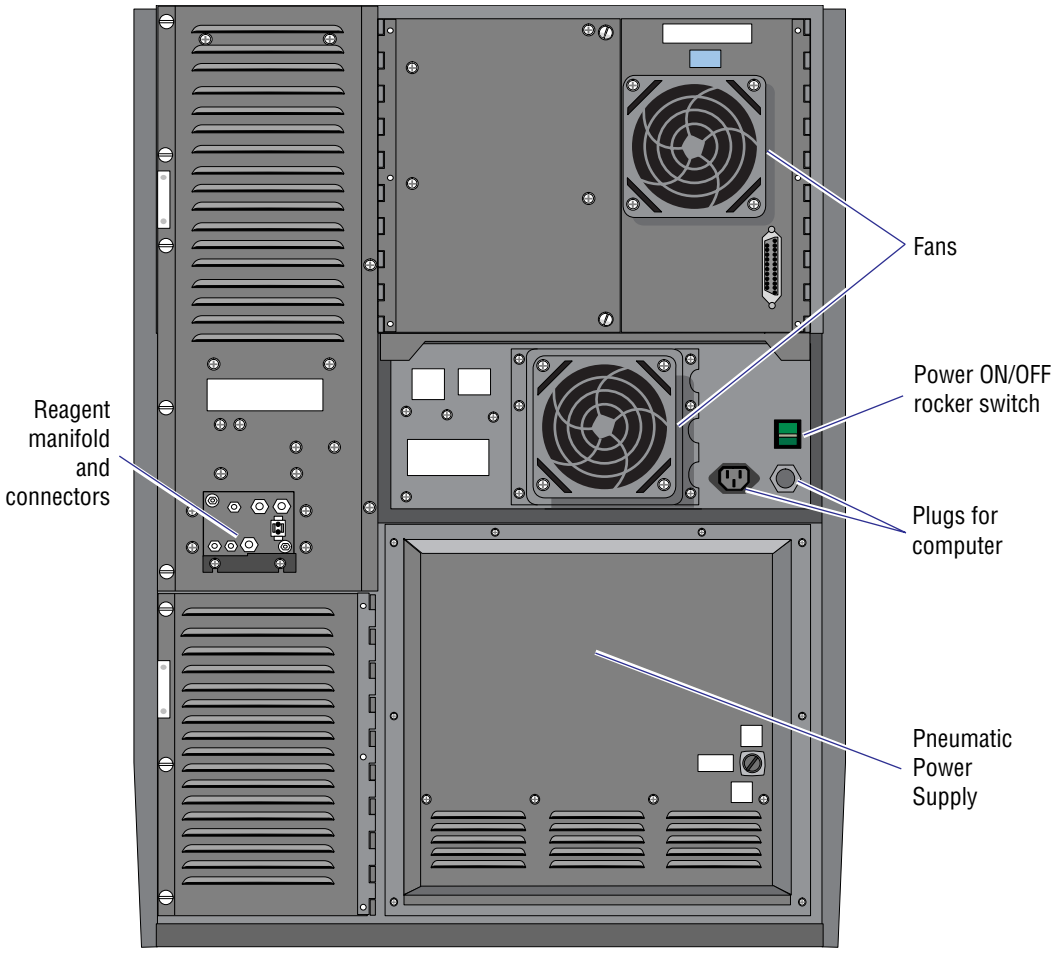
Flow Cell Module

- Contains the components necessary for analyzing the WBC Diff and Retics using VCS technology
- Also referred to as the Triple Transducer Module (TTM)
- Flow cell and laser are inside a protective housing

Mixing Module

- Associated with the VCS portion of the system
- Used to mix the whole blood sample with the reagents needed to prepare the sample for WBC differential analysis using VCS technology

Back View of the Main Unit



Module on the Back of the Main Unit

Pneumatic Power Supply

- Source of the vacuums and pressures used throughout the instrument
- The compressor/vacuum pump produces one service adjustable level of air pressure, 60 psi and one nonadjustable level of vacuum, >22" Hg

REAGENTS

Reagents Connected to the Main Unit

Reagent	Recommended	Function	CBC	DIFF	Retic	Open Container Stability
Diluent	ISOTON III diluent	• Isotonic solution that dilutes the sample • Stabilizes cell membranes • Conducts aperture current • Carries and focuses the VCS sample stream • Rinses the system between samples	✓	✓	✓	Same as shelf life (date printed on the container)
CBC Lytic Reagent	LYSE S III diff lytic reagent	• Disrupts erythrocytes • Frees hemoglobin • Reduces cellular debris • Converts hemoglobin to a stable cyanide-containing pigment	✓			60 Days
Diff Lytic Reagent	Erythrolyse II erythrocyte lytic reagent (also called PAK LYSE) One of the HmX PAK reagents	• Dilutes the sample for the diff • Lyses red blood cells • Reduces cellular debris		✓		60 Days
Diff Leukocyte Preservative	StabiLyse leukocyte preservative (also called PAK PRESERVE) One of the HmX PAK reagents	• Preserves white blood cells in their near-native state for differentiation using VCS technology		✓		60 Days
Cleaning Agent	COULTER CLENZ cleaning agent	• Cleans and rinses the diluter parts • Prevents protein buildup • Eliminates routine aperture bleaching	✓	✓	✓	90 Days

Special Reagents Used in Sample Preparation for Retic Analysis

Reagent	Recommended	Function	CBC	DIFF	Retic	Open Container Stability
ReticPrep Reagent A	New Methylene Blue dye, special formulation	• Stains any reticulum present in the RBCs			✓	60 Days
ReticPrep Reagent B	Clearing reagent (very dilute sulfuric acid)	• Removes hemoglobin from the cells without removing the precipitated stain-RNA complex • Keeps the cell membrane intact			✓	60 Days

Storage and Handling

- All reagents should be stored and used at the laboratory's ambient room temperature
- Active components may separate (layer) if the reagent has been frozen during transport or storage → inconsistency of components affects parameter results
- To ensure reagent integrity, treat each reagent container as if it were frozen sometime during transport or delivery to your institution
- Before using a reagent
  - Make sure the reagent is completely thawed
  - Gently mix by inversion
  - Allow sufficient time for any microbubbles to dissipate

    Note: Microbubbles may increase the background count and affect parameter results.

➤ Changing Reagents Summary

1 Record the New Reagent Information

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ System Set Up ▸▸ Reagents**
- Enter the lot number and the expiration date or open container stability date, as applicable
  - The date opened automatically changes to today's date when the lot number is entered (this is the reason the lot number should always be typed even if it is the same as the last container)
  - Six digits must be entered for the expiration date or open container stability date

    Note: Open container stability is stated on the reagent container, when applicable.
- Press F10 to save the information then F9 to return to the Main Menu

2 Replace the Reagent Container

- Turn the compressor off

  From the Main Menu, select **Special Functions ▸▸ Diagnostics ▸▸ Operator Options ▸▸ Fluidics Tests ▸▸ Compressor On/Off** then press Enter; press the Spacebar to select **Off** and press Enter again
- Open the new reagent container
- Remove the pickup tube assembly from the old container then transfer it directly to the new container and tighten

  Note: If the lower part of the assembly touches you or anything outside the container, flood that lower part of the assembly with distilled water then wipe it with a lint-free tissue.

3 Prime the Lines

- From the Main Menu, select **Diluter Functions ▸▸ Prime Reagents**
- Select the reagent that was changed then press Enter, the compressor turns on and the Prime Reagents program activates
- Once priming is complete, press F9 to return to the Main Menu and continue normal operation

➤➤

➤ Replacing a Waste Container Summary

1 Put the Instrument into Standby

- Move the Standby/Reset rocker switch to Standby, position O

2 Turn OFF the Main Power

- Use the Main POWER On/Off rocker switch located on the back of the Main Unit

3 Replace the Waste Container

- Using biohazardous precautions,
  - Remove the waste assembly from the full container
  - Transfer the waste assembly directly to an empty waste container and tighten
- Dispose of the biohazardous waste according to you laboratory's protocol

4 Turn ON the Main Power

- Use the Main POWER On/Off rocker switch located on the back of the Main Unit

5 Put the Instrument into Ready

- Move the Standby/Reset rocker switch to Ready, position I

➤➤

OBJECTIVES

When the subject is complete, you will be able to . . .

Startup

- Initiate a Startup.
- Recognize an out-of-limit result on the Startup Summary or System Status Values screen.
- Explain what to do if a Startup result is out of limits.

Clean Cycle

- Initiate a Clean Cycle.
- State the difference between a Clean Cycle and a Shutdown.

Shutdown

- Initiate a Shutdown.
- State how often a Shutdown must be performed.
- State the minimum time the COULTER CLENZ cleaning agent should stay in the instrument while in Shutdown.

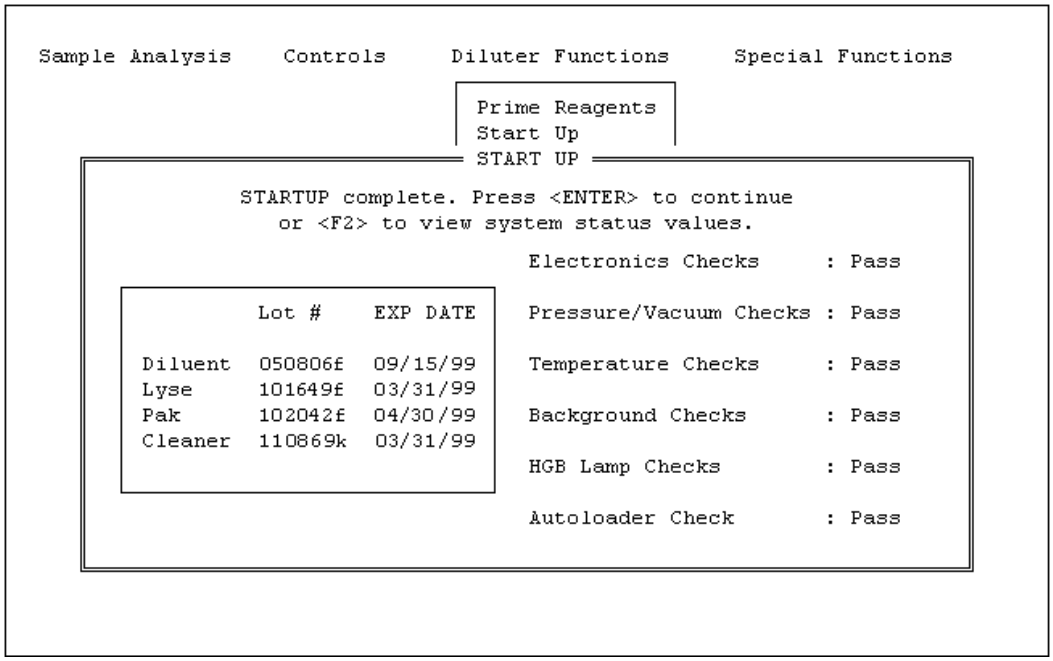
NOTES

STARTUP

- Must be completed before running patient specimens or controls anytime cleaning agent has replaced diluent in the aperture baths
- During a Clean Cycle, Startup is automatically initiated 30 minutes after completion of the Shutdown cycle
- Perform quality control checks before running patient specimens

STARTUP SCREENS

Summary Screen



- Also referred to as the First Start Up screen because it is the first display that automatically appears and prints
- Shows a reagent summary on the left and a system check summary on the right
- *Autoloader Check* prompt only appears on the Summary screen of a HmX Hematology Analyzer with Autoloader

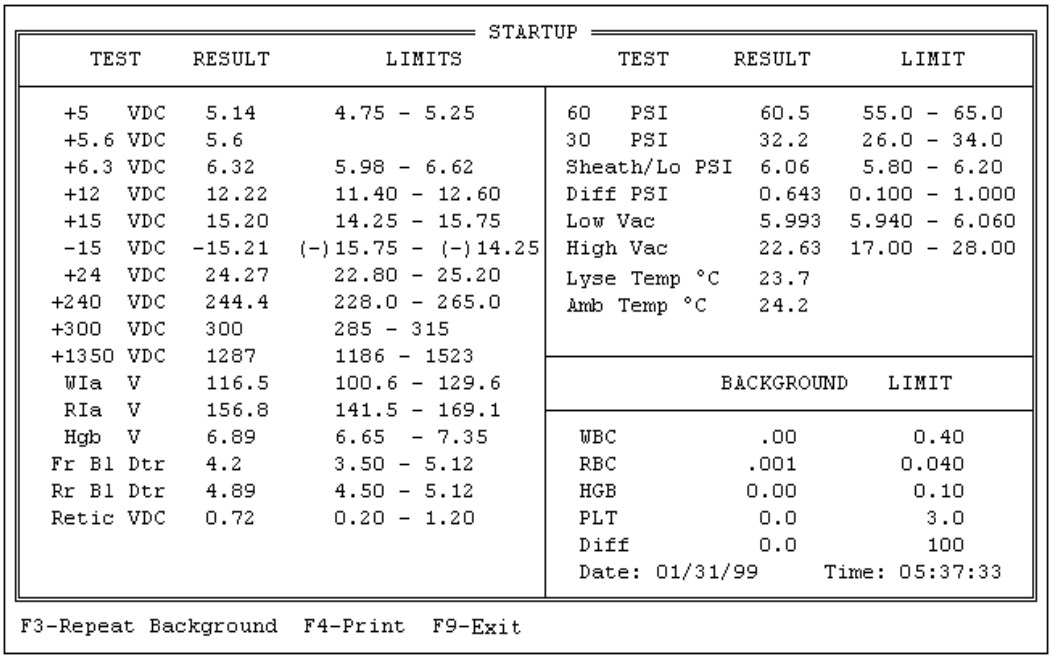
Reagent Summary

- The reagent summary lists each reagent lot number and expiration date (may be the open container expiration date, if applicable)
- If a date appears in red numbers, the expiration date is exceeded

System Check Summary

- Shows results on a Pass/Fail basis
- A check that fails appears in red

System Status Values Screen



STARTUP

TEST	RESULT	LIMITS
+5 VDC	5.14	4.75 - 5.25
+5.6 VDC	5.6	
+6.3 VDC	6.32	5.98 - 6.62
+12 VDC	12.22	11.40 - 12.60
+15 VDC	15.20	14.25 - 15.75
-15 VDC	-15.21	(-)15.75 - (-)14.25
+24 VDC	24.27	22.80 - 25.20
+240 VDC	244.4	228.0 - 265.0
+300 VDC	300	285 - 315
+1350 VDC	1287	1186 - 1523
WIa V	116.5	100.6 - 129.6
RIa V	156.8	141.5 - 169.1
Hgb V	6.89	6.65 - 7.35
Fr Bl Dtr	4.2	3.50 - 5.12
Rr Bl Dtr	4.89	4.50 - 5.12
Retic VDC	0.72	0.20 - 1.20

TEST	RESULT	LIMIT
60 PSI	60.5	55.0 - 65.0
30 PSI	32.2	26.0 - 34.0
Sheath/Lo PSI	6.06	5.80 - 6.20
Diff PSI	0.643	0.100 - 1.000
Low Vac	5.993	5.940 - 6.060
High Vac	22.63	17.00 - 28.00
Lyse Temp °C	23.7	
Amb Temp °C	24.2	

BACKGROUND		LIMIT
WBC	.00	0.40
RBC	.001	0.040
HGB	0.00	0.10
PLT	0.0	3.0
Diff	0.0	100

Date: 01/31/99 Time: 05:37:33

F3-Repeat Background F4-Print F9-Exit

- Also called the Second Start Up screen
- Allows an operator to review the detailed system check results
- Can only be viewed by pressing F2 when the Summary screen is displayed

Background Values

- If any Background result is outside of its limits,
  - Result is displayed in red
  - Press F3 **Repeat Background** to redo the background counts
  - If the condition still exists, refer to the Special Procedures and Troubleshooting (SPT) Manual

System Values

- If any of the electronic readings, pressures, vacuums, or temperature readings are outside of limits,
  - Value is displayed in red
  - Perform a **System Test** to confirm

    Special Functions ▸▸ Diagnostics ▸▸ Operator Options ▸▸ System Test
    Press Enter then F3 **Run** to initiate the test
  - If the condition still exists, refer to the Special Procedures and Troubleshooting (SPT) Manual

➤ STARTUP SUMMARY

1 Check Current Instrument Status

- If the power is off, turn it on
- If the Main unit is in Standby, switch it to Ready
- If the DMS monitor is dark, press Spacebar
- If Startup results are already displayed as the result of a Clean Cycle, go to step 3; if not, enter your three character Operator ID (optional)
  - From the Main Menu, press F5 **Options**
  - Type your three character Operator ID
  - Press Esc to return to the Main Menu

2 Perform Start Up

- From the Main Menu, select **Diluter Functions ▸▸ Start Up**
- Press Enter

3 Review Results

- Once the startup cycles are complete, review the Summary screen and, if necessary, the System Status Values screen
  - If the Background Check fails, press F2 to view the System Status Values screen and then press F3 **Repeat Background** to redo the background counts
  - If Fail appears for any other check, press F2 to view the System Status Values screen then perform a **System Test**

    Special Functions ▸▸ Diagnostics ▸▸ Operator Options ▸▸ System Test
    Press Enter then F3 **Run** to initiate the test then follow the prompts
- Startup results are not saved, but do print automatically
- Save the Summary and System Status Values screen printouts for documentation purposes

4 Perform Quality Control Checks

- Perform quality control checks before running patient specimens

➤ ➤

NOTES

SHUTDOWN

- Allowing cleaning agent to remain in the instrument a minimum of 30 minutes minimizes protein buildup in the instrument
- Perform either a Clean Cycle or a Shutdown once every 24 hours that the instrument is in use

Clean Cycle

- Consists of a Shutdown cycle followed 30 minutes later by a Startup cycle
- Perform quality control checks before running patient specimens

Shut Down

- When the Shutdown cycle is complete, leave the power ON → every 24 hours, the system does an autopurge cycle to purge the flow cell and the sample lines with diluent

  Note: If you turn the power OFF and the instrument is going to be idle for more than 48 hours, do the Prolonged Shutdown Procedure in the Operator's Guide, Chapter 5.
- Startup must be completed before running patient specimens or controls

➤ CLEAN CYCLE SUMMARY

1 Perform the Clean Cycle

- From the Access screen, press F3 **CLEAN** then press Enter

2 Let the Instrument Stand

- Cleaning agent remains in the instrument
- Leave instrument power on
- After 30 minutes, a Startup automatically begins

3 Review Results

- Once the startup cycles are complete, review the Summary screen and, if necessary, the System Status Values screen
  - If the Background Check fails, press F2 to view the System Status Values screen and then press F3 **Repeat Background** to redo the background counts
  - If Fail appears for any other check, press F2 to view the System Status Values screen then perform a **System Test**

    Special Functions ▸▸ Diagnostics ▸▸ Operator Options ▸▸ System Test
    Press Enter then F3 **Run** to initiate the test then follow the prompts
- Startup results are not saved, but do print automatically
- Save the Summary and System Status Values screen printouts for documentation purposes

4 Perform Quality Control Checks

- Perform quality control checks before running patient specimens

➤ ➤

➤ SHUTDOWN SUMMARY

1 Perform Shut Down

- Verify *SELECT FUNCTION* is displayed on the status line
- From the Main Menu, select **Diluter Functions ▸▸ Shut Down**
- Press Enter

2 Let the Instrument Stand

- Allow cleaning agent to remain in the instrument a minimum of 30 minutes
- Leave instrument power on

  Note: If the power is turned off and the instrument is going to be idle for more than 48 hours, do the Prolonged Shutdown Procedure in the Operator's Guide, Chapter 5.
- Perform the Startup procedures before running patient specimens or controls

➤ ➤

OBJECTIVES

When the subject is complete, you will be able to . . .

- Set up control files using the **Control set up** option.
- Enter data under the **Sample analysis set up** option to include flagging limits, display formats, and print options.
- Enter data under the **System set up** option including the communications definition if a host computer is in use.

NOTES

SET UP MENU OPTIONS

- From the Main Menu, select **Special Functions ▸▸ Set Up** to access this menu
- **Set Up** submenu consists of three options
  - **Control set up**
  - **Sample analysis set up**
  - **System set up**
- All the options available in the Set Up area of the DMS software are listed here
  - Each menu item is listed with a short description
  - For details, see Chapter 6 of the Operator's Guide

CONTROL SET UP

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up**
- For additional details, see Chapter 6 of the Operator's Guide
- Used to set up any of the 20 control files
- Sets up a variety of control files
  - CBC/DIFF file
  - Latex file
  - CBC file
  - RETIC file
- Also used to enable or disable the Auto-Stop function
  - Although available on both HmX instruments, the Auto-Stop function is more typically used with a HmX Hematology Analyzer with Autoloader
  - **Auto-Stop** option *ON*

    Instrument stops and a beeping alarm sounds when one of the following control error messages occur:

    Control Out of Range

    Control Expired

    File Full

    File not Found (CBC/DIFF only)

    Disk Drive C: Full
  - **Auto-Stop** option *OFF*

    Instrument continues analysis when a control error occurs

Setting Up Your Laboratory's Control Files

- Use the instructions under the Initial Set Up of Control Files Summary heading to set up your laboratory's control files

➤ INITIAL SET UP OF CONTROL FILES SUMMARY

Any Control File can be set up as a:

- LATRON control file (Latex file)
- 5C cell control file (CBC/DIFF file)
- Retic-C cell control file (RETIC file)

Setting Up a LATRON Control File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ Latex file**
- Move the cursor to a **NOT SETUP** file then press Enter

2 Type Manual Entries

- Enter the name of the file and your Operator ID
- Enter the Lot # and expiration date from the package insert

3 Verify Host Setting

- Make sure the Host transmit setting correlates with your laboratory's protocol
- To change, use the Spacebar to toggle between *ON* and *OFF*

4 Verify All Information

- Check all entries are correct then press F10 to save and exit the screen

Setting Up a 5C Cell Control File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ CBC/DIFF file**
- Move the cursor to a **NOT SETUP** file then press Enter

2 Upload the Assay Values

- Insert the 5C cell control diskette into the A: drive of the computer
- Press F5 **Upload Assay Values**
- Press the function key for the desired level of control

3 Type Manual Entries

- Select the Shift and manually type the Operator ID

4 Verify Host Setting

- Make sure the Host transmit setting correlates with your laboratory's protocol
- To change, use the Spacebar to toggle between *ON* and *OFF*

5 Verify All Information

- Check all entries are correct then press F10 to save and exit the screen

6 Set Up Another Level of Control, if applicable

- Move the cursor to a **NOT SETUP** file then press Enter
- Repeat steps 2 through 6

7 Remove the Diskette from Drive A: (after all needed set ups are complete)

Setting Up a RETIC File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ RETIC file**
- Move the cursor to a **NOT SETUP** file then press Enter

2 Type Entries from the Table of Expected Results

- Enter the Lot #, Expiration Date, Shift, and your Operator ID
- Enter the RBC and Retic% values from the assay sheet

  Note: If you report Retic number (RET #), it is important that you enter the correct RBC value from the assay sheet. If you enter the wrong number and then correct it after running controls, the DMS does not recalculate the incorrect RET # control results that are stored in the RETIC file.
- If your instrument has the MRV and IRF parameters enabled, enter 99.9 as the assigned values and expected ranges for these parameters. Use these values until you establish your own.

3 Verify Host Setting

- Make sure the Host transmit setting correlates with your laboratory's protocol
- To change, use the Spacebar to toggle between *ON* and *OFF*

4 Verify All Information

- Check all entries are correct then press F10 to save and exit the screen

➤➤

Note: This summary is only for initial set up of control files; therefore, it is not included in the Summary Pages. The next time you set up a control file, use the Control File Management Using the Default Limits/Expected Ranges from the Package Insert Summary unless you wish to maintain customized expected ranges in a 5C control file. In this case, use the Control File Management Using Your Laboratory's Custom Ranges Summary.

SAMPLE ANALYSIS SET UP

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Sample analysis set up**
- For additional details, see Chapter 6 of the Operator's Guide

Action limits

XB limits

- Sets Target Values and Limits, user defined

Definitive flag limits

- These word messages are generated based on user defined limits

High/low flag limits

Note: MRV and IRF parameters appear on this screen when these parameters are enabled.

- These flags are generated based on user defined limits

Laboratory Normal Ranges

Note: MRV and IRF parameters appear on this screen when these parameters are enabled.

- These limits are the default normal ranges for the HmX instruments
- The laboratory's normal ranges should be entered here for printing on the report

Location list

- Creates a list of common locations
- Operator may then choose from this list when assigning demographics in the Worklist or when editing in the Data Base

Physician list

- Creates a list of common physicians
- Operator may then choose from this list when assigning demographics in the Worklist or when editing in the Data Base

Display formats

Screen Labels

Note: MRV and IRF parameters appear on this screen when these parameters are enabled.

- Makes Primary Identifier selections and allows the operator to change the name of the label identifiers

Parameter Selection

- Enables or disables the Pct, PDW, and RET#
- Enables or disables the MRV and IRF

Reporting Units

Note: MRV and IRF parameters appear on this screen when these parameters are enabled.

- Selects the appropriate reporting units for the laboratory

Delete database

- Deletes all samples in the data base and resets the system

Delete host spooler

- Clears the buffer of results waiting to be transmitted to the host computer

Clear printer spooler queue

- Clears the buffer of results waiting to be transmitted to the printer

Print options

Auto Print Format

- Selects ticket or graphics format

Ticket Options

- Selects the options available for the ticket format (if applicable)
- These options include selecting the units (% or $10^3/\mu L$), normal ranges, parameter labels (WBC or RBC) or if Suspect and Definitive flags will print

Spooler Priority

- Sets which will print first (Manual, Auto, or Batch)

Graphics Options

- Selects the options available for the graphics format
- These options include the page format (width/font), which scatterplots will print, and determines if demographics, suspect and definitive flags, units, normal ranges and the diff box will print

Optional Printer

- Sets the options for a second printer

SYSTEM SET UP

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ System set up**
- For additional details, see Chapter 6 of the Operator's Guide

Shift

- Used to separate the control files into different shifts

Reagents

- Updates the reagent log

Institution

- Sets up the banner for the top of each printout

Communication def

- Host computer definition
- Configures the communication format for transfer of sample information to a host computer

IQAP ID#

- After entering the lab's participant number in this field, it will attach to the control files for IQAP reference

Set Date/Time

- Allows the operator to adjust the current date and/or time (for example, daylight savings time)

Supervisor Password

- Allows the operator to change the supervisor password

Optimize Hard Disk

- Sets the option to initiate the clean up of the DMS hard drive upon power up or resetting the unit
- See the Special Procedures and Troubleshooting (SPT) Manual for more information

OBJECTIVES

When the subject is complete, you will be able to . . .

LATRON Primer and Control

- Explain the storage and handling requirements.
- State the open-vial stability.
- Prepare and run LATRON primer and control.
- Recognize an out-of-limits result on the LATRON screen.
- Using the Operator's Guide, explain what to do if a LATRON primer or control result is out-of-limits.
- Explain the statistics associated with the control files and graphs.

5C Cell Control

- Explain the storage and handling requirements.
- State the open-vial stability.
- Define an event.
- State indications of instability or deterioration.
- Prepare and run 5C cell control.
- Recognize an out-of-limit result on the 5C control screen.
- Using the Operator's Guide, explain what to do if a 5C cell control result is out-of-limits.
- Explain the statistics associated with the control files and graphs.

Retic-C Cell Control

- Explain the storage and handling requirements.
- State the open-vial stability.
- Define an event.
- State indications of instability or deterioration.
- Prepare and run Retic-C cell control.
- Recognize an out-of-limit result on the Retic-C control screen.
- Using the Operator's Guide, explain what to do if a Retic-C control result is out-of-limits.
- Explain the statistics associated with the control files and graphs.

Interlaboratory Quality Assurance Program (IQAP)

- Explain the purpose of the Interlaboratory Quality Assurance Program (IQAP).

Control File Management

- Review numeric and graphic control data for errors and make appropriate corrections.
- Print and/or download control file data.
- Delete individual control runs.
- Delete a control file.
- Properly set up control files for running LATRON, 5C Cell Control, and/or Retic-C Cell Control.
- Explain how to make changes to an existing control file.

Additional QC Checks

- Explain the purpose of Mode-to-Mode Comparison.
- Explain the purpose of a linearity study.
- Explain the purpose of a diff comparison.

$\overline{X}_B$ Analysis

- Explain the purpose of $\overline{X}_B$ Analysis.
- Explain why only RBC indices are used in the $\overline{X}_B$ Analysis.
- Define the terms batch, target values, action limits, non-reportable results, and non-random sampling as they apply to the $\overline{X}_B$ Analysis.
- State the minimum number of samples for a valid $\overline{X}_B$ batch.
- Explain when an $\overline{X}_B$ batch mean table prints automatically.
- Describe the three areas of the $\overline{X}_B$ graph.
- Identify the problem parameter (or parameters) causing an $\overline{X}_B$ batch to be out-of-control.

LATRON PRIMER AND CONTROL

- Daily quality control check to verify performance of the VCS technology TTM components in both the DIFF and Retic modes
- Must be at room temperature prior to use
  - May be stored in the refrigerator but not necessary
  - If stored in the refrigerator product must be brought to room temperature before running
- Instrument aspirates approximately 1.5 mL

LATRON Primer

- Contains a surfactant to remove bubbles and clean the sample tubing and pathway from the tip of the aspirate probe to and through the flow cell (located inside the TTM)
- Does not need to be mixed
  - Vigorous mixing creates microbubbles that may cause increased counts
- Results are automatically saved in the Latex control file
- Can manually print the results if desired

LATRON Control

- Contains specific size latex particles with a predetermined Volume, Conductivity and Laser Light Scatter (measurements determined using VCS technology)
- Control is used to verify
  - Flow cell alignment
  - Gains for flow cell volume, conductivity and laser light scatter
  - CV for flow cell volume, conductivity and laser light scatter
- Latex particles settle to the bottom of the vial
  - Operator must gently invert the vial to resuspend particles
  - Vigorous mixing creates microbubbles that may affect results
- Control must not be exposed to direct sunlight or temperatures above 30°C (86°F); keep the vials away from windows and heaters
- Results are automatically saved in the Latex control file
- Can manually print the results if desired

Reviewing LATRON Control Results

On the Control Run Screen

- Mean Channel result for Volume, Conductivity, and Scatter
  - An **H** or **L** is displayed when a result is outside the limits (flag does not appear in red as its does on the Startup screen)
- CV results for Volume, Conductivity, and Scatter
  - An **H** is displayed when a result is above the upper limit

Using F6 Graph . . . a Control Run Screen Option

- Semi-quantitative graphs
- Mean channel and %CV graphs of the last 10 runs
- Press F6 as many times as necessary to scroll to the graphs you want to view

Using Review or Report, Latex . . . a Control Menu Option

- Comprehensive display of all runs, up to 100 runs per file
- Allows operator to view the Mean Channel and CV data
  - An **H** or **L** is displayed when a Mean Channel result is outside the limits
  - An **H** is displayed when a CV percentage result is above the upper limit
- Use the → and ← keys to move between DIFF and Retic data
- Use the **F6-Remove/Restore** option as a data management tool

Using Graphs . . . another Control Menu Option

- Levey-Jennings graphs
- Plot point for each result, up to 100 runs per file
- Ideal for spotting trends and shifts
- Use F6 **Additional Graphs** to move between DIFF and Retic graphs

➤ Running LATRON Primer and Control Summary

1 Status for Proceeding

- Ensure Startup is complete
- LATRON Primer and Control must be at room temperature

2 Run LATRON Primer

At the DMS

- From the Main Menu, select **Controls ▸▸ Control Run**
- If the LATRON file does not appear, press F2 **File** and select your current Latex file
- Press F3 **Run** then F4 **PRIMER**

At the Diluter

- Cycle LATRON Primer in the Secondary mode
  - Immerse the aspirator tip completely in the Primer
  - Do not place the tip against the side of the bottle
  - Press and release the sample bar
  - Listen for the beep or verify the status line message changes from *Aspirating* to *Diluting* **before** removing the bottle

Reviewing Primer Results at the DMS

- Check the count for both DIFF and Retic when the analysis is complete
- If both counts are ≤500,
  - Press Esc to remove the Primer Run window
  - Go to step 3 and run LATRON Control
- If either or both counts are ≥500,
  - Rerun the LATRON Primer
  - Evaluate the new count and troubleshoot if needed

3 Run LATRON Control

At the DMS

- If necessary, press Esc to close the Primer Run window
- Press F3 **Run** then press F3 **CONTROL (SECONDARY)**

At the Diluter

- Gently invert the LATRON Control bottle 5 to 8 times
- Cycle LATRON Control in the Secondary mode
  - Immerse the aspirator tip completely in the control
  - Do not place the tip against the side of the bottle
  - Press and release the sample bar
  - Listen for the beep or verify the status line message changes from *Aspirating* to *Diluting* **before** removing the bottle

Reviewing Control Results at the DMS

- Review results once the run appears on the Control Run screen
- If a result is out-of-limits, repeat the entire process beginning with LATRON Primer
- If the results of two control runs are unacceptable,
  - Follow your laboratory's protocol
    or
  - Refer to the When LATRON is Out of Limits table in the Operator's Guide
- Compare the results of this run with previous runs, as desired
  - To evaluate stored numerical data, select **Controls ▸▸ Review or Report**

    Note: Use the → and ← keys to move between DIFF and Retic data.
  - To evaluate Levey-Jennings graphs, select **Controls ▸▸ Graphs**



5C CELL CONTROL

- Material for quality control of CBC and DIFF parameters
- 5C Cell Control is assayed only for Primary mode
- Three levels of control with varying mixtures of normal and abnormal values: Normal, Abnormal I, and Abnormal II

Storage

- Control must be stored at 2 to 8°C (35 to 46°F)
- Storage of the control product in the cap down (inverted) position is not recommended
  - Might require additional mixing for complete resuspension of cellular components

Proper Handling

- Do not write an open tube date or initials in the white space between the bar code and the tube's cap
  - Bar-code reader may not be able to properly read the barcode if marks are present in this white space
  - When the bar-code label cannot be read, the control is stored as a sample in the Sample Analysis data base rather than in its proper control file

Prepare 5C Cell Controls according to the Package Insert Instructions

- Warm control vials at ambient temperature for 10 to 15 minutes before mixing
- Mix gently by hand using the 8 x 8 x 8 method twice
  - After the second mixing, check the sides and bottom of the tube to verify the control is mixed completely
  - Overmixing causes hemolysis which indicates product deterioration
- Do not use mechanical mixers or rotators
- Return to the refrigerator within 30 minutes to ensure stated open-vial stability

Stability

Open-Vial Stability

- Open-vial stability is 13 days or 13 events (whichever comes first)
- An event occurs anytime an operator completes the following sequence:
  - Removes a control vial from the refrigerator
  - Allows the control vial to stand at room temperature 10 to 15 minutes
  - Mixes the control vial gently by hand using the 8 x 8 x 8 method two times
  - Aspirates sample
  - Returns the control vial to the refrigerator within 30 minutes
- MCV and/or RDW parameters may show trending through the product's shelf life; however, this is inherent to the product and should not be considered an indicator of product instability

Indications of Instability

- A slight pink color to the supernatant is normal
- Inability to obtain expected values in the absence of known instrument problems or gross hemolysis (darkly colored supernatant) indicates deterioration of the control

Running 5C Cell Control

Control Run Option

- Using the **Control Run** option to process 5C cell controls is **not** recommended
- Only use this option to run
  - LATRON Primer and Control in the Secondary (Open-Vial) mode
  - Retic-C cell controls in the Secondary (Open-Vial) mode
  - Control vials with damaged bar-code labels

    Note: We recommend control vials with damaged bar-code labels be processed in the Primary (Closed-Vial) mode using the Cycling Commercial Cell Controls without Bar-Code Labels procedure in Chapter 2 of the Operator's Guide.
  - Commercial cell controls without bar-code labels

    Note: We recommend these controls be processed in the Primary (Closed-Vial) mode; see the Cycling Commercial Cell Controls without Bar-Code Labels procedure in Chapter 2 of the Operator's Guide.

Recommendations for Running 5C Cell Controls

- Control tubes may be run in any order
- Run cell controls in the Primary mode in **Sample Analysis**
  - Will not see the control results displayed on the Run Samples screen
  - Continues to display the last patient sample processed
  - Bar-code label on the control tube directs results to the correct file
- To use the Secondary (Open-Vial) mode for running 5C cell control, your laboratory must determine its own means and expected ranges for each parameter and set up a control file using these values

➤ Running 5C Cell Control Summary

1 Getting the DMS Ready

If *SELECT FUNCTION* is displayed

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

  Note: Preparation for cycling in the Primary mode with the DIFF mode ON begins automatically.

If *SELECT FUNCTION* is <u>not</u> displayed

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**
- Press F3 **Run**
- If DIFF mode is OFF, press F9 **STOP** then press F6 **DIFF ON/OFF**
- Is the *PRIMARY: SAMPLE ANALYSIS* prompt at the top of the F3-Run window?
  - If yes, press Esc
  - If no, press F2 **START PRIMARY**

2 Prepare Controls according to the Package Insert

- Warm controls at room temperature 10 to 15 minutes
- Mix each control tube using the 8 x 8 x 8 method twice
  - Do not use a mechanical mixer!
  - Do not write an open tube date or your initials in the white space between the bar code and the tube's cap

3 Run Controls in the same manner as Patient Samples

If using a HmX Hematology Analyzer with Autoloader

- Load the control tubes into a cassette
  - Load in any order
  - Verify the bar-code label is facing upward
- Place the cassette in the loading bay

If using a HmX Hematology Analyzer

- Scan the bar-code label of the control tube you wish to run

  Note: Control levels may be processed in any order.
- After a successful read, insert the control tube through the entry port into the carousel
- Repeat for each control tube

4 Review Results

If using a HmX Hematology Analyzer with Autoloader

- To verify control results are stored in the control file, select **Controls ▸▸ Review or Report**
- If the bar code was not facing up properly or if the bar code was not read because it is damaged, control results are stored in the patient data base
  - If the bar code was not facing up, position it correctly and run the control again
  - If the bar code is damaged, see the Cycling Commercial Cell Controls without Bar-Code Labels heading in the Operator's Guide Startup and Controls chapter

For both the HmX Hematology Analyzer with Autoloader and the HmX Hematology Analyzer

- If a result is out-of-limits,
  - Follow your laboratory's protocol

    or
  - Refer to the When CBC/DIFF Control is Out of Limits table in the Operator's Guide
- Compare the results of this run with previous runs, as desired
  - To evaluate stored numerical data, select **Controls ▸▸ Review or Report**
  - To evaluate Levey-Jennings graphs for the presence of shifts or trends, select **Controls ▸▸ Graphs**
- Print results from the Control Run screen, as desired

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RETIC-C CELL CONTROL

- Three levels: Level I, Level II and Level III
- Controls can only be analyzed after sample preparation is completed using the COULTER ReticPrep Reagent Kit
- Used for monitoring the Coulter method of reticulocyte analysis which uses VCS technology
- Cannot be used to control the manual method

Storage

- Control must be stored at 2 to 8°C (35 to 46°F)
- Store open vials upright in the rack provided
  - Storage of the control product in the cap down (inverted) position might require additional mixing for complete resuspension of cellular components

Proper Handling

Prepare according to the Package Insert Instructions

- Warm at room temperature (20 to 25°C) for 15 minutes before mixing
- Mix gently by hand using the 8 x 8 x 8 method once
  - After mixing, check the sides and bottom of the vial to verify the control is mixed completely
  - Overmixing causes hemolysis which indicates product deterioration
- Do not use mechanical mixers or rotators
- Return to the refrigerator within 30 minutes to ensure stated open vial stability

Stability

Open-Vial Stability

- Open-vial stability is 30 days or 30 events (whichever comes first)
- An event occurs anytime an operator completes the following sequence:
  - Removes a control vial from the refrigerator
  - Allows the control vial to stand at room temperature 15 minutes
  - Mixes the control vial gently by hand using the 8 x 8 x 8 method (one time)
  - Pipettes control sample from the vial
  - Returns the control vial to the refrigerator within 30 minutes

Indications of Instability

- A pink to light red supernatant is normal
- Moderate to marked hemolysis indicates deterioration of the control, usually due to overmixing

Running Retic-C Cell Control

- Use the **Control Run** option for processing
- Prepare a dilution using the COULTER ReticPrep Reagent Kit before analysis
- Run in the Secondary mode

Special Reagents Needed for Retic Analysis

Reagent	Recommended	Function	CBC	DIFF	Retic	Open Container Stability
ReticPrep Reagent A	New Methylene Blue dye, special formulation	• Stains any reticulum present in the RBCs			✓	60 Days
ReticPrep Reagent B	Clearing reagent (very dilute sulfuric acid)	• Removes hemoglobin from the cells without removing the precipitated stain-RNA complex • Keeps the cell membrane intact			✓	60 Days

➤ Running Retic-C Cell Control Summary

1 Prepare Controls according to the Package Insert

- Warm controls at room temperature 10 to 15 minutes
- Mix each control tube using the 8 x 8 x 8 method once
- Do not use a mechanical mixer

2 Preparing the Control

- For each control tested, label two 12 x 75 mm test tubes "A" and "B"
- Place 4 drops of Reagent A into tube "A"
- Dispense 50 µL of the well mixed control into tube "A"
- Gently mix tube "A"
  - Let the tube stand for a minimum of 5 minutes and a maximum of 60 minutes

3 Getting the DMS Ready

- From the Main Menu, select **Controls ▸▸ Control Run**
- Press F2 **File** and select the desired file
- Press F3 **Run**, F3 **CONTROL (SECONDARY)**

4 Run Controls in the Same Manner as Patient Samples

- Gently mix the "A" tube
- Pipette 2 µL of the control/stain mixture from tube "A" into the bottom of tube "B"
- Place tube "B" with the control/stain aliquot at an angle under the tip of the Reagent B dispenser
- Dispense 2 mL of Reagent B into tube "B"
- **Do not mix**
- Wait 30 seconds then analyze the retic preparation you just made
  - Immerse the aspirator tip into the retic preparation
  - Press and release the sample bar
  - Listen for the beep or verify the status line message changes from *Aspirating* to *Diluting* **before** removing the tube

5 Review Results

- If a result is out-of-limits,
  - Follow your laboratory's protocol
    or
  - Refer to the When Retic Control is Out of Limits table in the Operator's Guide
- Compare the results of this run with previous runs, as desired
  - To evaluate stored numerical data, select **Controls ▸▸ Review or Report**
  - To evaluate Levey-Jennings graphs for the presence of shifts or trends, select **Controls ▸▸ Graphs**

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REVIEWING CONTROL RESULTS

Using Control Menu Options to Review 5C Cell Control or Retic-C Results

Note: MRV and IRF parameters appear on the retic screens when these parameters are enabled.

On the Control Run Screen

- Compares control results to expected ranges
  - An **H** or **L** is displayed when a result is outside the limits (flag does not appear in red as it does on the Startup screen)

Using F6 Graph . . . a Control Run Screen Option

- Semi-quantitative graphs
- Displays results of the last 10 runs
- Press F6 as many times as necessary to scroll to the graphs you want to view

Using Review or Report, CBC/DIFF (or RETIC) . . . a Control Menu Option

- Comprehensive display of all runs, up to 100 runs per file
- Use the → or ← key to view additional parameters
- Allows operator to review all parameter results
  - Values for Assay and Limits are entered when a control file is set up
  - Parameter results are listed individually and by average (MEAN); ongoing 2SD and CV values are also provided
  - If the CV result is greater than 20%, a +++++ code is displayed

    Note: +++++ code replacing basophil parameter results is typical.
  - Out-of-control results have an **H** or **L** next to the result
- Use the **F6-Remove/Restore** option as a data management tool

Using Graphs . . . another Control Menu Option

- Levey-Jennings graphs
- Plot point for each result, up to 100 runs per file
- Ideal for spotting trends or shifts

TABLE OF QUALITY CONTROL MATERIALS

Recommended QC material	Function	CBC	DIFF	Retic	Open Vial Stability
LATRON Primer	Cleans and prepares the pathway for running LATRON Control		✓	✓	30 Days
LATRON Control	Checks the functioning of the VCS Technology		✓	✓	30 Days
5C Cell Control	Checks recovery of CBC and Diff parameters	✓	✓		13 Days/Events
Retic-C Cell Control	Checks recovery of Retic parameter			✓	30 Days/Events
S-CAL Calibrator	Adjusts the accuracy of the CBC parameters	✓			1 Hour
LIN-C Cell Control	Checks the linear recovery of the CBC parameters	✓			7 Days

IQAP (Interlaboratory Quality Assurance Program)

- The Coulter Interlaboratory Quality Assurance Program is a free service offered to all regular users of 4C, 4C PLUS, 5C and Retic-C cell controls as well as users of LIN-C linearity controls
- It complements a laboratory's in-house quality control program by providing peer group comparisons for each lot number of a given control product run on the same instrument type
- The assessment is completely independent of the assay value issued when the product was supplied
- A separate IQAP manual (PN 4206266) is available with information on enrollment, data submission, understanding the IQAP report, and troubleshooting IQAP problems

CONTROL FILE MANAGEMENT

- Once a control file is initially set up, the file:
  - Never reverts back to a **NOT SET UP** status
  - Is considered an existing control file
- Two types of existing control files
  - Control file that contains control run data (may be either current or old data)
  - Control file with no stored control run data because the file is either newly set up or has had all its control runs deleted from it

Existing Control Files Currently In Use

Reasons an Operator may need to Access a Control File that is Currently in Use

- To correct a typographical error
- To edit an assigned value (obtained from the Table of Expected Results) to a running mean (established by the laboratory)

➤ MAKING CHANGES TO A CONTROL FILE CURRENTLY IN USE

Use only if you want to make a change in a control file that is currently in use. This summary should be used when changing individual items in a previously set up control file, such as entering a running mean or correcting a typographical error. If you want to make a set up change (such as changing the lot number), use the appropriate Control File Management Summary.

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up**
- Select the type of file and then select the existing file you want to change

2 Answer the Questions

- *Recompute Existing Runs ?*
  - Press Spacebar to answer *Yes* then press Enter
  - *Yes* automatically adjusts the statistics and graphs to the change
  - Operator must toggle to *Yes* to look at the screen even if no changes are made
- *Use Default Limits / Expected Range ?*
  - To maintain laboratory custom ranges, press Enter to answer *No*
  - To use Expected Ranges (from the package insert), press Spacebar to answer *Yes* then press Enter

3 Make the Changes

- Enter the new information

4 Verify All Changes

- Check all entries are correct then press F10 to save and exit the screen

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Existing Control Files No Longer In Use

Reason an Operator may need to Access an Existing File that is No Longer in Use

- To overwrite the file with the set up information of another lot number

  Note: Management of these control files differs depending on whether the laboratory wants to use the default limits/expected ranges (from the Table of Expected Results on the Beckman Coulter package insert) or the laboratory wants to use their own custom ranges (expected ranges established by the laboratory).

  To evaluate the difference of control file management for a laboratory using the default limits/expected ranges from the package insert versus a laboratory using their own custom ranges, see a comparison of procedures on the next page.

Management of Existing Control Files No Longer In Use . . . a Comparison

Using Default Limits/Expected Range (from the package insert)

1. Control file is initially set up using a **NOT SETUP** file
2. Run controls for the month
3. Using the **Review or Report** option, review stored control data and use the **F6-Remove/Restore** option to remove any invalid runs
4. Print the control file
5. Download the file for IQAP, if applicable
6. Delete all the control runs using the **F8-Delete File** option
7. Set up a new lot in this file either now or in the future

Using Custom Ranges (established by your laboratory)

1. Control file is initially set up using a **NOT SETUP** file

   Operator types over the default limits/ expected ranges with their laboratory's own established ranges
2. Run controls for the month
3. Using the **Review or Report** option, review stored control data and use the **F6-Remove/Restore** option to remove any invalid runs
4. Print the control file
5. Download the file for IQAP, if applicable
6. Set up the new lot number of control
   a. Answer the following questions:

   Question 1:
   Recompute Existing Runs ?

   - Press Spacebar to toggle to *Yes* then press Enter
   - *Yes* automatically adjusts the statistics and graphs to the change (operator must toggle to *Yes* to look at the screen even if no changes are made)

   Question 2:
   Use Default Limits/ Expected Range ?

   - Pressing Enter to answer *No* maintains the laboratory's custom ranges

   b. Set up the new lot information
7. Before starting to run the new control, access the **Review or Report** option and delete all the control runs using the **F8-Delete File** option

➤ CONTROL FILE MANAGEMENT SUMMARY (Using the Default Limits/Expected Range from the Package Insert)

Note: To maintain customized expected ranges in a control file, use the Control File Management Using Your Laboratory's Custom Ranges Summary.

Review the Numeric Control Data and Graphs, if applicable

1 Locate the Control File

- From the Main Menu, select **Controls ▸▸ Graphs**
- Press F2 **File** and select the file for review then press Enter

2 Review the Graphs

- Using F6 **Additional Graphs** review all graphs noting any trends, shifts or plot points inside the red area
- Press Esc then R to switch to the **Review or Report** option

3 Review Numeric Data

- Review % CV of all parameters (press → to see additional parameters)
- If a CV is high, use Page Down and examine each run for results mistakenly run into the file (the graph review should correlate)
- Delete any mistakes from the file using F6 **Remove/Restore**

Print / Download the Control File Data

- From the Review or Report screen, press F4 to automatically print all parameters and all runs
- If desired, press Esc then G then F4 to print all graphs
- Download IQAP data, as needed

Delete the Control File Data

1 Go to Control Review Screen

- From the Main Menu, select **Controls ▸▸ Review or Report**
- Press F2 **File** and select the file you want to delete then press Enter

2 Delete the Control File Data

- Press F8 **Delete File**
- Press Spacebar to toggle to *Yes*
- Press Enter to confirm and delete the control file data

Set Up the New Lot Number of Control

Any Control File can be set up as a:

- 5C cell control file (CBC/DIFF file)
- LATRON control file (Latex file)
- Retic-C cell control file (RETIC file)

Setting Up a 5C Cell Control File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ CBC/DIFF file**
- Move the cursor to a **NOT SETUP** file or an inactive file where the control run data is deleted then press Enter

2 Upload the Assay Values

- Insert the 5C cell control diskette into the A: drive of the computer
- Press F5 **Upload Assay Values**
- Press the function key for the desired level of control

3 Type Manual Entries

- Select the Shift and manually type the Operator ID

4 Verify All Information

- Make sure the Host transmit setting correlates with your laboratory's protocol
- Check all entries are correct then press F10 to save and exit the screen

5 Set Up Another Level of Control, if applicable

- Move the cursor to a **NOT SETUP** file or an inactive file where the control run data is deleted then press Enter
- Repeat steps 2 through 5

6 Remove the Diskette from Drive A: (after all needed set ups are complete)

Setting Up a LATRON Control File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ Latex file**
- Move the cursor to a **NOT SETUP** file or an inactive file where the control run data is deleted then press Enter

2 Type Manual Entries

- Enter the name of the file and your Operator ID
- Enter the Lot # and expiration date from the package insert

3 Verify All Information

- Make sure the Host transmit setting correlates with your laboratory's protocol
- Check all entries are correct then press F10 to save and exit the screen

Setting Up a RETIC File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ RETIC file**
- Move the cursor to a **NOT SETUP** file or an inactive file where the control run data is deleted then press Enter

2 Type Entries from the Table of Expected Results

- Enter the Lot #, Expiration Date, Shift, and your Operator ID
- Enter the RBC and Retic % values from the assay sheet

  Note: If you enter the wrong RBC value and then correct it after running controls, the DMS does not recalculate the incorrect RET # control results stored in the RETIC file.

3 If the MRV and IRF Parameters Are Enabled

- Enter 99.9 for MRV and IRF assigned values and expected ranges if customized values are not established

4 Verify All Information

- Make sure the Host transmit setting correlates with your laboratory's protocol
- Check all entries are correct then press F10 to save and exit the screen

➤➤

➤ CONTROL FILE MANAGEMENT SUMMARY (Using Your Laboratory's Custom Ranges)

Use this summary only if you want to maintain the customized expected ranges previously entered in an existing control file (typically a 5C control file).

Review the Numeric Control Data and Graphs, if applicable

1 Locate the Control File

- From the Main Menu, select **Controls ▸▸ Graphs**
- Press F2 **File** and select the file for review then press Enter

2 Review the Graphs

- Using F6 **Additional Graphs** review all 20 graphs noting any trends, shifts or plot points inside the red area
- Press Esc then R to switch to the **Review or Report** option

3 Review Numeric Data

- Review % CV of all parameters (press → to see additional parameters)
- If a CV is high, use Page Down and examine each run for results mistakenly run into the file (the graph review should correlate)
- Delete any mistakes from the file using F6 **Remove/Restore**

Print / Download the Control File Data

- From the Review or Report screen, press F4 to automatically print all parameters and all runs
- If desired, press Esc then G then F4 to print all 20 graphs
- Download IQAP data, as needed

Changing the Existing File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ CBC/DIFF file**
- Select the existing file you want to change

2 Answer the Questions

- *Recompute Existing Runs ?*
  - Press Spacebar to answer *Yes* then press Enter
  - *Yes* automatically adjusts the statistics and graphs to the change (operator must toggle to *Yes* to look at the screen even if no changes are made)
- *Use Default Limits / Expected Range ?*
  - Press Enter to answer *No* (maintains your laboratory's custom ranges)

3 Upload the 5C Control Assay Values

- Insert the 5C cell control diskette into the A: drive of the computer
- Press F5 **Upload Assay Values**
- Press the function key for the desired level of control

4 Type Manual Entries

- Select the Shift and manually type the Operator ID

5 Verify All Information

- Make sure the Host transmit setting correlates with your laboratory's protocol
- Check all entries are correct then press F10 to save and exit the screen

6 Set Up Another Level of Control, if applicable

- Select another **CBC/DIFF file** you want to change
- Repeat steps 2 through 6

7 Remove the Diskette from Drive A: (after all needed set ups are complete)

IMPORTANT Risk of misleading control results. Old control runs remaining in a newly set up control file are included in the statistical data for the new file. Delete all controls runs before starting to run the new control.

Delete the Control File Data

1 Go to Control Review Screen

- From the Main Menu, select **Controls ▸▸ Review or Report**
- Press F2 **File** and select the file you want to delete then press Enter

2 Delete the Control File Data

- Press F8 **Delete File**
- Press Spacebar to toggle to *Yes*
- Press Enter to confirm and delete the control file data

➤➤

ADDITIONAL QC CHECKS

Mode-to-Mode Comparison

- Ensures both the Primary and Secondary aspiration modes recover similar values
- Minor differences between the Primary and Secondary modes are due to differences in the flow characteristics of the aspiration pathways
- 5C cell control is assayed for Primary mode only
- Recommended procedure is located in Chapter 2 of the Operator's Guide
- The mode to mode limits stated in the Reference manual are established using 10 normal bloods measured in triplicate (3 consecutive runs each); these limits should not necessarily be used as limits for a single sample used to check differences between modes

Linearity Checks

- Linearity limits apply only to WBC, RBC, Hgb, and Plt
- Uses various concentrations of blood to verify the reportable range from very low to very high values
- Linearity can be performed using whole blood or a commercial product such as COULTER LIN-C linearity control
- Coulter publishes performance specifications in the Reference Manual, however, a laboratory may be required by a regulatory agency to perform an independent procedure
- When running very low concentrations in the Primary mode, blood detectors must be disabled prior to the run

  Special Functions ▸▸ Diagnostics ▸▸ Operator Options ▸▸ BSV Tests ▸▸ Blood Detector ON/OFF

Diff Comparison

- Perform manual differentials as a measure of good QC practice or as recommended by your laboratory, state, and federal protocols
- Manual differentials are performed to verify the automated differential
- Checks the full differential including:
  - Reagents
  - Mixing process in the mixing chamber
  - Sheath and sample pressures

  Note: LATRON control checks the VCS technology and the integrity and alignment of the flow cell.
- For details, see the Differential Comparison Procedure in Appendix B of the Reference Manual

$\overline{X}_B$ Analysis

- $\overline{X}_B$ $\overline{X}$ = Mean
  B = Bull (for Brian S. Bull, M.D.)
- Pronounced x-bar-bee
- Appears on the DMS screen as XB
- XB will default to ON if instrument is reset

$\overline{X}_B$ Analysis, a Quality Control Tool

- $\overline{X}_B$ Analysis is a quality control method that allows a laboratory to continuously monitor the performance of their automated hematology system and thus control the quality of their results
- Any method used for quality control must use a material that meets two requirements
  - Material must be stable
  - Material must be similar in content to the patient samples that will be analyzed
- $\overline{X}_B$ Analysis meets these requirements
  - Uses the patient samples themselves as a material so the similarity requirement is met
  - Uses the red blood cell indices (MCV, MCH, and MCHC) so the stability requirement is met
- No additional cost to the laboratory because the method uses patient sample results

RBC Indices

- MCV, MCH, and MCHC are relatively stable parameters
  - RBC indices are very tightly controlled by the body because the red cells function best within a very narrow range of size and hemoglobin content
  - Other parameters such as the white blood cells and platelets tend to have a wider physiological range and are, therefore, not as predictable as the red cell indices
- Are typically stable for an individual patient from day to day
- Are stable for a patient population over time
  - Many hospitalized patients have been investigated and it has been found that there is no significant day-to-day or week-to-week variability in the mean of their indices

Target Values

- A constant for each RBC index (MCV, MCH, or MCHC)
- Target values should reflect the entire patient population of the laboratory
  - The patient population of a general hospital usually includes samples from all patient age groups, disease states and many hematologically “normal” samples
  - A patient population that includes only one age group or only one disease state will yield different target values than a patient population that includes all groups

- As long as the patient population remains constant, the target values of each index also remain constant
- If the patient population changes, the mean of an index may also change and needs to be reevaluated

Dr. Bull's Target Values

- Default starting values
  - MCV = 89.5
  - MCH = 30.5
  - MCHC = 34.0
- Each laboratory should begin with these target values and then evaluate and adjust them for their own patient population

Getting Started

Enter Target Values and Action Limits

- Target values and action limits must be entered in the DMS:

  Special Functions ▸▸ Set Up ▸▸ Sample Analysis Set Up ▸▸ Action Limits ▸▸ XB Limits
- Use either the suggested target values or the target values established by your laboratory
- 3% action limits are suggested

Turning the XB Option ON

- $\overline{X}_B$ Analysis is done automatically by the DMS only if the option is turned on
- On the Run Samples screen, check the Status line
  - **XB↑** indicates the $\overline{X}_B$ Analysis is turned ON
  - **XB↓** indicates the $\overline{X}_B$ Analysis is turned OFF
- To change the setting,
  - **Sample Analysis ▸▸ Run Samples**; press F5 **Optns** then F2 to change **XB ON/OFF** setting
- $\overline{X}_B$ Analysis is automatically turned OFF when the Run Samples F7 **Display Only** option is ON

Using the $\overline{X}_B$ Analysis

When XB function is turned on, the

- DMS stores the RBC results of all patient samples as they are cycled
- $\overline{X}_B$ Analysis is performed on small sets of 20 patient results at regular intervals (each patient set is referred to as a batch)

- Stored results (RBC, Hgb, Hct, MCV, MCH, and MCHC) are displayed on the Current XB Batch table: **Sample Analysis ▸▸ XB ▸▸ Current XB Batch**
- Of the displayed results, only the MCV, MCH, and MCHC parameters are used in the analysis

- Having the other RBC parameters displayed allows the operator to judge the validity of the sample results when an out-of-limits situation occurs
- When the batch of 20 patient samples is completed, the DMS performs the $\overline{X}_B$ Analysis and determines the batch mean of each index (MCV, MCH, and MCHC)
  - Method for obtaining the batch mean is rather complicated, but the calculation is done automatically by the DMS
  - This batch mean is not a simple average value of the patients' indices, but a type of "weighted moving average" that statistically allows the small batch of 20 patient samples to appear as if it were a larger set of approximately 100 samples
- DMS then compares each batch mean to its expected target value
  - Results are displayed on the XB Batch Means table: **Sample Analysis ▸▸ XB ▸▸ XB Batch Means**
  - If the batch mean is inside the expected action limits, the batch is said to be IN
    - Instrument is functioning properly
    - **XB**↑ status message remains white to indicate the last completed batch was inside the action limits
  - If the batch mean is outside the expected action limits, the batch is said to be OUT
    - One or more of the three indices did not come close enough to the expected target values
    - An **H** or **L** flag appears beside the out-of-limit results
    - **XB**↑ status message turns red to indicate the last completed batch was outside the action limits
    - **XB**↑ status message will return to white when the next batch of XB is IN

Reviewing XB Data

Current XB Batch

- Stores RBC parameter results when $\overline{X}_B$ Analysis is turned on
- When a batch of 20 samples is collected, the DMS performs the $\overline{X}_B$ Analysis and calculates the batch mean for MCV, MCH, and MCHC
  - The system automatically excludes samples that do not yield numeric results such as partial aspirations
- Option primarily used for reviewing individual sample results to identify sample related problems such as non-reportable results or non-random sampling
  - Once the batch is completed (20 patient samples are accumulated), individual samples cannot be deleted
  - Deleted sample results and partial aspiration codes are displayed and consume one of the 20 sample spaces; however, these results are not used in the $\overline{X}_B$ batch mean calculation
    - A maximum of 5 samples may be deleted from the current batch prior to completion
    - A minimum of 15 samples are required to calculate a batch mean

XB Batch Means Option

- Used to view the calculated batch means for the last batch of 20 samples collected
- Also provides the percent difference between the calculated batch mean and its corresponding target value
- If a percent difference is outside the action limit, the parameter is flagged with an **H** or **L**
- May be used to identify the affected RBC indices and to determine the direction and amount of the change
- After the first 20 batches are completed, this option always displays the last 20 completed batches
  - Provides a history of recent batch means which may serve as an aid during the troubleshooting process
  - After the 20th batch is completed, the table begins to rollover where the oldest batch results are deleted to make space for the last completed batch results

XB Graphs

- Three graphs, one for each index
- Used to view the batch means in relationship to the target value and action limit of the corresponding index
- Graphs are good for identifying trends and shifts
- Each graph contains three areas
  - A central white line representing the actual target value
  - A black area representing the acceptable range
  - An upper and lower red line represent action limit boundaries
- After the first 20 batches are completed, this option always displays the plot points for the last 20 completed batches
  - Provides a history of recent batch means which may serve as an aid during the troubleshooting process
  - After the 20th batch is completed, the graph begins to rollover where the oldest batch results are deleted to make space for the last completed batch results

What Causes a Batch to go "Out-of-Limits"?

- Change in the patient population
  - One or more types of patients added or removed from the total patient population mix
  - Non-reportable results where a batch of patients is biased by short samples, physiologically impossible results and/or erroneous data accumulated before a reagent or instrument problem was identified and solved
  - Non-random patient sampling where a batch of patients is biased by several abnormal patients of one particular type (oncology, neonatal and so forth)
- An instrument problem may exist
- A calibration problem may exist
- A reagent problem may exist

Troubleshooting when an XB Batch is "Out"

Consider a Change in the Patient Population

- Is there a change in the overall patient population?
  - One or more new patient types were added to the population
  - One or more patient types are no longer part of the population
  - Possible seasonal change of the patient population (hospitals or clinics in resort areas)
- If a change in the overall patient population is probable,
  - Future XB batch results will also be outside the limits
  - New target values based on the current population must be established and entered in the computer
- If a change in the overall patient population is not probable,
  - It is possible that the patient population has not really changed but just appears to have changed because the batch of 20 patients does not truly represent the total population
  - Use the **Current XB Batch** option to check for non-reportable results and/or non-random sampling:

    Sample Analysis ▸▸ XB ▸▸ Current XB Batch

Checking for Non-Reportable Results

- An XB batch may go outside the ±3% action limits because the batch is biased by physiologically impossible results caused by a reagent or instrument problem, or by physiologically abnormal specimen

Consider an Instrument Problem Exists

- Consider an instrument problem only if
  - A change in the patient population is doubtful
  - All the results in the Current XB Batch are reportable (physiologically possible)
  - Random sampling is verified
- Instrument problems cause XB batch results to go "out" and stay out
  - Can be a gradual change
    - If caused by a calibration drift, should go back within limits after calibration
    - If caused by a component going bad over time, should go back within limits after the component is replaced
  - Can be a sudden change
    - If caused by a component failure, should go back within limits after the component is replaced
    - If caused by any number of instrument problems, should go back within limits after the problem is fixed

Assessing the Situation

- Review the XB Graphs and XB Batch Means options to identify the out-of-limits RBC indices and note the direction of the change (increased or decreased)
  - Generally two of the three RBC indices are affected
  - It is also important to note any index that may still be inside the limit but shows a significant change

Identifying the Parameter Causing the Problem

- There are two ways to identify the parameter causing the problem
  - Knowledge and analysis of the involved parameters
  - Use of a table showing the relationship between the RBC Indices and their associated parameters

Analysis of Involved Parameters

- MCV parameter is derived from the RBC Histogram
  - Although derived from the histogram, the size of the individual red cells is determined using the Coulter Principle which is a direct measurement
- $MCH = \frac{Hgb}{RBC} \times 10$
- $MCHC = \frac{Hgb}{Hct} \times 100 \text{ where the } Hct = \frac{RBC \times MCV}{10}$
- RBC indices are based on three directly measured parameters: MCV, RBC, and Hgb

By Use of a Parameter Relationship Table

- Table relates the RBC Indices and their associated parameters

	MCV LOW	MCV HIGH	MCH LOW	MCH HIGH	MCHC LOW	MCHC HIGH
MCV	dec	inc	---	---	inc	dec
RBC	---	---	inc	dec	inc	dec
Hgb	---	---	dec	inc	dec	inc
Hct	dec	inc	---	---	inc	dec

- Once the operator identifies the affected RBC indices and notes the direction of the change (increased or decreased), the table reveals the suspected parameters (MCV, RBC, or Hgb)

NOTES

OBJECTIVES

When the subject is complete, you will be able to . . .

- Explain the purpose of calibration.
- Tell when one needs to verify calibration.
- Perform the preliminary procedures.
- Perform the calibration procedure using the Special Procedures and Troubleshooting (SPT) Manual.

NOTES

CALIBRATION

- A procedure used to standardize the instrument by determining its deviation from calibration references and applying any necessary correction factors
- Never use calibration to adjust for an instrument problem
- Verify the ambient room temperature is typical for the laboratory and within the instrument's operating range (16 to 32°C; 60 to 90°F)
- In the normal process of tracking data for an extended period of time, your laboratory can make a specific decision to recalibrate a given parameter
- **Never** adjust to a specific value based on an individual sample result

When to Calibrate

- At installation, before you begin analyzing samples
- After you replace any component dealing with
  - Dilution preparation, such as the BSV
  - Primary measurement, such as an aperture
- If your Beckman Coulter Representative suggests you calibrate

When to Verify Calibration

- As dictated by your laboratory procedures, local, or national regulations
- When controls show evidence of unusual trends
- When controls exceed the manufacturer's defined acceptable limits
- When average ambient room temperature changes more than 10°F (5.5°C) from the room temperature during your last calibration

PRELIMINARY CALIBRATION PROCEDURES

- Procedures designed to ensure the instrument is operating optimally
- Recommend a thorough review of data stored in current control and $\overline{X}_B$ files to note any possible bias that might require a change in a calibration factor
- Thorough review of IQAP data is also recommended

Procedures to be Completed Prior to Calibration

- Check reagent volumes to ensure sufficient volume to complete calibration procedures
- Ensure the instrument is clean
  - If the instrument is routinely shut down with COULTER CLENZ cleaning agent for at least 30 minutes every 24 hours it is in use, initiate the **Clean Cycle** option
  - If the instrument is **not** routinely shut down with COULTER CLENZ cleaning agent for at least 30 minutes every 24 hours the instrument is in use, the apertures must be bleached as instructed in the SPT's Cleaning Procedures chapter
- Bleach the aspiration system using the **Clean Needle** option
  Special Functions ▸▸ Diagnostics ▸▸ Operator Options ▸▸ Fluidic Tests ▸▸ Clean Needle
- Do a Start Up
- Run commercial cell controls

CALIBRATION PROCEDURE

- Beckman Coulter recommends using S-CAL Calibrator and performing the procedure in the Primary mode (Closed-Vial mode)
- Carefully follow the instructions in Chapter 1 of the Special Procedures and Troubleshooting (SPT) Manual anytime calibration is performed
- From the Main Menu, selecting **Special Functions ▸▸ Calibration ▸▸ CBC Calibration** accesses the CBC Calibration screen

CBC Calibration Screen

CBC CALIBRATION

RUN	WBC	RBC	HGB	MCV	PLT	MPV	N =10
DEL	9.07	4.256	12.67	85.9	204.6	10.64	
2	8.62	4.145	12.46	86.4	203.3	10.65	
3	8.80	4.181	12.60	85.6	206.0	10.67	
4	8.75	4.128	12.50	85.7	212.5	10.65	
5	8.72	4.141	12.46	85.8	208.6	10.62	
6	8.60	4.161	12.50	85.4	200.3	10.83	
7	8.83	4.202	12.56	85.4	212.8	10.67	
8	8.88	4.175	12.65	86.0	214.3	10.71	
9	8.99	4.187	12.55	85.7	204.8	10.60	
10	8.75	4.178	12.56	85.7	197.7	10.60	
11	8.61	4.157	12.65	86.7	193.9	10.79	
MEAN	8.75	4.165	12.55	85.8	205.4	10.68	
NEW CAL FAC	1.174	1.301	1.266	0.912	1.301	1.066	
OLD CAL FAC	1.155	1.297	1.261	0.922	1.291	1.054	
%CV	1.4	0.6	0.6	0.5	3.3	0.7	
FAC. % DIFF	1.66	0.35	0.41	-1.10	0.77	1.13	
DELTA DIFF	0.15	0.014	0.05	0.9	1.6	0.12	EXP 04/20/99
REF. VALUES	8.90	4.180	12.60	84.9	207.0	10.80	LOT 5737

Terms and Formulas used in the CBC Calibration Procedure

MEAN

- Average of the 10 S-CAL calibrator runs
  - Run 1 of the 11 runs is automatically deleted

NEW CAL FACTOR

- Calibration Factor needed to recover the S-CAL Calibrator Reference value
- It is calculated and displayed regardless of whether or not you need to change it

$$NEW\ CAL\ FAC\ = \frac{Reference\ Value \times Old\ Cal\ Factor}{S\text{-}CAL\ Mean\ Value\ (n=10)}$$

OLD CAL FACTOR

- The current Calibration Factor in use by the analyzer

% CV

- Indicates the reproducibility of the S-CAL Calibrator run
- Checked to ensure valid data is used as you are deciding whether or not to recalibrate

FACTOR % DIFF

- The percentage difference between the NEW CAL FAC and the OLD CAL FAC

DELTA DIFF

- Absolute difference between the REFERENCE VALUE and the S-CAL Calibrator MEAN

REFERENCE VALUES

- The assigned value for each parameter

OBJECTIVES

When the subject is complete, you will be able to . . .

Basics of Running a Sample

- State the proper anticoagulant to use for specimens processed on a HmX Hematology Analyzer with Autoloader or HmX Hematology Analyzer.
- State the minimum volume of blood needed for the Primary mode, Secondary mode, Predilute mode, and Retic mode.
- State the maximum storage time prior to running a specimen for CBC, Differential, and Retic analysis.
- State the proper storage temperature.
- Properly place a bar-code label on a specimen tube.
- Properly insert a bar-code labeled specimen tube into a cassette or the cap-pierce station, as applicable.
- Explain the purpose of a Worklist.
- Explain the purpose of the blood detecting system.

Cycling Options

- Use the correct **F3-Run** option to designate the appropriate operation mode for the required task.
- Identify when it is necessary to use **F9-STOP** prior to changing the instrument's state.
- Set up the options you want under **F5-Optns**.
- Explain the purpose of the **F7-Display Only** option.
- Print results automatically.
- Explain how an operator can quickly verify the communication link between the DMS and HmX instrument is intact.

Running Whole Blood Patient Samples

- Run samples in the Primary mode.
- Briefly describe the cassette transport or rotary cap-piercing operational sequence, as applicable.
- Run samples in the Secondary mode.
- Run samples with and without differentials.

Processing a STAT Specimen (HmX Hematology Analyzer with Autoloader only)

- Interrupt Primary mode operation to run a STAT specimen.
- Resume operation in the Primary mode without skipping any patient samples.

Predilute Mode

- State when this mode should be used.
- State the dilution ratio required for processing samples.

Run Samples Screen

- Explain F1 through F5 and F9 on the Run Samples screen.
- Name and define each area on the Run Samples screen.
- Label the WBC populations on a normal DF1 scatterplot.
- Use F11 to display Suspect and Definitive flag messages.

Retic Mode

- State the volumes used in the reticulocyte procedure for:
  - Whole blood
  - Reagent A
  - Incubated blood/stain mixture for the final preparation
  - Reagent B
- State the minimum and maximum incubation times for the blood/stain mixture.
- State the waiting time once Reagent B is added to the proper volume of the incubated blood/stain mixture.

Worklist (Optional)

- Define the *PART ASP*, *NO MATCH*, and *NO READ* error messages.
- List indications the system gives when
  - Three consecutive *PART ASP*, *NO MATCH*, or *NO READ* errors occur.
  - Any combination of 10 errors accumulate on the Worklist.
- List the steps an operator must take to resume operation when the above errors occur.
- Explain what the colors behind the **WL** on the Status line indicate.
- Explain what the ↑ or ↓ next to **WL** on the Status line indicates.

SAMPLE HANDLING

Specimen Collection

Using Evacuated Collection Tubes

- Collect venous blood specimens in K_3EDTA or K_2EDTA
  - A proper proportion of whole blood to anticoagulant is critical
  - K_3EDTA is the preferred anticoagulant because all performance claims and validation studies are based on its use
  - K_2EDTA shows no significant differences for CBC and differential results generated by instruments using VCS technology
- Specimen tubes cycled in the Primary mode must contain a minimum 1.0 mL of a properly collected specimen

Using Microcollection Devices

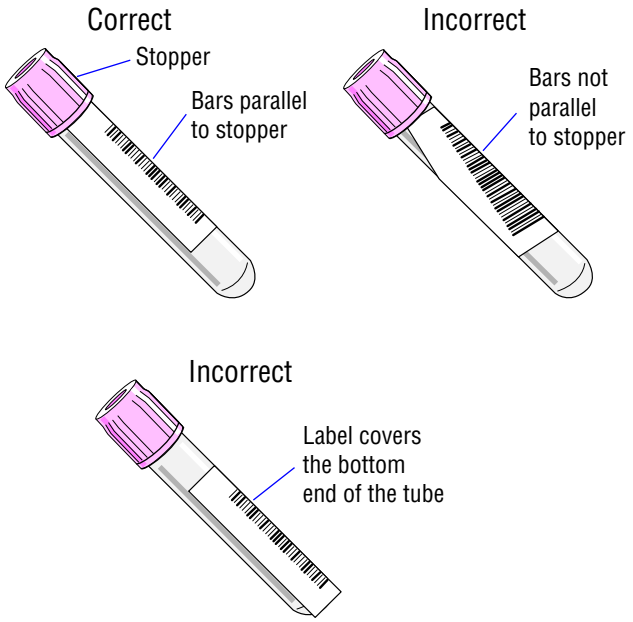
- Anticoagulated capillary specimens collected in a microcollection device may be directly aspirated into the HmX instrument using the Secondary mode
- Proper collection is critical to avoid micro-clots and/or debris
- Review and follow the directions in the package insert provided by the manufacturer

Storage

- Run a CBC/Diff specimen within 24 hours of collection
- Store specimens capped at room temperature
- Run a Retic specimen within 8 hours of collection if stored at room temperature or within 24 hours if refrigerated (2-8°C; 36-46°C)

BAR-CODE LABELING

IMPORTANT Risk of misidentification. Do not use the tilde (~) character in demographics, including Specimen or Patient ID.



Proper Placement of a Bar-Code Label on a Specimen Tube

- Bar-code labels must be within specifications as stated in the Reference Manual
- The bars on the label must be parallel to the stopper
  - If the label is skewed more than 5 degrees, the scanner may not read it
- Do not cover the bottom of the tube with the bar-code label
  - On a HmX Hematology Analyzer with Autoloader, this space is needed for the scanner to distinguish the specimen tube label from the cassette label
  - On a HmX Hematology Analyzer with rotary cap-pierce, proper label placement prevents jamming in the carousel

Tube Adapters for Single Specimen, Rotary Cap-Pierce Instruments Only

- Tube adapters are used for 2 mL and 3 mL specimen tubes
  - 2 mL adapter is for 10.25 mm x 47 mm specimen tubes
  - 3 mL adapter is for 10.25 mm x 64 mm specimen tubes
  - Although they appear similar, the base differs to provide different stop points for the specimen tubes
- An operator may scan the bar-code labeled specimen tube before or after placing the tube into the adapter

CYCLING OPTIONS

F3-Run Options

From the Main Menu, select **Sample Analysis ▸▸ Run Samples ▸▸ F3-Run**

F2 START PRIMARY

- Closed-Vial mode

F3 SECONDARY

- Open-Vial mode

F4 PREDILUTE CBC

- Open-Vial mode only
- For processing a 1:3 sample dilution (1 part blood to 2 parts diluent) in the Secondary mode

F5 RETIC

- Open-Vial mode only
- Sample preparation is required before processing in the Secondary mode

F6 DIFF ON/OFF

- To toggle this option ON or OFF, the *SAMPLE MODE ?* prompt must appear at the top of the **F3-Run** window
- If the *SAMPLE MODE ?* prompt is not displayed, press **F9 STOP**

  Note: If an operator attempts to change the DIFF status without pressing **F9 STOP**, the DMS beeps and will not make the requested change.

F7 PURGE

- For troubleshooting purposes

F8 RINSE

- For troubleshooting purposes

F9 STOP

- Stops the system as soon as the current cycle is complete
- Returns the system to *SELECT FUNCTION*
- Must be used when changing the DIFF mode from ON to OFF
- If an operator attempts to change the DIFF mode without using **F9 STOP** first, the instrument signals with an audible beep

F5-Optns

From the Main Menu, select **Sample Analysis ▸▸ Run Samples ▸▸ F5-Optns**

F2 **XB**

- Three indicators:
  - ON / OFF current usage of $\overline{X}_B$ Analysis
  - N number of patient samples accumulated in the current batch
  - IN / OUT status of the last completed batch of 20 patient samples
- If **Display Only** is ON, then XB is automatically turned OFF
- If instrument is reset, ON is the default setting

F3 **CAP PIERCER: 10mm-13mm (Single Specimen, Rotary Cap-Pierce System Only)**

- Allows the operator to select the correct size carousel slot based on the size of the specimen tube
  - Majority of the tubes are 10-13 mm tubes (Control tubes are 10-13 mm)
  - 16 mm tubes are the fat 7 mL specimen tubes
- Do not run 10-13 mm tubes in the 16 mm tube slot
- Run control and calibrator vials in the 10-13 mm slot
- If instrument is reset, 10 mm - 13 mm is the default setting

F4 **DB**

- Must be on to store patient results
- Data base can store up to 5000 complete sets of results including retic results
- When the data base is full, the newest sample results replace the oldest sample results in a process referred to as wraparound
- If **Display Only** is ON, then the data base is automatically turned OFF
- If instrument is reset, ON is the default setting

F5 **Print**

- If the setting is NONE, the operator must manually print results from display screen or data base
- If the setting is ALL, NORMAL, or ABNORMAL, results are automatically printed as they are run
  - Results are considered abnormal when any of the parameter codes or messages listed in Chapter 4 of the Operator's Guide are generated
- If instrument is reset, NONE is the default setting

F6 **Host**

- Turn this on if your laboratory is interfaced to a host computer and you want the results to transmit automatically to the host as the samples are run
- If **Display Only** is ON, then the host is automatically turned OFF
- If instrument is reset, OFF is the default setting

F7 Display Only

- Useful for cycling samples when an operator does not want results sent to the host computer or stored in XB or the data base
  - Especially helpful when troubleshooting
- Quick way to turn XB, DB, and Host off then back on simultaneously
  - Position of the arrows on the Status line easily verifies the current state of these options
- If instrument is reset, OFF is the default setting

F8 Operator

- Operator identification
- Up to three alphanumeric characters can be entered
- If instrument is reset, OPR is the default setting

F11 B&W screen print

- Used to make a large black and white printout of the results displayed on the screen
- Can only be used for the current sample

F12 Color screen print

- Used to make a large color printout of the results displayed on the screen if a color ribbon or ink cartridge is installed
- Can only be used for the current sample

Note: When at the Main Menu, an operator can access a window displaying the **F5-Print**, **F6-Host**, and **Operator** options by pressing F5 **Options**.

To continue HmX Hematology Analyzer training, proceed to page 6-9.

To continue HmX Hematology Analyzer with Autoloader training, go to page 6-15.

NOTES

RUNNING WHOLE BLOOD PATIENT SAMPLES (Using a HmX Hematology Analyzer)

Running Patient Samples in the Primary Mode

- Lower door must remain closed during operation
- Aspirator probe is retracted

Scanning the Bar-Code Label on the Specimen Tube

- Uses a laser bar-code scanner
- Reading the bar-code label provides positive identification of the patient sample
- Green LED glowing means a specimen tube may be safely inserted; red LED glowing means WAIT
- When a specimen tube is labeled with a bar code, the operator must hold the specimen tube in front of the bar-code reader
  - If the bar-code label is successfully read, the green LED flashes and there is an audible beep
  - If the bar-code label is not successfully read, the red LED flashes; wait and try again
- When a bar-code label is not used, the operator manually types the Sample ID then presses Enter

Specimen Transport Overview

- Using the diagram at the cap-pierce entry port as a guide, position the specimen tube with the stopper slanted downward → insert the specimen tube through the entry port → the red LED glows and the carousel rotates the specimen tube to the cap-piercing station
  - Hand detectors will not allow the carousel to activate until the operator's hand is initially moved out of the way
- At the cap piercing station, the needle pierces the specimen tube's stopper
- While 185 µL of sample is being pulled out of the specimen tube, blood detectors are activated to verify sample integrity
- Once aspiration is complete, the needle retracts and the carousel rotates the specimen tube depositing it onto the exit tray for removal by the operator
- As sample analysis occurs, the red LED goes out and the green LED glows to indicate another specimen tube can be processed

Running Patient Samples in the Secondary Mode

- Aspirator probe is extended allowing the probe to be submerged in a specimen tube that has its stopper removed
- Blood detectors are not active; operator is responsible for checking sample integrity
- Since the aspirator probe is self-cleaning, the operator must remove the specimen tube promptly once the aspiration process is complete

Steps for Changing the Carousel Slot

Note: Since the majority of specimen tubes are 10 to 13 mm tubes, the carousel is most likely using the 10mm-13mm slot. Before beginning this sequence, note the appearance of this solid black 10mm-13mm slot.

1. From the Main Menu, select **Sample Analysis ▸▸ Run Samples ▸▸ F5-Optns**
2. Press F3 **CAP PIERCER** to change from **10mm-13mm** to **16mm**
3. To activate the change,
   a. Press Esc to exit **F5-Optns**
   b. Press F3 **Run** then F2 **START PRIMARY**
   c. Carousel rotates to the 16mm slot which has a yellow bar with a 16mm label
4. To return to the 10mm-13mm slot,
   a. Press F5 **Optns** then F3 to change the option from **16mm** back to **10mm-13mm**
   b. Press Esc to exit **F5-Optns**
   c. Press F3 **Run** then F2 **START PRIMARY**
   d. Carousel rotates to the solid black 10mm-13mm slot

➤ Running Whole Blood Patient Samples in the Primary Mode Summary (Using a HmX Hematology Analyzer)

1 Getting the DMS Ready

If not using the Worklist

- If *SELECT FUNCTION* is displayed,
  - ▸ From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

    Note: Preparation for cycling in the Primary mode with the DIFF mode ON begins automatically.
  - ▸ To continue operation with the DIFF mode ON, go to step 2
  - ▸ To operate with DIFF mode OFF, go to Finish DMS Preparation
- If *SELECT FUNCTION* is **not** displayed,
  - ▸ From the Main Menu, select **Sample Analysis ▸▸ Run Samples**
  - ▸ Go to Finish DMS Preparation

If using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Worklist**
- Enter appropriate identifiers
- Press F3 **Comments** to enter additional demographics then press F10 **Save/Esc**
- If *SELECT FUNCTION* is displayed,
  - ▸ Press Ctrl + W

    Note: Preparation for cycling in the Primary mode with the DIFF mode ON begins automatically.
  - ▸ To continue operation with the DIFF mode ON, go to step 2
  - ▸ To operate with DIFF mode OFF, go to Finish DMS Preparation
- If *SELECT FUNCTION* is **not** displayed,
  - ▸ Press Ctrl + W
  - ▸ Go to Finish DMS Preparation

Finish DMS Preparation

- Press F3 **Run**
- If the DIFF mode needs to be changed, press F9 **STOP** then F6 **DIFF ON/OFF**
- If the *PRIMARY: SAMPLE ANALYSIS* prompt is at the top of the F3-Run window, press Esc; if not, press F2 **START PRIMARY**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

2 Process the Sample

With Bar-Code Labels

- Mix the specimen gently but thoroughly
- Hold the tube's bar-code label in front of the reader
- Listen for the beep and verify the green LED is glowing
- Confirm the ID appears in the Enter ID field
- Properly insert the specimen tube into the carousel

Without Bar-Code Labels

- Type the sample ID at the keyboard then press Enter
- Mix the specimen gently but thoroughly
- Verify the green LED is glowing
- Properly insert the specimen tube into the carousel

3 When the Cycle is Complete

- Verify the sample ID entry and results before reporting
- Remove the specimen tube from the exit tray
- If using the Worklist, periodically check for accumulation of Status messages

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➤ Running Whole Blood Patient Samples in the Secondary Mode Summary (Using a HmX Hematology Analyzer)

1 Getting the DMS Ready

If not using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

If using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Worklist**
- Press F2 **Sec**
- Enter appropriate identifiers
- Press F3 **Comments** to enter additional demographics then press F10 **Save/Esc**
- Press Ctrl + W

- Press F3 **Run**
- If the DIFF mode needs to be changed, press F9 **STOP** then F6 **DIFF ON/OFF**
- Press F3 **SECONDARY**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

2 Process the Sample

With Bar-Code Labels

- Mix the specimen gently but thoroughly
- Hold the tube's bar-code label in front of the reader
- Listen for the beep and verify the green LED is glowing
- Confirm the ID appears in the Enter ID field
- Aspirate the sample into the system

Without Bar-Code Labels

- Type the sample ID at the keyboard then press Enter
- Mix the specimen gently but thoroughly
- Aspirate the sample into the system

3 When the Cycle is Complete

- Verify the sample ID entry and results before reporting
- If using the Worklist, periodically check for accumulation of Status messages

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To continue HmX Hematology Analyzer training, go to page 6-19.

NOTES

RUNNING WHOLE BLOOD PATIENT SAMPLES (Using a HmX Hematology Analyzer with Autoloader)

Running Patient Samples in the Primary Mode

- Lower door must remain closed during operation
- Aspirator probe is retracted
- Up to five specimen tubes can be loaded into a cassette, barcode labels up
- Up to five cassettes can be stacked in the loading bay

Specimen Transport Overview

- Once operation is initiated, a platform lowers the bottom cassette onto the rocker bed → the rocker bed rocks back and forth mixing the sample as the cassette advances to the cap piercing station → when the first specimen tube is detected, the cassette stops moving forward but continues to rock
- The bar-code scanner reads the cassette bar-code label and the specimen tube bar-code label (if present) for positive identification of the sample
  - If the cassette bar-code label is not read, the Autoloader stops
  - If the cassette bar-code label is read, the rocker bed locks in the forward piercing position
- The needle pierces the specimen tube's stopper
- While 185 µL of sample is being pulled out of the specimen tube, blood detectors are activated to verify sample integrity
- Once aspiration is complete, the needle retracts and the rocker bed begins to rock again
- The bar-code scanner reads the cassette bar-code label and the specimen tube bar-code label (if present) a second time
  - If both bar-code scans are identical, the Autoloader continues to rock and moves the cassette until the next available specimen tube is detected
  - If the bar-code scans are not identical, the Autoloader stops and no data is available for that sample → when restarted, the cassette advances to the next specimen tube where the process of piercing and aspiration repeats
  - In either case, if additional cassettes are stacked in the loading bay when the cassette reaches the fourth pierce position, a platform lowers the bottom cassette onto the rocker bed
- When all specimens tubes in the cassette are processed, the rocker bed continues to rock and move the cassette along the bed to the unloading bay where it is stacked for removal by the operator

Running Patient Samples in the Secondary Mode

- Aspirator probe is extended allowing the probe to be submerged in a specimen tube that has its stopper removed
- Blood detectors are not active; operator is responsible for checking sample integrity
- Since the aspiration probe is self-cleaning, the operator must remove the specimen tube promptly once the aspiration process is complete

NOTES

➤ Running Whole Blood Patient Samples Summary (Using a HmX Hematology Analyzer with Autoloader)

Running Whole Blood Samples in the Primary Mode

1 Getting the DMS Ready

If not using the Worklist

- If *SELECT FUNCTION* is displayed,
  - ▸ From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

    Note: Preparation for cycling in the Primary mode with the DIFF mode ON begins automatically.
  - ▸ To continue operation with the DIFF mode ON, go to step 2
  - ▸ To operate with DIFF mode OFF, go to Finish DMS Preparation
- If *SELECT FUNCTION* is **not** displayed,
  - ▸ From the Main Menu, select **Sample Analysis ▸▸ Run Samples**
  - ▸ Go to Finish DMS Preparation

If using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Worklist**
- Enter appropriate identifiers
- Press F3 **Comments** to enter additional demographics then press F10 **Save/Esc**
- If *SELECT FUNCTION* is displayed,
  - ▸ Press Ctrl + W

    Note: Preparation for cycling in the Primary mode with the DIFF mode ON begins automatically.
  - ▸ To continue operation with the DIFF mode ON, go to step 2
  - ▸ To operate with DIFF mode OFF, go to Finish DMS Preparation
- If *SELECT FUNCTION* is **not** displayed,
  - ▸ Press Ctrl + W
  - ▸ Go to Finish DMS Preparation

Finish DMS Preparation

- Press F3 **Run**
- If the DIFF mode needs to be changed, press F9 **STOP** then F6 **DIFF ON/OFF**
- If the *PRIMARY: SAMPLE ANALYSIS* prompt is at the top of the F3-Run window, press Esc; if not, press F2 **START PRIMARY**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

2 Load Cassettes

- Load the specimen tubes into the cassette making sure any bar-code labels are visible through the top of the cassette
- Place up to 5 cassettes in the loading bay

3 When the Run is Complete

- Verify all sample ID entries before reporting results
- Remove cassettes from the unloading bay
- If using the Worklist, periodically check for accumulation of Status messages

Interrupting Primary Mode Operation to Run a STAT

- Press F3 **Run** then F3 **SECONDARY** (the bed continues to rock and the current cycle continues to completion)
- When the Enter ID field appears, type the sample ID then press Enter
- Run the STAT specimen in the Secondary mode
- Press F3 **Run** then F2 **START PRIMARY** to resume operation in the Primary mode (Autoloader picks up where it left off)

Running Whole Blood Samples in the Secondary Mode

1 Getting the DMS Ready

If not using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

If using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Worklist**
- Press F2 **Sec**
- Enter appropriate identifiers
- Press F3 **Comments** to enter additional demographics then press F10 **Save/Esc**
- Press Ctrl + W

- Press F3 **Run**
- If the DIFF mode needs to be changed, press F9 **STOP** then F6 **DIFF ON/OFF**
- Press F3 **SECONDARY**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

2 Process the Sample

- Type the sample ID at the keyboard then press Enter
- Mix the specimen gently but thoroughly
- Aspirate the sample into the system

3 When the Cycle is Complete

- Verify the sample ID entry and results before reporting
- If using the Worklist, periodically check for accumulation of Status messages

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PREDILUTE MODE

- Diluted samples are aspirated through the Secondary mode
- Dilution ratio must be 1 part blood to 2 parts diluent
- Minimum sample to be used is 50 µL of whole blood to 100 µL of diluent
- Must make dilutions from properly collected specimen tubes
- System generates CBC results only
- WBCs are not in their “near-native state” when diluted
- Computer will automatically calculate the diluted results correctly
- Predilute mode cannot be used to verify overrange values as results will recalculate to (+++++)

➤ Running Patient Samples in the Predilute Mode Summary

For HmX Hematology Analyzer with Autoloader and HmX Hematology Analyzer

1 Getting the DMS Ready

If not using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

If using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Worklist**
- Press F2 **Sec**
- Enter appropriate identifiers
- Press F3 **Comments** to enter additional demographics then press F10 **Save/Esc**
- Press Ctrl + W

- Press F3 **Run**
- Press F4 **PREDILUTE CBC**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

2 Make the Dilution

- Make an accurate 1:3 dilution of the specimen (Use a minimum of 50 µL of well-mixed, fresh whole blood and 100 µL of diluent)
- Mix the dilution gently but thoroughly

3 Process the Dilution

- Type the sample ID at the keyboard then press Enter
- Mix the dilution gently again
- Aspirate the dilution into the system using the Secondary mode's aspirator tip and sample bar

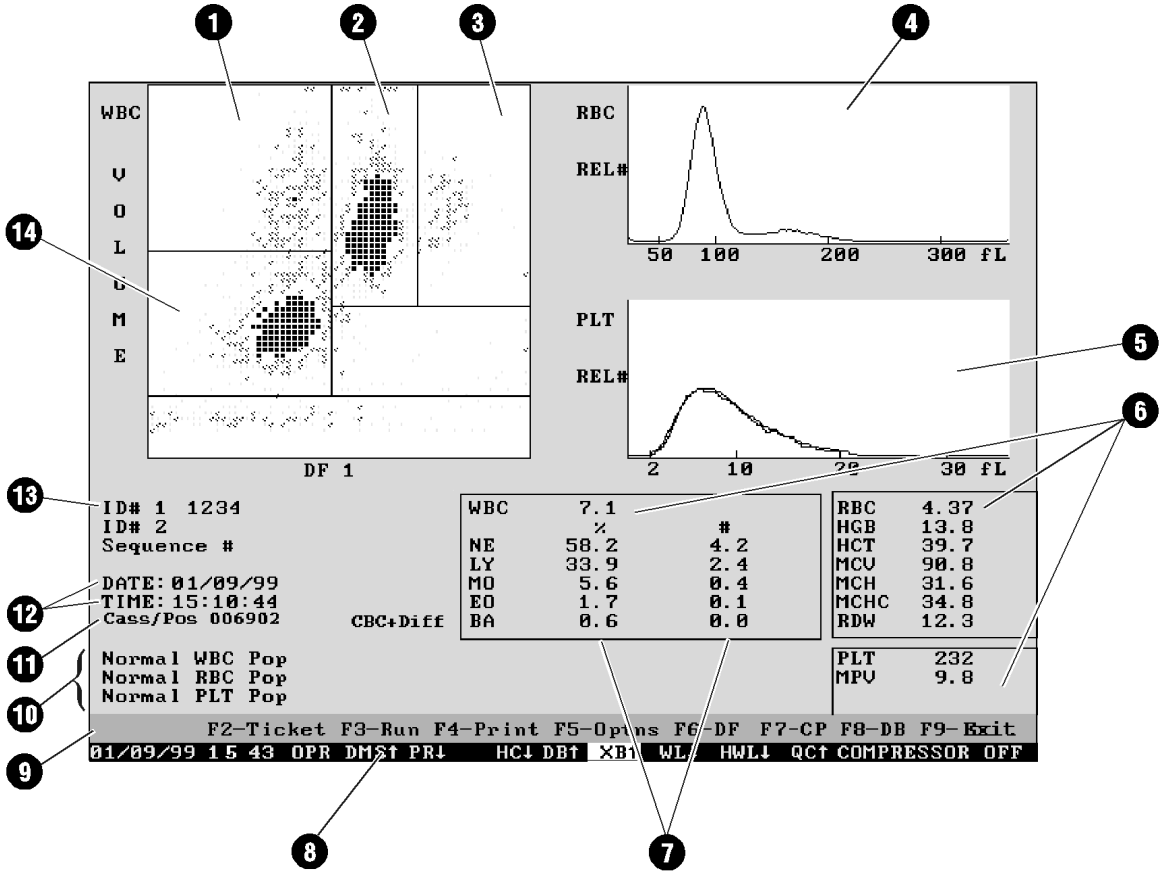
4 When the Cycle is Complete

- The instrument automatically multiplies the diluted results by 3
- Verify the sample ID entry and results before reporting
- If using the Worklist, periodically check for accumulation of Status messages

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RUN SAMPLES SCREEN

Screen Display



1. Monocyte Population
2. Neutrophil Population
3. Eosinophil Population
4. RBC Histogram
5. PLT Histogram
6. CBC Parameter Results
7. Diff Results
8. Status Line
9. Option (Functions) Line
10. Condition Messages
11. HmX Hematology Analyzer with Autoloader only
    Cass/Pos number **or**
    S = Secondary PrD = Predilute RET = Retic

    HmX Hematology Analyzer only
    Mode P = Primary S = Secondary
    PrD = Predilute RET = Retic
12. Date and Time of Analysis
13. Barcode or Keyboard Entry Identification
14. Lymphocyte and Basophil Populations

Run Samples Screen Areas

Demographics Area

IMPORTANT Risk of misidentification. Do not use the tilde (~) character in demographics, including Specimen or Patient ID.

- ID # 1
  - Alpha and/or numeric characters read from a bar-code label
  - If no barcode is read on a HmX Hematology Analyzer with Autoloader system, then dashes (- - - - - - - - - - -) are displayed
  - Alpha and/or numeric characters can be manually typed to identify a specimen to be processed in the Secondary mode (also in the Primary mode if you laboratory is using a HmX Hematology Analyzer)
- ID # 2 (Optional)
  - Enter using the Worklist
- Sequence # (Optional)
  - Enter using the Worklist
- DATE / TIME of the run
- Cass / Pos (HmX Hematology Analyzer with Autoloader only)
  - Four digit cassette / two digit position number identifying an individual specimen tube location
  - **S** indicates the sample was processed in the Secondary mode
  - **PrD** indicates the sample was processed in the Predilute mode
  - **RET** indicates the sample was processed in the Retic mode
- Mode (HmX Hematology Analyzer only)
  - **P** indicates the sample was processed in the Primary mode
  - **S** indicates the sample was processed in the Secondary mode
  - **PrD** indicates the sample was processed in the Predilute mode
  - **RET** indicates the sample was processed in the Retic mode

CBC Result Area

- WBC, RBC, and Plt results are displayed
- **F7-CP** option
  - Used to access the CBC results for each count period for troubleshooting purposes

Scatterplot

- Displays the major white blood cell groups
  - Lymphocytes
  - Monocytes
  - Neutrophils
  - Eosinophils

- Basophils (available on DF3 view only)

- **F6-DF** option is used to look at the different scatterplot views

Condition Messages

- Population condition messages appear in the lower left corner
  - *Normal WBC Pop* or *Abnormal WBC Pop*
  - *Normal RBC Pop* or *Abnormal RBC Pop*
  - *Normal Plt Pop* or *Abnormal Plt Pop*
- DMS generates condition messages from an internal algorithm and from Suspect and/or Definitive messages (not from the **H** and **L** flags)
- F11 **Cell Classification** displays the specific Suspect and/or Definitive messages
  - Only present if Suspect and/or Definitive messages are generated

NOTES

RETIC MODE

Sample Preparation Requirements

ReticPrep Reagent Kit

- Kit contains two ready to use reagents
  - Reagent A Buffered New Methylene Blue
  - Reagent B Sulfuric Acid with stabilizers
    - Not the concentrated form of sulfuric acid
    - Even though solution is dilute, handle with care
    - Avoid eye or skin contact
- Store at room temperature (20-25°C)
- Open container stability is 60 days
- Do not use the kit if Reagent B is cloudy

Equipment

- 12 x 75 mm disposable test tubes
- Disposable pipette tips
- 50 µL air displacement pipette for measuring the proper whole blood sample volume
- 2 µL air displacement pipette for measuring the proper volume of the incubated blood/stain mixture
- 2 mL dispenser (available from Beckman Coulter) for delivering the proper volume of Reagent B

Volume Requirements

- 50 µL of whole blood
- 4 drops of Reagent A
- 2 µL of the incubated blood/stain mixture
- 2 mL of Reagent B

Time Requirements

- Incubation time for the blood/stain mixture
  - Minimum 5 minutes
  - Maximum 60 minutes
- Run the dilution **exactly** 30 seconds after adding Reagent B to the blood/stain mixture

Reporting Retic Results

- Retic % is directly measured using VCS technology
- Retic # is computed based on the Retic % and the RBC count
- Retic # cannot be obtained at the time of the run
- Retic results may be printed alone or combined with CBC results

MRV and IRF Parameters

- MRV (Mean Reticulocyte Volume)
  - Average cell volume of the reticulocytes
  - Determined by the VCS technology algorithm from the retic scatterplot
  - Reported in femtoliters (fL)
- IRF (Immature Reticulocyte Fraction)
  - Parameter is based on the following factors:
    - Degree of stained reticulum (using New Methylene Blue dye) is proportional to the degree of RNA present in the cell and, therefore, the amount of light scatter produced
    - Younger retics scatter more light due to the greater quantity of RNA and typically show slightly larger volumes
  - Parameter is a ratio
    - Calculated using the count of the highest light scatter reticulocytes to the total count of the reticulocytes
    - Reported as a percentage

➤ Running Samples in the Retic Mode Summary

For HmX Hematology Analyzer with Autoloader and HmX Hematology Analyzer

1 Preparing the Sample

- For each patient tested, label two 12 x 75 mm test tubes with the patient's ID then label one tube "A" and the other "B"
- Place 4 drops of Reagent A into each of the tubes labeled "A"
- Dispense 50 µL of the well-mixed, whole-blood specimen into the tube labeled "A"
- Gently mix tube "A"
- Let the tube stand for a minimum of 5 minutes and a maximum of 60 minutes

2 Getting the DMS Ready

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**
- Press F3 **Run**
- Press F5 **RETIC**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

3 Process the Sample

- Gently mix tube "A"
- Pipette 2 µL of the patient/stain mixture from tube "A" into the bottom of tube "B"
- Place tube "B" with the patient/stain aliquot at an angle under the top of the Reagent B dispenser
- Dispense 2 mL of Reagent B into tube "B"
  - ▸ Do not mix
- Wait 30 seconds
- While waiting, type the Sample ID# at the keyboard and press Enter
- At **exactly** 30 seconds, analyze the specimen
  - ▸ Immerse the aspirator tip into the retic preparation
  - ▸ Press and release the sample bar
  - ▸ Listen for the beep or verify the status line message changes from *Aspirating* to *Diluting* **before** removing the tube

4 Reporting Retic Absolute Number

- Locate your Retic result in **Data Base Query**
- Press F3 **Edit** and enter the patient's RBC value
- The system automatically calculates the Retic #
- An **E** appears beside the RBC value and an **e** beside the Retic # value

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NOTES

WORKLIST (Optional)

- A Worklist is a list of work to be done
- Worklists are used to preassign identifiers and/or demographics
- Preassigning a Worklist is mainly done by laboratories that do not use bar-code labels → the Worklist offers an alternative that still allows positive identification of their patient samples (particularly if using a HmX Hematology Analyzer with Autoloader)

Status Messages

- Status messages appear on the Worklist in the far right column, the Status column
  - *PART ASP*
  - *NO MATCH*
  - *NO READ*

    Note: *NO READ* error messages only occur if a HmX Hematology Analyzer with Autoloader is being used and ID# 1 is set up as the primary identifier

WL on the Status Line

- **WL**↓ indicates there are no preassigned entries on the Worklist
- **WL**↑ indicates there are preassigned entries on the Worklist
- Yellow background indicates the Worklist is full (300 entries)
- Red background indicates a pattern of error messages left on the Worklist:
  - Three consecutive *PART ASP*, *NO MATCH*, or *NO READ* errors are on the Worklist
  - 10 *NO READ*, *NO MATCH*, and/or *PART ASP* error messages have accumulated on the Worklist
  - Whether triggered by three or ten error messages, the operator responds in the same manner

Operator Response

1. Press Alt + End to stop the alarm
2. Go to the Worklist and locate the error messages
   a. If any one error is occurring frequently, determine the cause of the error and take appropriate action
   b. If it appears the messages represent the accumulation of old errors, tag and delete some or all of the samples showing error messages
      - Use F7 to tag individual samples or F8 to tag all
      - Use F6 to delete tagged samples from the Worklist
3. Rerun specimens as necessary

   Note: If the Worklist is used to rerun any of these specimens, the preassigned data for any specimen with a *PART ASP* error message must be entered again.

PARTial ASPiration

- A sample processed in the Primary mode that fails the blood detection criteria is considered a partial aspiration
- When the system detects a partial aspiration, the
  - Analyzer replaces all numeric results with the (· · · · ·) code and sends this information to the DMS
  - DMS searches the Worklist for a match based on the primary identifier → once the DMS matches the sample results to the preassigned data on the Worklist:

    In the data base,
    - The (· · · · ·) codes and the preassigned data are stored in the data base together
    - *PART ASP* appears in the Sample status field

    On the Worklist,
    - Original preassigned entries remain to identify the specimen that experienced this error
    - An aspiration error is documented by placing the *PART ASP* message in the Status column to remind the operator that the specimen has been run before

Note: If the Worklist is used to rerun this specimen, the preassigned data must be reentered before rerunning the specimen.

3 Consecutive Partial Aspirations

- If there are three consecutive *PART ASP* errors messages:
  - The system stops
  - A beeping alarm sounds
  - An error message, *3 CONSECUTIVE PARTIAL ASPIRATIONS*, appears at the bottom of the screen
  - The background of the **WL** Status Line indicator turns red
- Operator response is needed to resume operation

  Note: See the Operator Response heading at the beginning of this subject.

NO MATCH

- *NO MATCH* means that the primary identifier of the cycled sample does not match any of the entries on the Worklist
- Sample results are saved to the data base with a *NO MATCH* message in the Sample status field
- On the Worklist,
  - A new entry with a *NO MATCH* message in the Status column is added to document the error
  - Original preassigned data entries remain on the Worklist so this data is still available for matching

3 Consecutive *NO MATCH* Messages

- If there are three consecutive *NO MATCH* error messages:
  - The system stops
  - A beeping alarm sounds
  - An error message, *3 CONSECUTIVE NO MATCHES*, appears at the bottom of the screen
  - The background of the **WL** Status line indicator turns red
- Operator response is needed to resume operation

  Note: See the Operator Response heading at the beginning of this subject.

NO READ

Note: *NO READ* error messages only occur if a HmX Hematology Analyzer with Autoloader is being used

- A *NO READ* error message occurs only when ID# 1 is a primary identifier and the bar code on the specimen tube is either not read or a specimen tube is processed without a bar-code label
  - ID# 1 is the bar-code number from the specimen tube
  - If the bar-code label on the specimen tube is not read, there is no way to match the sample results to an entry on the Worklist
- When the bar-code label on a specimen tube is not read,
  - The Analyzer replaces all numeric results with the (· · · · ·) code and sends this information to the DMS
  - In the data base,
    - ID# 1 field contains (- - - - - - - - - - -)
    - Dots (· · · · ·) appear in all parameter fields
    - *NO READ* appears in the Sample status field
  - Original preassigned data entries remain on the Worklist so this data is still available if the bar-code label is read successfully when the specimen is cycled again

3 Consecutive *NO READ* Messages

- If there are three consecutive *NO READ* error messages:
  - The system stops
  - A beeping alarm sounds
  - An error message, *3 CONSECUTIVE NO READS*, appears at the bottom of the screen
  - The background of the **WL** Status line indicator turns red
- Operator response is needed to resume operation

  Note: See the Operator Response heading at the beginning of this subject.

10 NO READ, NO MATCH, PART ASP

- If any combination of 10 *PART ASP*, *NO MATCH* and/or *NO READ* errors accumulate on the Worklist:
  - The system stops
  - A beeping alarm sounds
  - An error message appears at the bottom of the screen
  - The background of the **WL** Status line indicator turns red
- Operator response is needed to resume operation

  Note: See the Operator Response heading at the beginning of this subject.

OBJECTIVES

When the subject is complete, you will be able to . . .

Data Base Query

- Define the functions of the **Data Base Query** option.
- Explain the areas of the Data Base Query screen.
- Define each function key of the Data Base Query screen.
- List the sort screen options.
- Describe the process of sorting samples in the data base.
- Use **F3-Edit** and **F12-DB** to review sample results.
- Add demographics and/or edit a result.
- Use the print function to generate a line list printout of sorted sample results.
- Print single sample results from the data base in a graphics and/or ticket format.
- Tag and batch print results from the data base in graphics format.
- Tag and batch archive results from the data base.
- Tag and batch transmit results from the data base to a host computer, if applicable.

Basics of CBC Analysis

- State the Coulter Principle.
- State the relationship of resistance, cell volume, and pulse height.
- Define coincidence correction, threshold, count periods, and voting.
- Explain the concepts of channelization and pulse editing.
- Explain how pulses are used to develop a histogram.

RBC Parameters

- List the individual parameters that comprise the RBC Profile.
- Identify which parameters in the RBC Profile are directly measured, derived from the RBC histogram, or computed.
- Compute Hct, MCH, and MCHC results.
- Explain the process of building an RBC histogram.
- Explain the area of the RBC histogram used to determine the MCV parameter.
- Explain the area of the RBC histogram used to determine the RDW parameter.
- Describe a typical RBC histogram.

Plt Parameters

- List the individual parameters that comprise the Plt Profile.
- Identify which parameters in the Plt Profile are directly measured, derived from the Plt histogram, or computed.
- Explain the purpose of sweep flow.
- Explain the process of building a Plt histogram.

- Explain the area of a typical Plt histogram used to determine the Plt count and MPV parameter result.
- Describe a typical Plt histogram.
- State the conditions that generate a “no-fit” platelet result.

WBC Parameters

- List the individual parameters that comprise the WBC Profile.
- Identify which parameters in the WBC Profile are directly measured, derived from the scatterplot, or computed.

VCS Technology Basics

- Explain how Erythrolyse II and StabiLyse prepare the Diff sample for analysis.
- Differentiate between DF1, DF2, and DF3 by the technology represented on the X-axis.
- Label the four areas of the DF5 scatterplot.

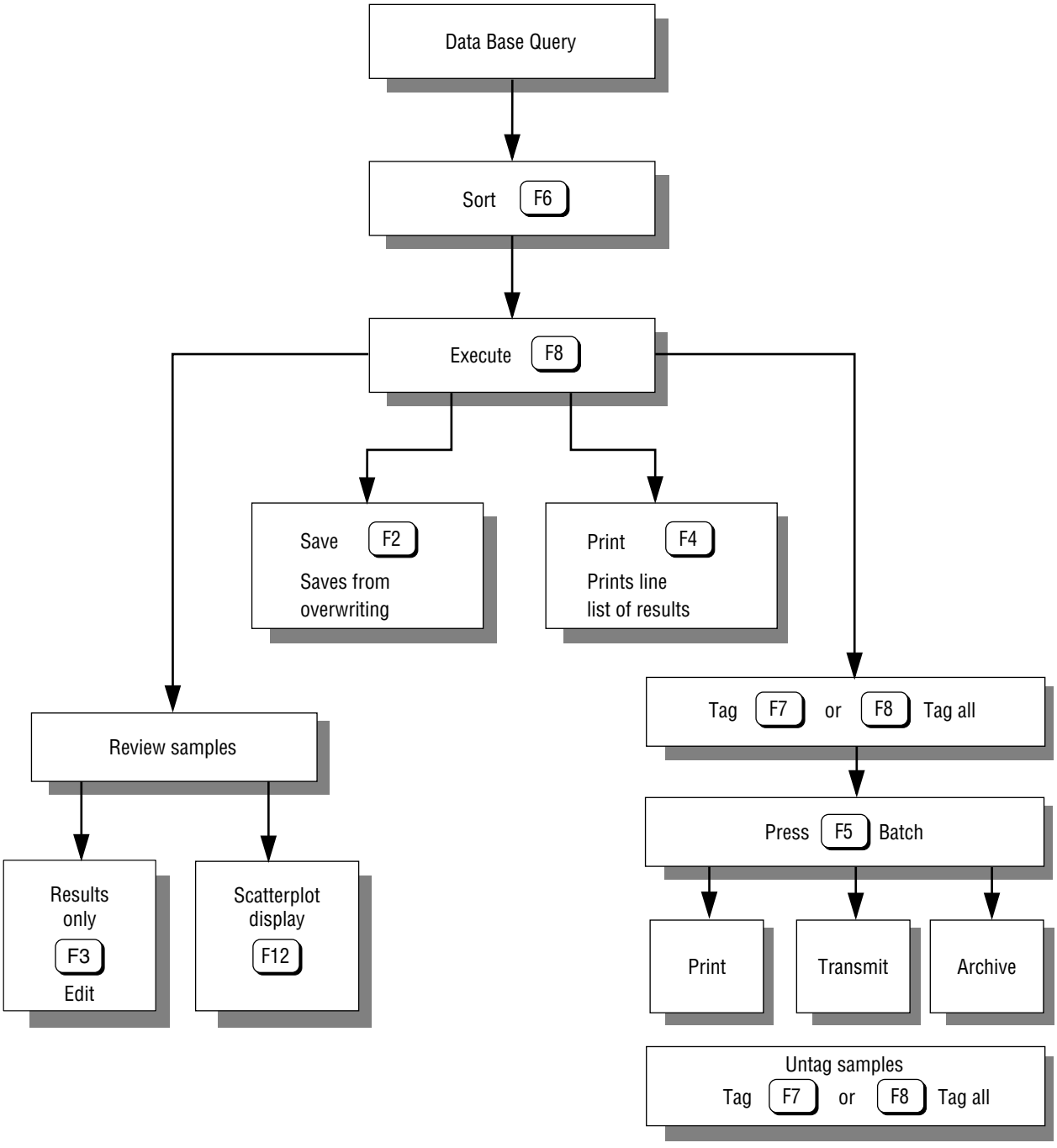
Flagging

- List the two types of Operator Definable flags.
- Differentiate between Suspect and Definitive flags.

DATA BASE QUERY

- Option allows an operator to sort, retrieve, review, print, transmit, archive to diskette and mark for saving previously stored sample results
- Archive feature allows an operator to copy patient result data from the DMS onto a diskette and retrieve it on another computer in a spreadsheet format

  Note: Spreadsheet program must be compatible with the WKS format.
- Data base stores results of up to 5,000 samples; sample 5,001 overwrites the oldest sample not marked for saving



➤ DATA BASE QUERY QUICK REFERENCE GUIDE

If the *SELECT FUNCTION* prompt isn't displayed, press F9 **STOP**

From the Main Menu, select **Sample Analysis ▸▸ Data Base Query**

To Sort Sample Data

1 Press F6 **Sort** if the Sort window is not displayed

2 Select the group of samples you want to review or work on

- To find samples run during a certain time period, enter the DATE and TIME in the appropriate FROM and TO fields (enter "00" for seconds)
- To locate a specific sample,
  - DATE and TIME field entries may be cleared, if desired (use F6 **Clear**)
  - Use ↓ or Enter to go to ID# 1 or ID# 2 (if the sample was identified using the Worklist)
  - Type the sample identification **exactly** as it was entered when the sample was run
- Sort Rules for setting up a successful sort:
  - You can sort by ID# 1 or ID# 2, but not both
  - Pay close attention to case, commas and spaces when sorting by name
  - Time requires entry of hour, minute, and seconds
  - If you select a time period, that time period applies for all days you included
  - You can sort samples based on whether they are flagged (**F**), not flagged (**N**), or both (**B**)

3 Press F8 **Execute** to activate the sort

- Selected samples, including date and time run, should appear almost immediately
- If the samples you selected do not appear, check the sort criteria you entered against the requirements stated in the Sort Rules above

To View Sample Results

1 Execute a sort

2 Use ↑ or ↓ to highlight the desired sample

3 Press F12 **DB** → the Run Samples display of this sample's results, scatterplot, and histograms appears

To Save Sample Data

1. Execute a sort
2. Use ↑ or ↓ to highlight the sample to be saved
3. Press F2 **Save** → an **S** appears in the Output field to indicate the sample is saved from overwriting

   Note: Use this same routine to unsave a sample.

To Edit Sample Data

1. Execute a sort
2. Use ↑ or ↓ to highlight the sample to be edited
3. Press F3 **Edit** to view all the identifiers that were entered from the Worklist and/or read from a bar-code label as well as the individual parameter results
   - Press End or ↑ to view any suspect and/or definitive messages, or an overrange result estimate

     Note: Overrange result estimate may be used to determine the dilution ratio needed to bring the overrange result within the linear or operating range.
   - Press Home or ↓ to return to the parameter result screen
4. Edit results according to the following guidelines and press Enter to save each individual input then press F10 **Save/Esc** when completely finished
   - Any field except the primary identifier, sequence number or the date and time a sample is processed may be edited provided a *NO READ, NO MATCH*, or *PART ASP* message **does not** appear in the Status field
     - *NO READ* error messages only occur on a HmX Analyzer with Autoloader
   - You may edit parameter results that are replaced with (+ + + + +), (- - - - -), or (: : : : :) codes
   - Editing a principle parameter requires changing all dependent (computed) parameters
     - Edit the WBC and the diff absolute numbers must also be changed
     - Edit the RBC and the Hct, MCH, MCHC, and Retic # must also be changed
     - Edit the Hgb and the MCH and MCHC must also be changed
     - Edit the MCV and the Hct and MCHC must also be changed
     - Edit the Retic % and the Retic number must also be changed
   - **E** overrides an **H** or **L** flag and appears in the same position

- When an operator edits any parameter field, an *Edited Data* message appears in the Population Condition field on the following:
  - Sample analysis display
  - Sample analysis data base displays
  - With RBC and Retic # if transmitted to a host computer
  - Printouts
- When an operator edits any parameter, all Suspect and Definitive messages for that sample are deleted from the data base and all displays

To Output Sample Data: Print and Reprint / Transmit and Retransmit

To Print a Single Sample Data Set

1 Execute a sort

2 Use ↑ or ↓ to highlight the desired sample

3 Press F12 **DB** → the Run Samples display of this sample's results, scatterplot, and histograms appears

- For a ticket format printout, press F2 **Ticket**
- For a graphics format printout, press F4 **Print**

To Batch Print or Transmit a Group of Samples

1 Execute a sort

2 Tag the desired samples using F7 **Tag** or F8 **Tag All**

3 Press F5 **Batch**

4 Use ↑ or ↓ to highlight Print or Host and Spacebar to toggle to *Yes*

Note: Batch print (in graphics format), transmit, and archive functions must be done independently. When one of these three options is selected, the other two are automatically disabled.

5 Verify the needed peripheral (printer and/or host computer) is online

6 Press F8 **Execute** to start the process

- As needed, use F7 **Abort** to stop the process and F6 **Resume** to begin where it left off when stopped

7 Press Esc to return to the data base

To Print a Condensed Line List

1 Execute a sort

2 Press F4 **Print** to produce a condensed line list printout of all sorted samples

To Archive a Group of Stored Sample Results

1. Execute a sort
2. Tag the desired samples using F7 **Tag** or F8 **Tag All**
3. Press F5 **Batch**
4. Move the cursor to the Archive field then press F2 **Choice List**
5. Use the Spacebar to select either New (process all tagged samples not yet archived) or All (process all tagged samples, archived or not) then press Enter
6. In the Filename: field, type A:\ then up to eight characters (of your choice)
7. Insert a formatted diskette into the DMS diskette drive and press F8 **Execute**
8. When the *Batch is Inactive* message appears, remove the diskette from the drive

To Review Archived Files

1. Insert the diskette into a computer with a spreadsheet program that is compatible with the WKS format
2. Retrieve the desired file
   - If a column label is condensed either widen the column or move the active cell cursor to the label to see the complete label
   - One character codes include:

Fields	**Key for One Character Codes**
C/P Edit	0 = Not edited 1 = Edited
ID1	0 = Not edited 1 = Edited
Status	0 = Blank / Not used / Matched 1 = Partial Aspiration 2 = No Match 3 = No Read
WBC / RBC / Plt Population	0 = Normal 1 = Abnormal 2 = Edited
All Suspect and Definitive flags **except** Imm Grans / Bands	blank = No flag 1 = Flag
Imm Grans / Bands Suspect Flag	blank = No flag 1 = IMM GRANS / BANDS 1 2 = IMM GRANS / BANDS 2
Mode of Aspiration	1 = Primary (Closed-Vial) mode 2 = Secondary (Open-Vial) mode

3. Close the file and remove the diskette from the diskette drive

➤ ➤

NOTES

BASICS OF CBC ANALYSIS

Sample Dilutions

- During aspiration, whole blood is pulled through the BSV
- Once aspiration is complete, the center section of the BSV rotates to segment the whole blood sample into three portions
  - Whole blood contained in the center section's 1.6 µL drillout is used to make the RBC bath dilution
  - 28.0 µL of whole blood contained inside the right section and WBC loop is used to make the WBC bath dilution
  - 28.0 µL of whole blood contained in the center section's WBC Diff loop is used to make the sample preparation needed for differential analysis using VCS technology
- Two dilutions are made for CBC analysis: WBC and RBC

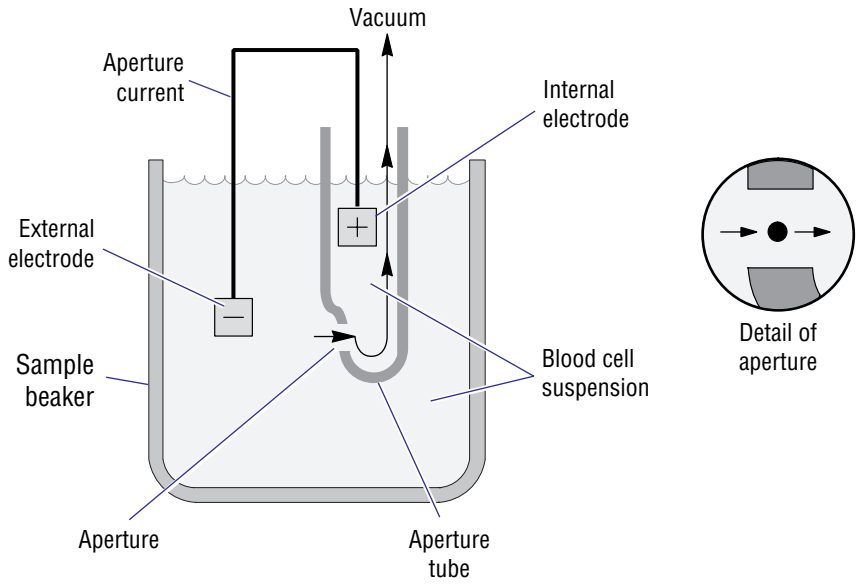
RBC Dilution

- 10.0 mL of diluent dispensed by the RBC dispenser pushes the 1.6 µL of whole blood segmented by the BSV into the RBC aperture bath to make the final dilution
- Information obtained from the RBC dilution includes:
  - RBC count which is determined using the Coulter Principle
  - RBC histogram from which MCV and RDW parameter results are derived
  - Plt histogram from which the Plt count and MPV parameter results are derived

WBC Dilution

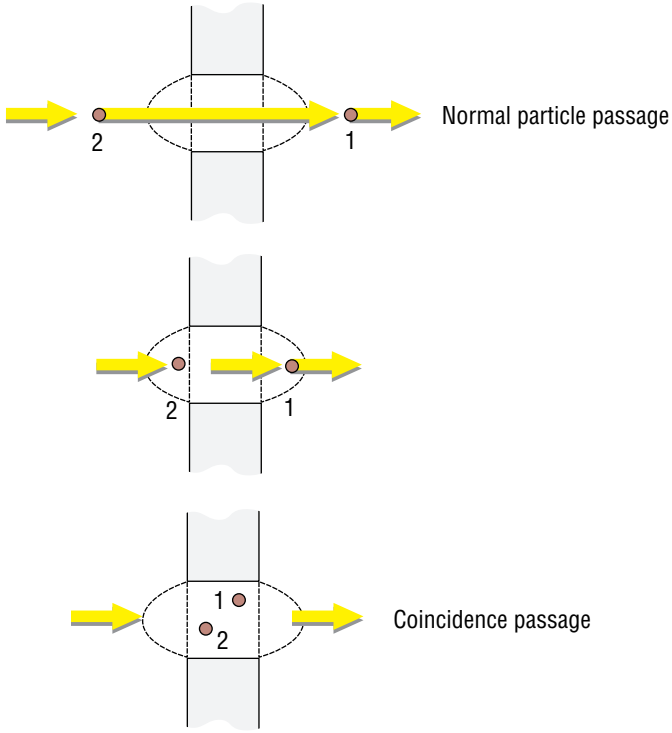
- 6.0 mL of diluent dispensed by the WBC diluent dispenser pushes the 28.0 µL of whole blood segmented by the BSV towards the WBC aperture bath → approximately three inches before this dilution enters the bath, 1 mL of CBC lytic reagent is added to make the final dilution entering the WBC bath
- Information obtained from the WBC dilution includes:
  - WBC count which is determined using the Coulter Principle
  - Hemoglobin which is directly measured using a photometric technique based on Beer's Law

The Coulter Principle



- The Coulter Principle is an electronic method for counting and sizing particles; it is based on the fact that cells, which are poor conductors of a weak current, will interrupt the current flow
- The cells are suspended in a conductive diluent and are pulled through the aperture by a low vacuum; this creates a momentary increase in resistance to the electronic flow
- This resistance creates a pulse that is sensed and counted by the instrument as a particle; the amount of resistance (the amplitude or height of each pulse) is directly related to the size (volume) of the particle that produced it

Coincidence Correction



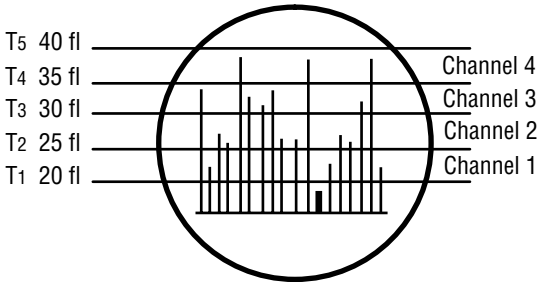
- Occasionally, more than one cell goes through the aperture at the same time and the analyzer counts only one pulse
- Phenomenon is referred to as coincidence
- Since the frequency of coincidence is statistically predictable for a particular count, the system computer makes the correction

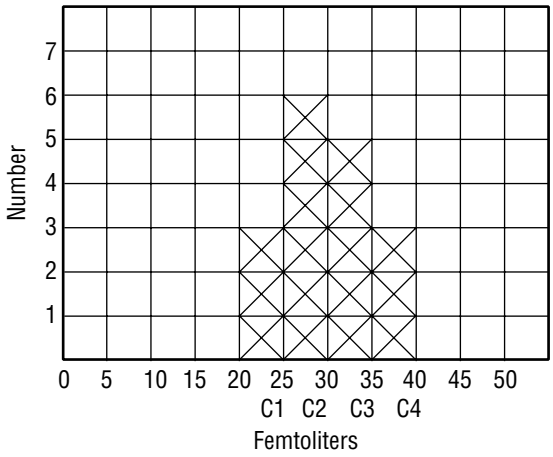
Count Periods

- Raw data for determining the WBC and RBC counts is collected over three consecutive time periods of 4-seconds each
  - May be referred to as count periods or triplicate counting
  - After the computer corrects for coincidence, it votes on the results of the three count periods
- Extended sensing may be initiated when additional data is needed for development of these size distribution curves called histograms

Thresholds

- Thresholds are electronically set size limits
- Particles, such as debris, are excluded from the analysis
- Particles equal to or above the threshold are analyzed
- WBC lower threshold is 35 fL; no upper threshold for counting
- RBC lower threshold is 36 fL; no upper threshold for counting
- Plt lower threshold is 2 fL and upper threshold is 20 fL





Histogram Development

- The lowest threshold (T_1) is sometimes referred to as the Base Threshold
- A succession of thresholds are used to sort particles by size and produce a size distribution curve
- The area between two adjacent thresholds is called a Channel
  - Channel 1 (C1) is between T_1 and T_2
  - Channel 2 (C2) is between T_2 and T_3, and so forth
  - Each threshold represents a size
  - For a particle to be counted in a particular channel, it must be larger than or equal to the lower threshold but smaller than the upper threshold
- To produce a particle size distribution curve or histogram, the number of particles is plotted on the Y-axis as Relative Number of particles and the particle size (or volume) on the X-axis as Femtoliters (fL)
- Histogram information may be used to determine the mean (average) size of the particles, the dispersion of particles around the mean size, and subpopulations within a main population

Pulse Editing

- When cells pass through the aperture near the edge or at an angle rather than at the center, atypical pulses result
- Pulse editing is used in the construction of the RBC histogram to eliminate these atypical pulses from influencing size measurements

Parameters Derived from a Histogram

- HmX instruments use the Coulter Principle and extended sensing to develop RBC and Plt histograms
  - Three individual histograms are developed
  - MCV and RDW parameter results are derived from each RBC histogram
  - Plt and MPV parameter results are derived from each Plt histogram
  - The three results generated for each parameter go through the voting process

Voting

- Each count period generates three WBC, RBC, Plt, MCV, RDW, and MPV parameter results that go through the voting process
- Voting is a comparison of the three results with each other to verify at least two of the three results are in close agreement
- The final reported result is the average the two or three parameter results that agree
  - Exception → if count period 1 does not agree with count periods 2 and 3, a total voteout (- - - - -) is automatically generated since this disagreement may be due to a partial blockage of the aperture

Total Voteout

- Occurs when none of the three results agree or anytime count period 1 does not agree with count periods 2 and 3
- Parameter result and any result computed using the affected parameter is replaced with a (- - - - -) or (· · · · ·) code
- For more information about flagging, see Chapter 4 of the Operator's Guide

Single Count Period Voteout

- Occurs when a result from count period 2 or 3 disagrees with the other two results

  Note: Anytime count period 1 does not agree with count periods 2 and 3, a total voteout (- - - - -) is automatically generated.
- If only two of the values are in agreement, the computer disregards the third and reports the average of the two similar results
  - ***V** parameter code appears next to the parameter result
  - **V** appears next to any parameter result computed using the affected parameter
- For more information about flagging, see Chapter 4 of the Operator's Guide

NOTES

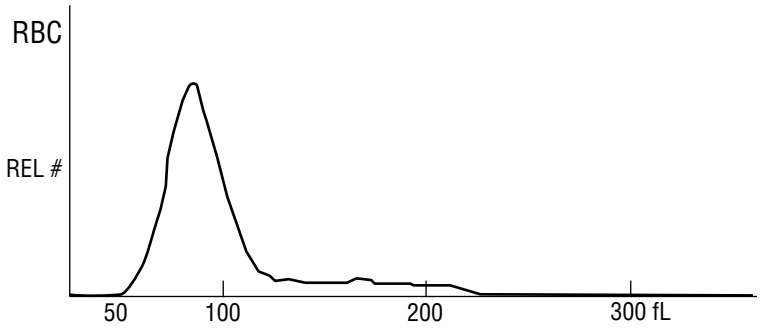
RBC PARAMETERS

RBC Count

- RBC count is obtained from the RBC dilution
- RBC dilution contains red blood cells, white blood cells, and platelets
  - Thresholds are used to separate the platelet pulses, which are smaller, from the red and white blood cell pulses
  - Particles 36 fL or larger are counted as red blood cells
  - Since white blood cells fall in the red blood cell size range, they are counted and sized as RBCs
  - WBCs are not gated out of the count because any interference is usually insignificant because there are normally very few WBCs (thousands) in comparison to the number of RBCs (millions)

RBC Histogram

- RBC histograms reflect the native size of the red blood cells and any other particle in the red blood cell size range
- RBC histograms provide the information for determining two different descriptors of the red cell population
  - MCV (Mean Cell Volume)
  - RDW (Red cell Distribution Width)



Characteristics of a Typical RBC Histogram

- Typical RBC histograms have two populations of cells:
  - The main population of red cells begins above 50 fL and is visually a symmetrical, bell-shaped curve (a narrow Gaussian distribution)
  - The smaller population (called the "toe") appears to the right of the main population
    - Represents white blood cells, doublets, triplets, agglutinates, and artifacts
    - The white blood cells included in the RBC histogram are normally insignificant in number
- Graphic display begins at 24 fL and ends at 360 fL
- RBC base threshold is 36 fL
- MCV is derived from the data between 36 fL and 360 fL
- RDW is a measure of the distribution (variation or spread) in the size in a population of red cells

RDW (Red cell Distribution Width)

- RDW is a percent measurement of the variability (CV) in the size of the red cells based on the main population
- $$\%CV = \frac{SD}{Mean} \times 100$$
- Generally, as the red cell variation increases, the width of the distribution curve broadens and visible asymmetry of the distribution curve is more likely
- Width of the distribution curve, however, does not always indicate a true increase in size variation
- CV more accurately detects red cell variation than a visual inspection of the histogram (unless two distinct populations of cells are present)

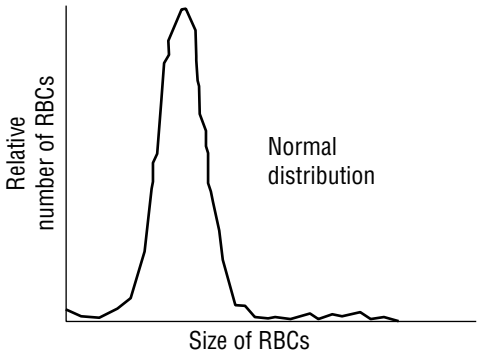
  Note: A primarily microcytic population of red cells (decreased MCV) tends to have a tighter distribution (lower SD) than a normocytic population. A primarily macrocytic population of red cells (increased MCV) tends to have a wider distribution (higher SD) than a normocytic population.

  Because a CV is calculated by dividing the SD by the population's mean (MCV), a primarily microcytic, normocytic, and macrocytic population of red cells can have a normal RDW even though visually the distributions appear different.

Flagging the RDW

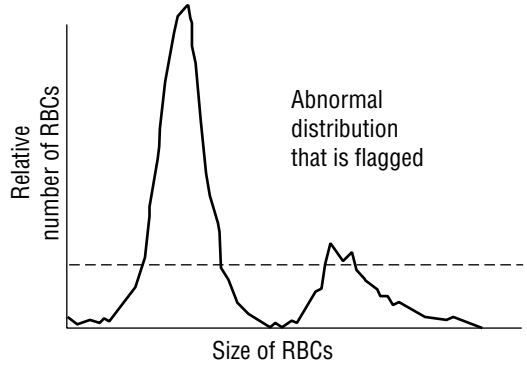
Normal Distribution

- RDW is a percent measurement of the variability (CV) in the size of the red cells based on the main population
- Since the areas to the left and to the right of the main population vary (sometimes significantly due to interferences or a patient abnormality), the computer disregards both extremes of the distribution curve to eliminate misleading data
- Analyzing only the main population provides a more stable measurement



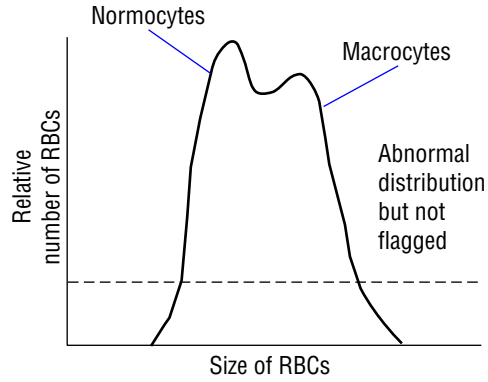
Abnormal Distribution

- An **R** flag next to the RDW result indicates the distribution exhibits two distinct, well-separated populations of red cells (sometimes referred to as excessive asymmetry)
- Indicates the secondary population (usually WBCs or giant platelets) was excluded from the calculation
- The **R** flag next to the RDW result alerts the operator that the RDW result is inaccurate; review a blood smear



Abnormal Distribution

- Width of the distribution curve correlates with an increased variation in the size of the red cells
- RDW result derived from an RBC histogram such as this would be increased, indicating the presence of anisocytosis
- Even though there is a dip in the distribution it is not distinct and well-separated as in the previous illustration
- An **H** flag appears next to the RDW result when the result exceeds the laboratory-set patient high action limit



Hemoglobin

- Hgb value is determined from the WBC dilution
- The addition of CBC lytic reagent disrupts the red cells to free the hemoglobin which is then converted to a stable pigment
- Converted hemoglobin is directly measured using a photometric technique based on Beer's Law

Computed Parameters

- Hct, MCH, and MCHC are computed parameters

RBC Parameters

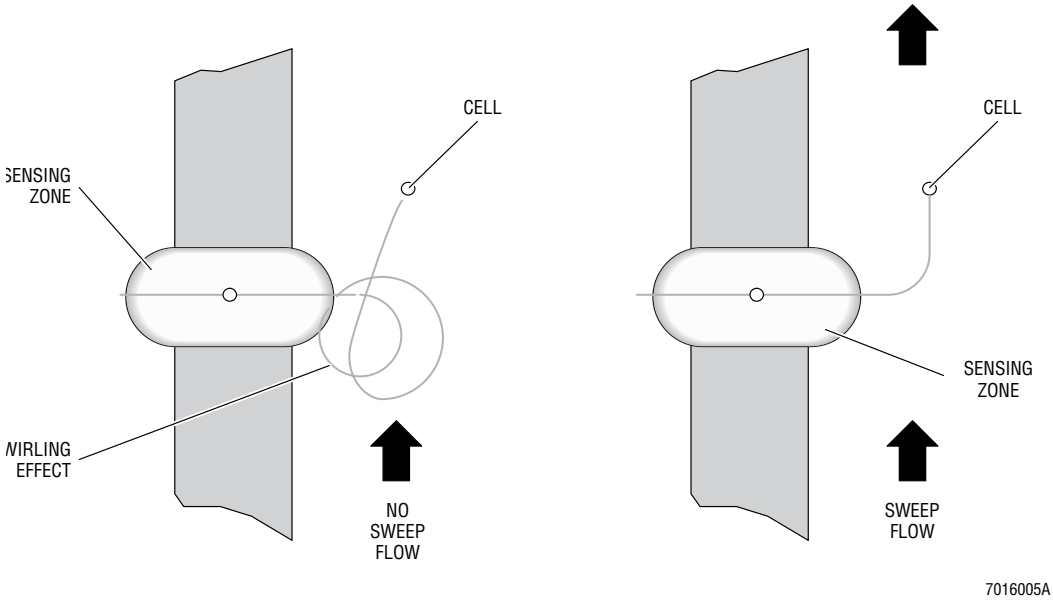
Category	Parameter	Source of Data
Directly Measured	RBC (Red Blood Count)	Coulter Principle
	Hgb (Hemoglobin Concentration)	Photometric Measurement
Derived from the RBC Histogram	MCV (Mean Corpuscular Volume)	RBC Histogram (Developed using the Coulter Principle)
	RDW (Red Cell Distribution Width)	RBC Histogram (Developed using the Coulter Principle)
Computed	Hct (Hematocrit)	$\frac{RBC \times MCV}{10}$
	MCH (Mean Corpuscular Hemoglobin)	$\frac{Hgb \times 10}{RBC}$
	MCHC (Mean Corpuscular Hemoglobin Concentration)	$\frac{Hgb \times 100}{Hct}$

PLATELET PARAMETERS

- Platelet parameters are obtained from the RBC dilution

Sweep Flow

- Sweep flow is a steady stream of diluent that flows behind the RBC aperture during sensing periods
- Without sweep flow (Figure A), there is a characteristic swirling of the dilution at the outlet of the aperture so that red (or white) cells caught up in these eddies behind the aperture may reenter the sensing zone and produce small pulses that could be counted as platelets
- With sweep flow (Figure B), the steady stream of diluent prevents the formation of these eddies

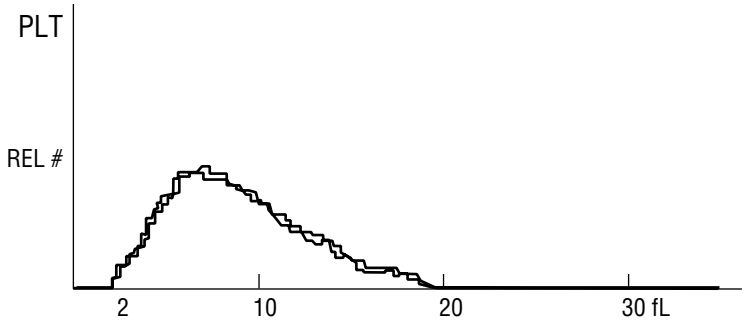


Plt Histogram

- Platelet histograms reflect the native size of the platelets and any other particle in the platelet size range
- Platelet histograms provide the information for determining three different descriptors of the platelet population using 64 channels
  - Platelet count
  - MPV (Mean Platelet Volume)
  - PDW (Platelet Distribution Width) results are not displayed but are used as an internal check in the platelet algorithm

    Note: Pct (Plateletcrit) is also a platelet parameter. Pct is computed using the Plt count and MPV results. No clinical significance has been identified for the Pct pararmeter.

    Pct and PDW are not FDA approved parameters; therefore, these parameter labels and results are not routinely displayed in the United States, but may be routinely displayed in other countries.



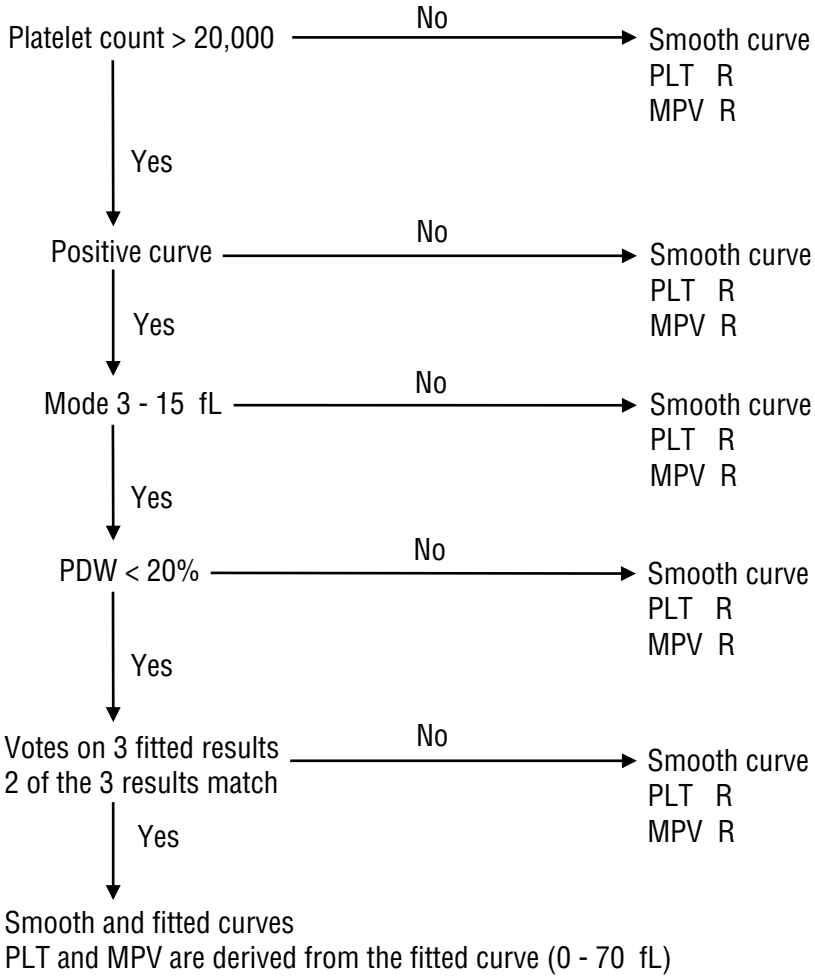
Characteristics of a Typical Platelet Histogram

- Typical platelet histograms are log normal and have two curves:
  - A smooth curve extending from 2 to 20 fL
    - The term “smooth” does not refer to the “look” of the curve but rather to the use of the moving average technique to smooth out the peaks and valleys of the raw data
    - The smooth curve represents the actual particles counted (raw data) during the sensing part of the instrument’s cycle
  - A fitted curve extending from 0 to 70 fL, but displayed from 0 to 36 fL (display is physically cut off at 36 fL)
    - This fitted curve represents the line of best fit for a log normal distribution based on the best part of the raw data collected between 2 and 20 fL
- Fitted platelet results (including Plt count, MPV, and PDW) are derived from the area under the fitted curve from 0 to 70 fL

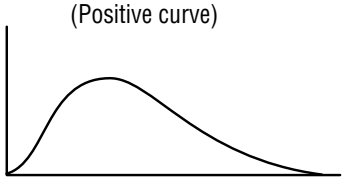
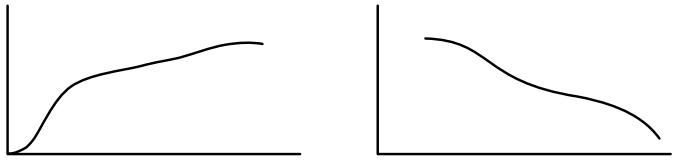
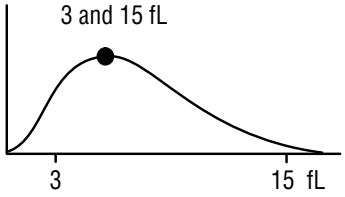
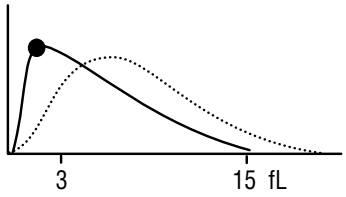
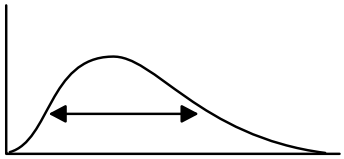
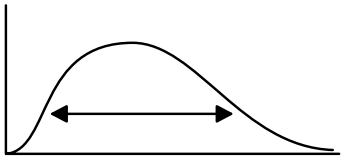
Advantages of the Fitting Process

- To include small or large platelets, if present
- To eliminate interference, if present

Criteria for Fit and No-Fit



Criteria for Fit and No-Fit (continued)

Criteria for PLT-Fit	Criteria for PLT No-Fit
> 20,000 Platelet count	< 20,000 Platelet count
Log normal curve (Positive curve) 	Non-positive curve 
Mode between 3 and 15 fL 	Mode <3 fL or >15 fL 
PDW < 20% 	PDW > 20% 
Platelet count: Average of fitted results	Total voteout of fitted curve results

Platelet Parameters

Category	Parameter	Source of Data
Derived from the Plt Histogram	Plt (Platelet Count)	Plt Histogram (Developed using the Coulter Principle)
	MPV (Mean Platelet Volume)	Plt Histogram (Developed using the Coulter Principle)
	PDW (Platelet Distribution Width)	Plt Histogram (Developed using the Coulter Principle)
Computed	Pct (Plateletcrit)	$\frac{Plt\ count \times MPV}{10}$

WBC PARAMETERS

WBC Count

- Total WBC count is obtained in the WBC bath after the addition of CBC lytic reagent
- All particles in the WBC dilution 35 fL or greater are counted as white blood cells
- Any interference detected at the 35 fL WBC threshold generates an ***R** flag next to the WBC count result, indicating failure of the lower WBC threshold check

WBC Differential

- WBC scatterplot is developed using VCS technology
- A five part differential that includes neutrophil %, lymphocyte %, monocyte %, eosinophil %, and basophil % is derived from this scatterplot
- Neutrophil #, lymphocyte #, monocyte #, eosinophil #, and basophil # are computed based on the cell percentage derived from the scatterplot (obtained from the VCS portion of the instrument) and the WBC count *(obtained from the CBC portion of the instrument)*

WBC Parameters

Category	Parameter	Source of Data
Directly Measured	WBC (White Blood Count)	Coulter Principle
Derived from the WBC Scatterplot	NE % (Neutrophil Percentage)	VCS Measurements
	LY % (Lymphocyte Percentage)	VCS Measurements
	MO % (Monocyte Percentage)	VCS Measurements
	EO % (Eosinophil Percentage)	VCS Measurements
	BA % (Basophil Percentage)	VCS Measurements
Computed	NE # (Neutrophil Absolute Number)	$\frac{Neut\% \times WBC}{100}$
	LY # (Lymphocyte Absolute Number)	$\frac{Lymph\% \times WBC}{100}$
	MO # (Monocyte Absolute Number)	$\frac{Mono\% \times WBC}{100}$
	EO # (Eosinophil Absolute Number)	$\frac{Eos\% \times WBC}{100}$
	BA # (Basophil Absolute Number)	$\frac{Baso\% \times WBC}{100}$

VCS TECHNOLOGY BASICS

Applications of the VCS Technology

Differential Analysis

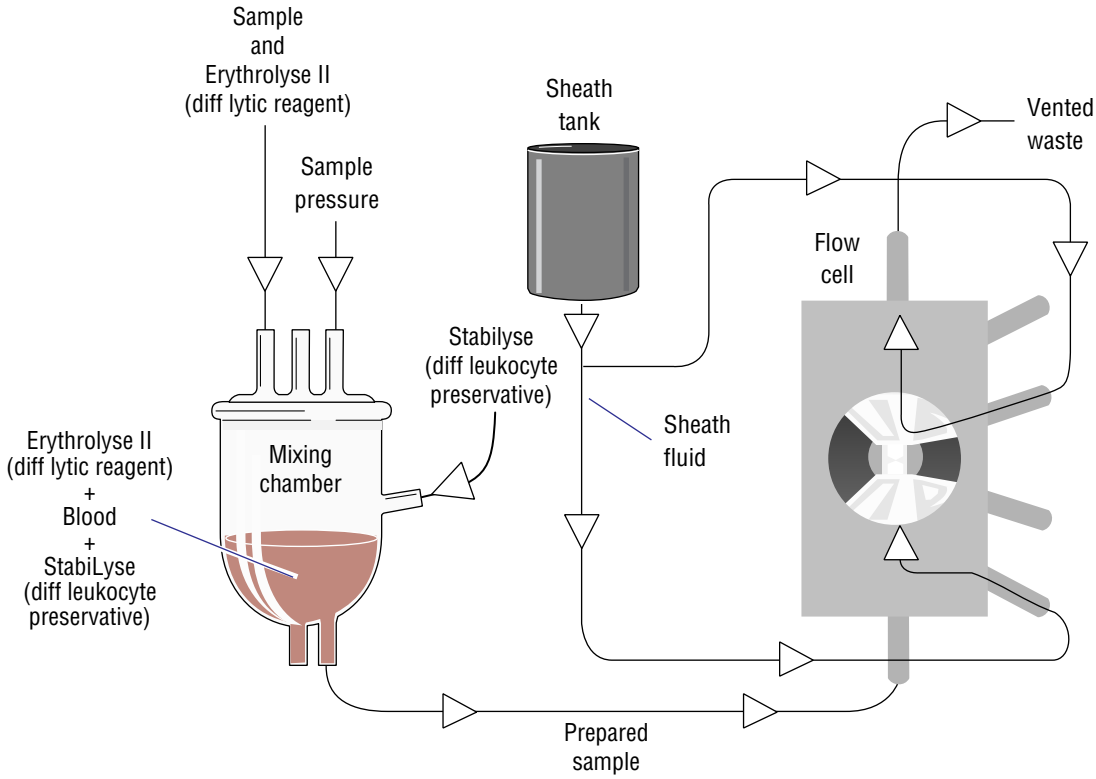
- First application of the VCS technology was to develop a scatterplot from which a five part differential could be derived
- Neutrophil %, lymphocyte %, monocyte %, eosinophil %, and basophil % are derived from the WBC scatterplot

Reticulocyte Analysis

- Early in 1994, Coulter Corporation adapted the VCS technology to perform reticulocyte analysis
- Uses the same VCS technology but a different reagent system
- Analyzes RBCs rather than WBCs

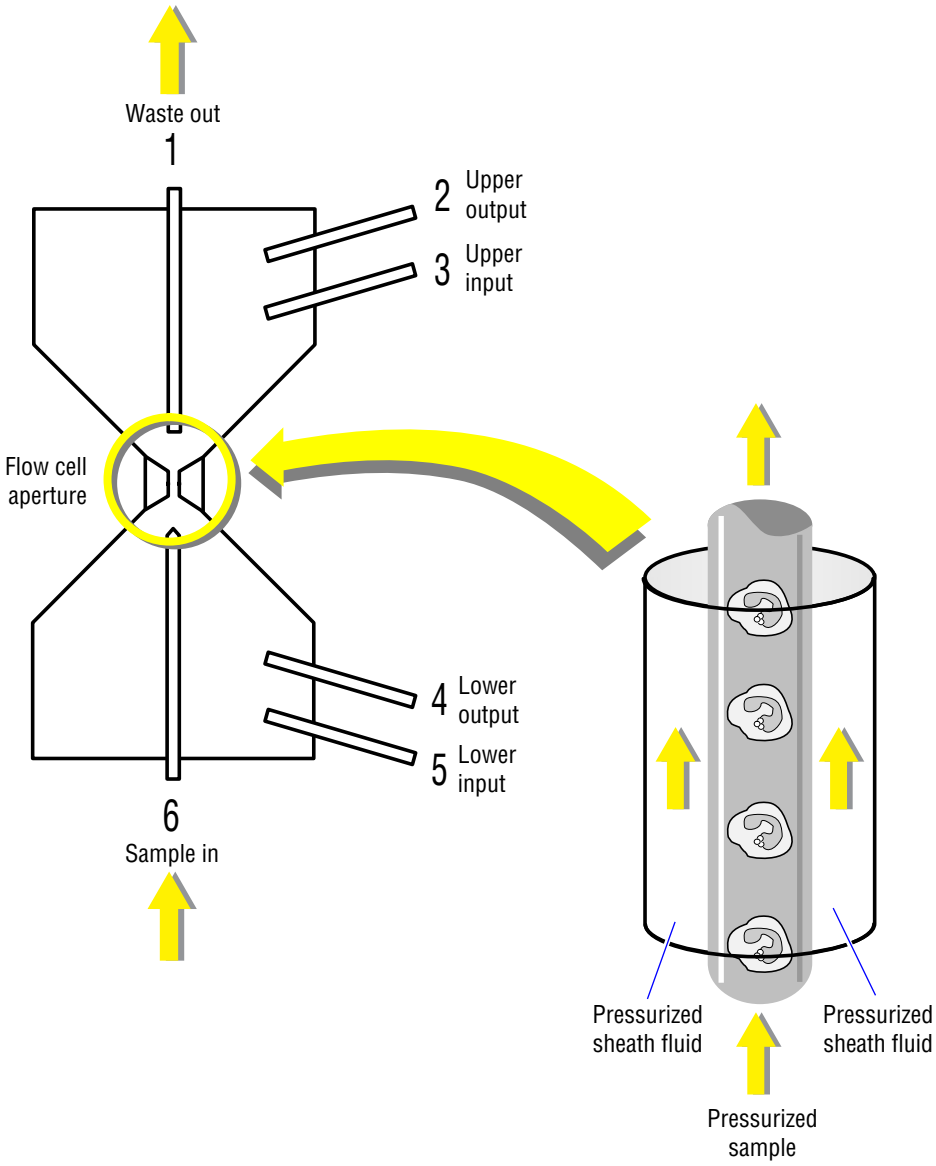
Differential Analysis

Preparing the Sample



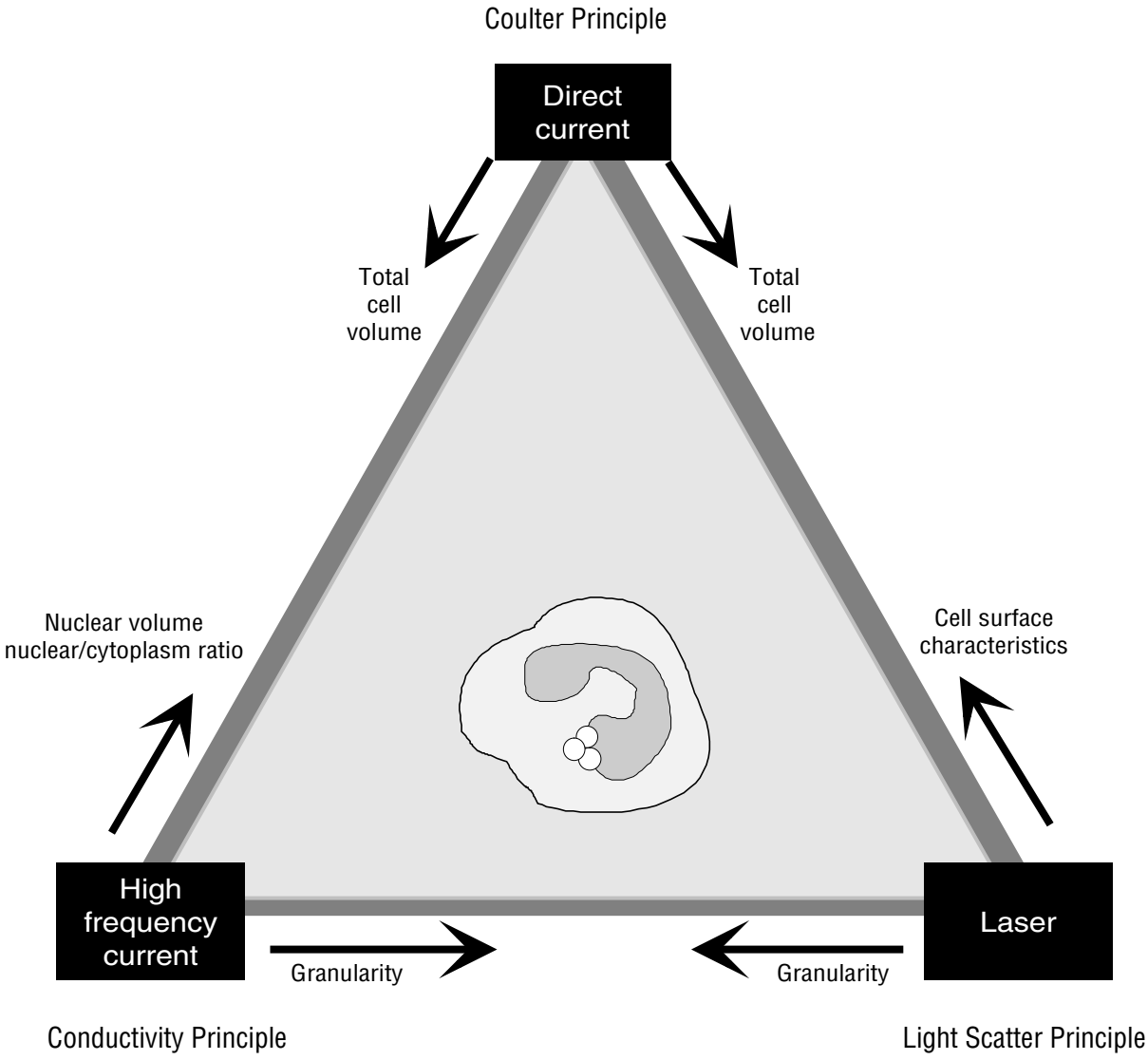
- The Erythrolyse II reagent pumps dispense the Diff lytic reagent to pick up and deliver the Diff portion of the whole blood sample to the Mixing Chamber
- As the Mixing Chamber rotates to mix this dilution of whole blood and Erythrolyse II reagent, a cytochemical reaction occurs that ruptures the red cell membranes, destroys platelets, and dissolves any cellular debris
- At just the right moment during mixing, the StabiLyse reagent pump dispenses Diff Leukocyte Preservative to stop the reaction and stabilize the WBC subpopulations
- Prepared sample is sent to the flow cell for analysis
- White blood cells are analyzed in their near-native state

Analyzing the Sample



- Sample pressure, applied to the Mixing Chamber, pushes the sample through the Flow Cell which is located inside the Triple Transducer Module (TTM)
- As the sample stream is pushed into the Flow Cell, the white blood cells are hydrodynamically focused through the aperture of the Flow Cell
- Cells are analyzed one at a time as they pass through the aperture, three measurements are taken simultaneously: volume, conductivity, and laser light scatter

VCS Measurements



Volume

- "V" in VCS technology refers to the volume of the WBC in its near-native state
- Plotted on the Y-axis
- Uses the Coulter Principle
  - Uses direct or low frequency current (DC)
  - Measures impedance (resistance) which is proportional to the volume of the cell
  - Does not penetrate the cell; but wraps around the nonconductive white blood cell

Conductivity

- "C" in VCS technology refers to the conductivity of the WBC in its near-native state
- Plotted on the Z-axis
- Uses high frequency current (RF) which can penetrate the cell to evaluate internal constituents such as:
  - Nucleus
  - Granularity
  - Other physical components of the cell
  - Chemical composition of the cytoplasm influences the flow of high frequency current
- Low frequency current (DC) and high frequency current (RF) are applied at the same time
  - Use the same pair of electrodes as used for direct current flow
  - Because the currents are different frequencies, the same electrodes can be used

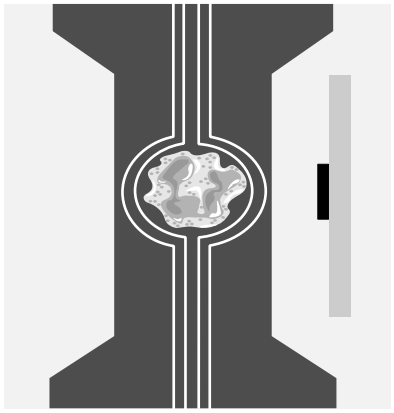
Opacity

- Opacity is derived by eliminating volume from the conductivity measurement to allow an even more accurate measurement of cellular content
- Opacity provides a measure of a cell's content without influence from volume

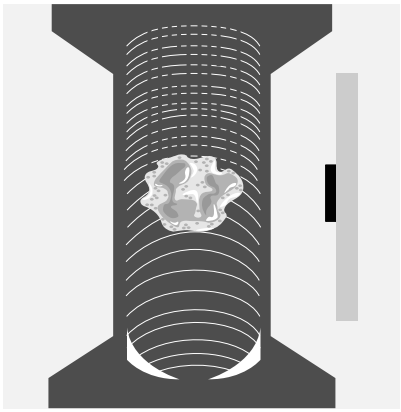
Scatter

- "S" in VCS technology refers to the Light Scatter the WBC produces in its near-native state
- Plotted on the X-axis
- Cell surface has irregular peaks and valleys that scatters the laser light as it strikes the cell
- Scatter measurement provides information about cell shape and surface characteristics, morphology, and granularity
  - Eosinophils scatter the most laser light because their granules are located close to the cell surface making the surface very irregular or "bumpy"
  - Neutrophils scatter a moderate amount of laser light because their granules are dispersed throughout the cytoplasm making their cell surface only moderately "bumpy"
  - Basophils have low light scatter because their granules are located deep within the cell near the nucleus making the cell surface similar to that of a lymphocyte or monocyte
  - Lymphocytes and monocytes have low light scatter because they have few, if any, granules which makes their cell surface very smooth

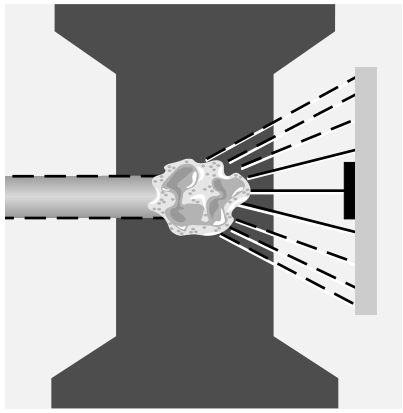
Simultaneous Analysis



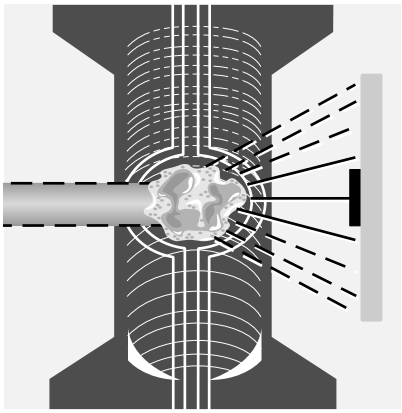
Volume



Conductivity



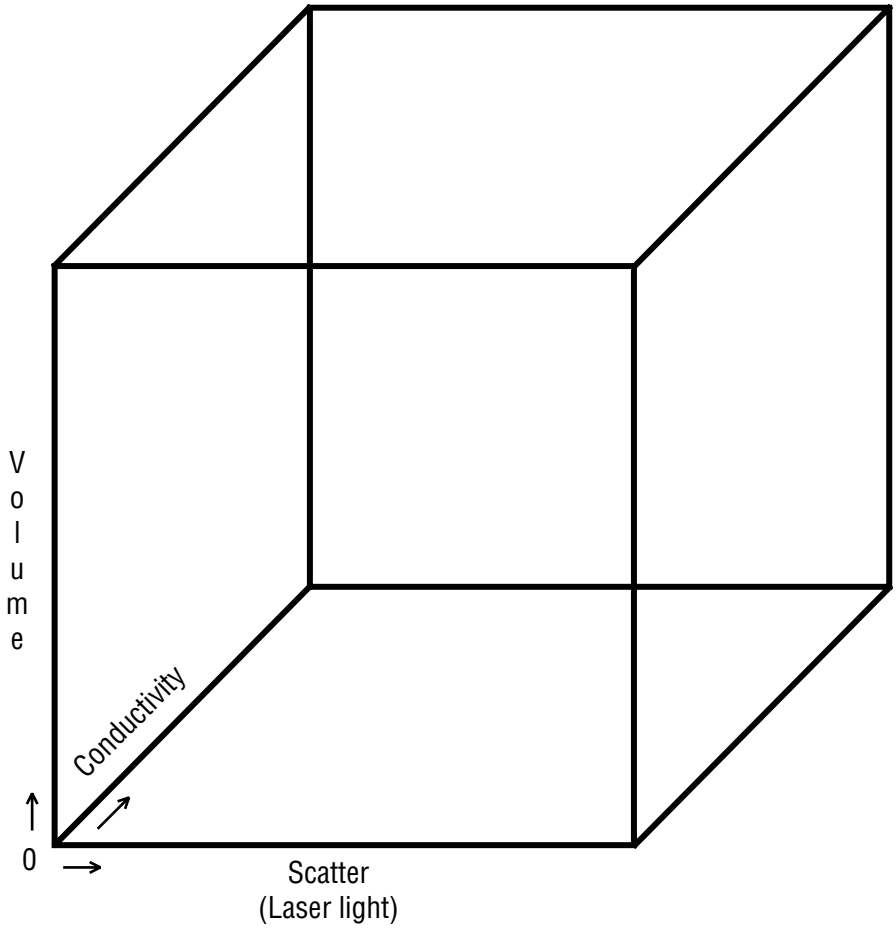
Scatter



VCS simultaneous measurement

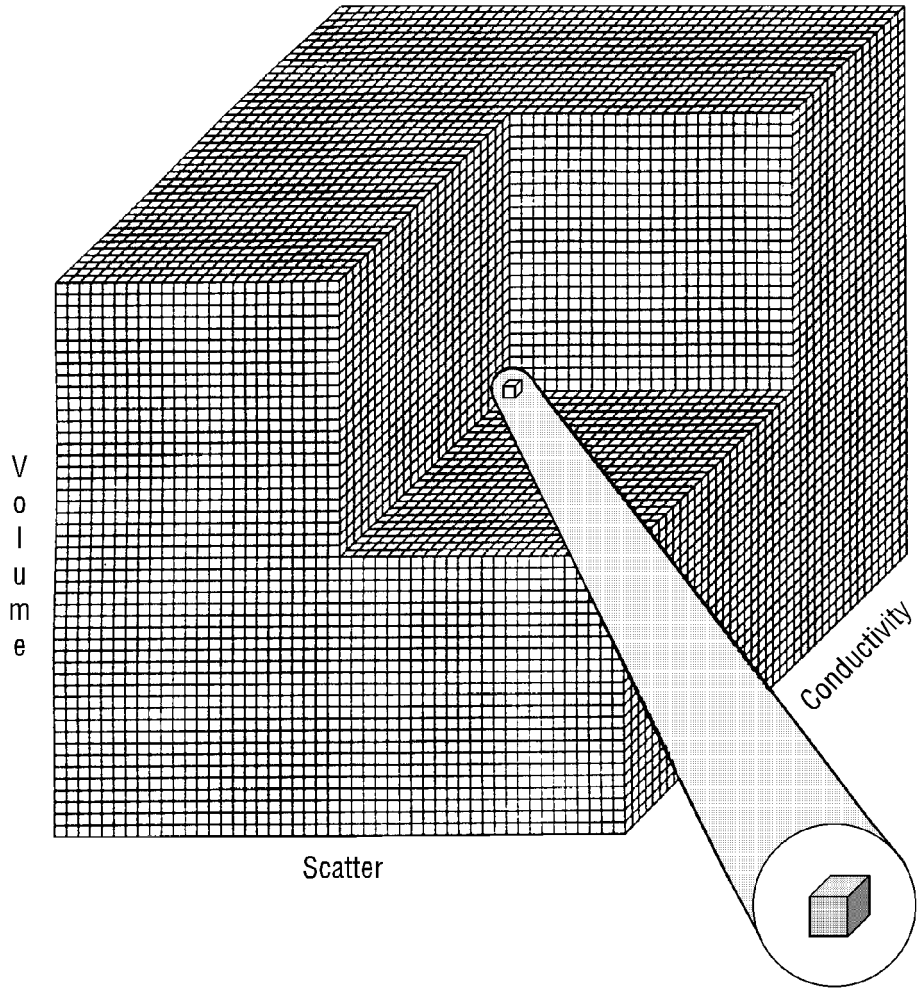
- Each cell is analyzed by all three technologies at the same time
- Diff sample analysis continues until either 8192 cells are accumulated or a maximum of 30 seconds elapse

VCS Cube

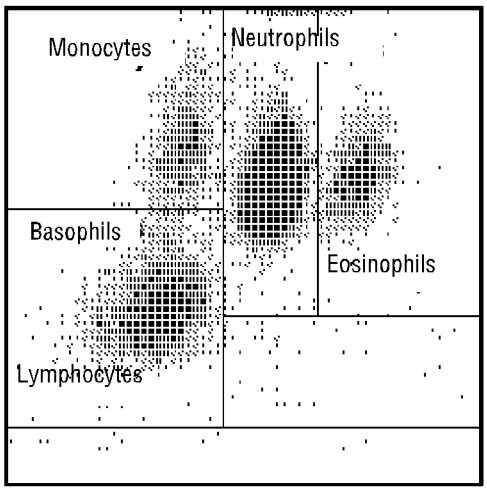


- Three measurements on each cell plotted in three dimensions
- Think of the XYZ coordinates as VCS coordinates
- Each axis is divided into 256 channels (divisions / categories)
  - 256 channels for Volume
  - 256 channels for Conductivity
  - 256 channels of laser light Scatter
- 256 x 256 x 256 = approximately 16.7 million different VCS coordinates within the cube

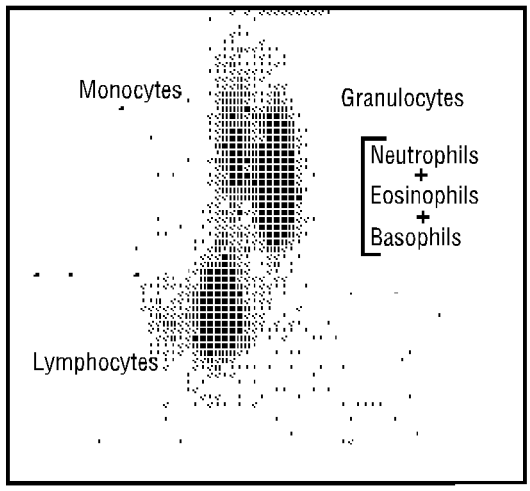
Cubes Within the Cube



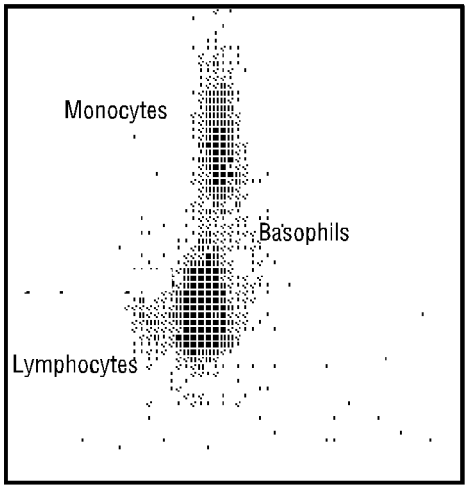
- The smaller cubes within the cube also have VCS coordinates
  - For example, the call-out cube has a certain amount of Volume, a certain amount of Conductivity, and a certain amount of Scatter that match a certain type of cell . . . considering the call-out cube's original location, neutrophils would probably fall within these coordinates
- More than one cell could have the same VCS coordinates
- Cells are first identified based on their location within the cube, then population percentages are determined


Monocytes
Neutrophils
Basophils
Eosinophils
Lymphocytes

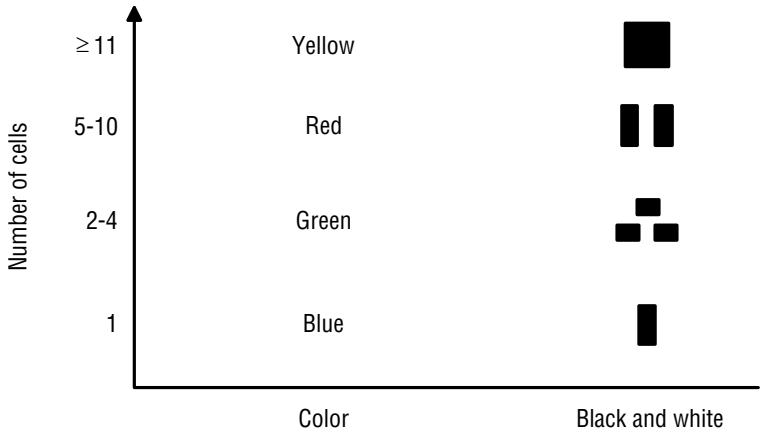
DF1


Monocytes
Granulocytes
Neutrophils
+
Eosinophils
+
Basophils
Lymphocytes

DF2


Monocytes
Basophils
Lymphocytes

DF3


Number of cells
≥ 11
5-10
2-4
1
Yellow
Red
Green
Blue
Color
Black and white

Labeled Scatterplot

- Y-axis is always VOLUME
- X-axis can be SCATTER or CONDUCTIVITY
  (dependent upon which side of the cube is being viewed)
- There are no units on any axis

Discriminator Lines

- Moveable, will vary from sample to sample
- Not used by the computer, more an operator benefit
- Locate the lowest horizontal discriminator line
  - Above this line, cells are called WBCs and included in the differential
  - Below the line, cells are considered non-white cells and are excluded from the differential

DF1 View

- DF1 is VOLUME vs. LIGHT SCATTER
- Note that basophils hide behind the lymphocytes on this view
- Aged/degenerating cells can be identified accurately and are included in the differential

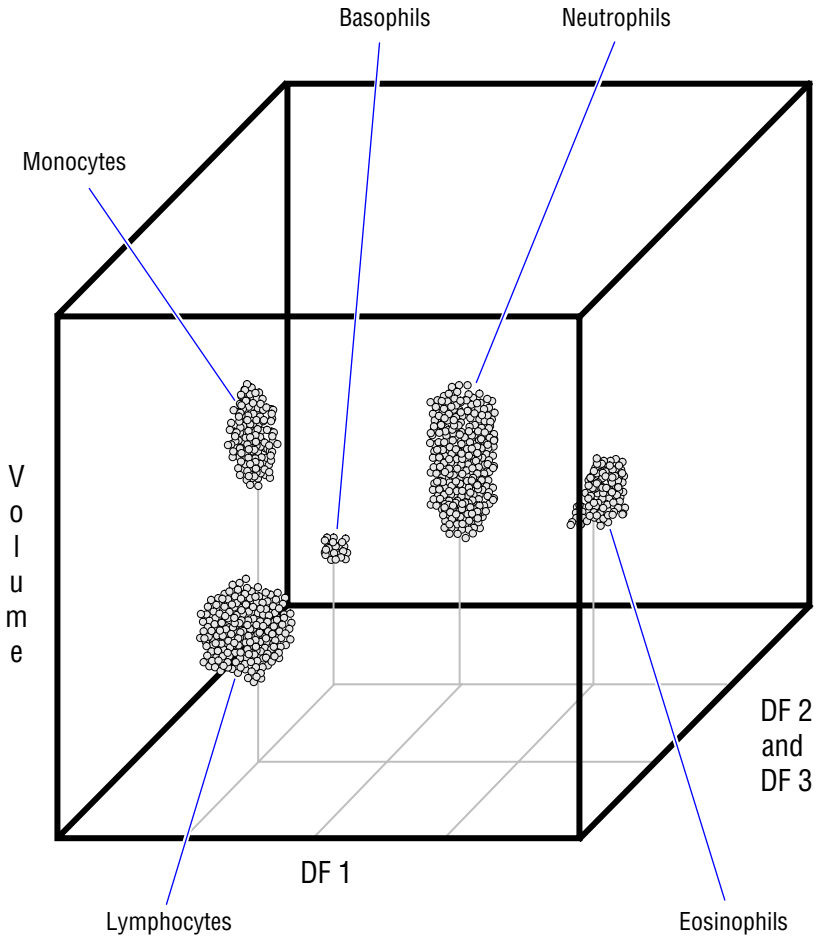
DF2 View

- DF2 is VOLUME vs. CONDUCTIVITY
- Note that the basophils hide behind the eosinophils and neutrophils on the DF2 view

DF3 View

- DF3 scatterplot view displays the same information as the DF2 view, but gates out the neutrophil and eosinophil populations for a clear view of the basophil population

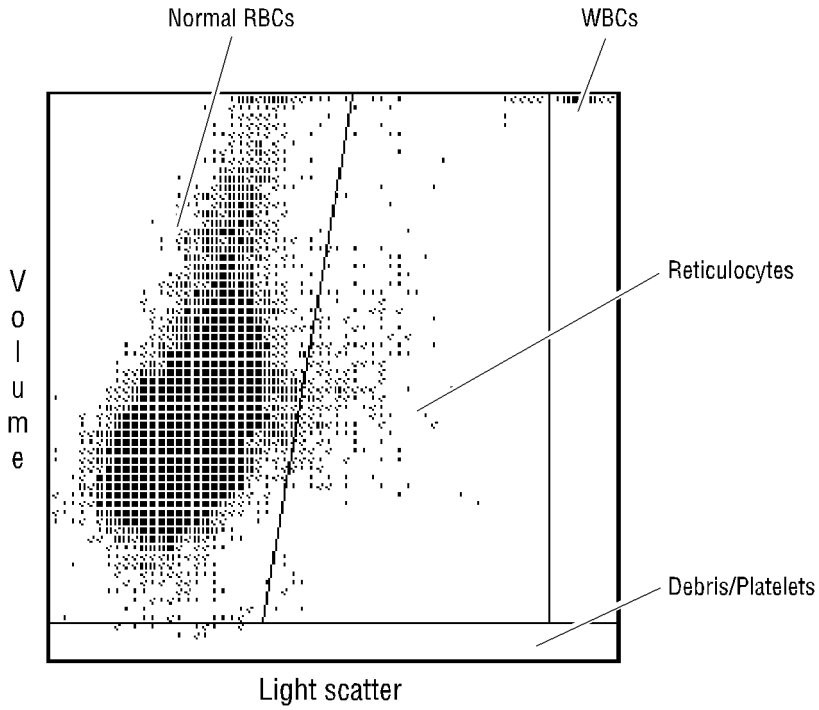
Cube View



- Notice the lymphocytes and monocytes tend to have the same Scatter and Conductivity; the factor that separates them is Volume
- Notice granulocytes all tend to have the same Conductivity; the factor that separates them is their Scatter (mainly due to their different granules)
- Notice the lymphocytes and basophils tend to have the same Scatter and Volume; factor that most separates them is their Conductivity

Reticulocyte Analysis

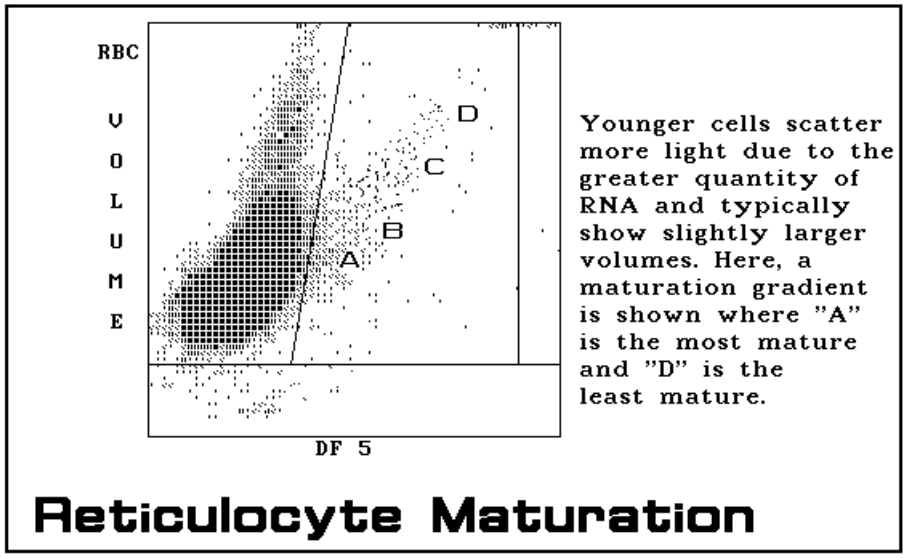
Typical Normal Retic Pattern - DF5 View



- Same VCS technology but using a different reagent system
- Retic analysis continues until either 32,768 RBCs are accumulated or a maximum of 30 seconds elapse
- DF5 is the Retic version of DF1 (VOLUME vs. SCATTER)
  - The younger the red cell, the higher the volume
  - The younger the red cell, the higher the light scatter because there's more stained and precipitated RNA present
  - DF5 scale is different than DF1 so that's why the WBCs fall in the upper right corner
- DF6 is VOLUME vs. CONDUCTIVITY

MRV and IRF Parameters

- MRV (Mean Reticulocyte Volume)
  - Average cell volume of the reticulocytes
  - Determined by the VCS technology algorithm from the retic scatterplot
  - Reported in femtoliters (fL)
- IRF (Immature Reticulocyte Fraction)



  - IRF parameter is based on the following factors:
    - Degree of stained reticulum (using New Methylene Blue dye) is proportional to the degree of RNA present in the cell and, therefore, the amount of light scatter produced
    - Younger retics scatter more light due to the greater quantity of RNA and typically show slightly larger volumes
  - Parameter is a ratio
    - Calculated using the count of the highest light scatter reticulocytes to the total count of the reticulocytes
    - Reported as a percentage

FLAGGING

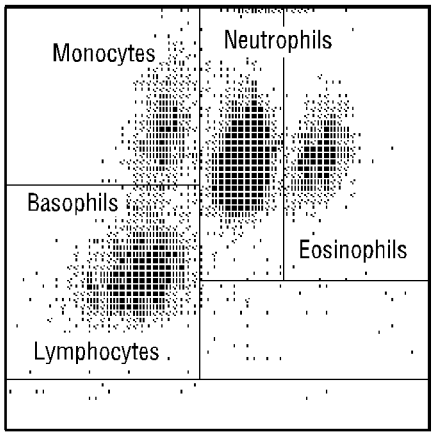
Flagging Categories

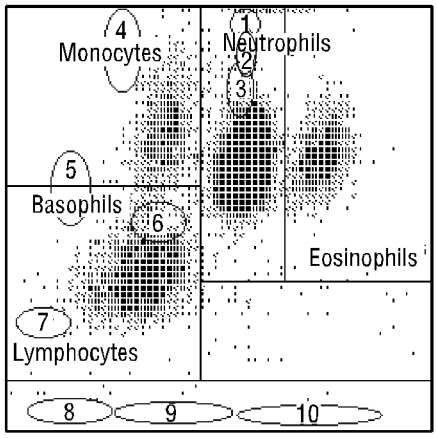
- There are four categories of flags that may be generated while processing patient samples
  - Condition Messages
  - Suspect Messages
  - Operator Definable Flags
    - Definitive Messages
    - High/Low Flags
  - Parameter Codes
- Each is designed to alert the operator that an abnormality may be present
- When a flag appears, the operator should take action according to the review protocol established by their laboratory for that flag

Condition Messages

- Condition messages describe the sample's condition as either a normal or abnormal population
- Commonly referred to as a "population message"
- System generates these population messages from one or a combination of the following:
  - Suspect messages
  - Definitive messages
- Messages appear in the lower left corner of the Run Samples screen

Suspect Messages





1. Suspect blasts (myeloblasts)
2. Suspect immature granulocytes
3. Suspect bands
4. Suspect blasts (monoblasts)
5. Suspect blasts (lymphoblasts)
6. Suspect variant lymphocytes
7. Suspect variant lymphocytes
8. Suspect nucleated red blood cells
9. Suspect giant platelets
10. Suspect platelet clumps

- Word messages generated by the DMS
- Computer generates these messages according to a complex internal algorithm which cannot be changed by the operator
- Used to flag an abnormal cell distribution or population (possible abnormal morphology)
- Use F11 **Cell Classification** to view message on the screen

Operator-Definable Flags

- There are two types of Operator-Definable flags
  - Definitive messages
    - Definitive word messages are associated with specific parameters
    - Possible flags are listed in the Operator's Guide
    - Morphology messages that have 1+, 2+, or 3+ are defined when you set the 1+ because after that, the computer determines the 2+ and 3+ so that results are standardized
  - High (**H**) and Low (**L**) flagging limits (may be referred to as High and Low Action Limits)
    - High (**H**) and Low (**L**) flags do not produce an *Abnormal Pop* condition message
- Use F11 **Cell Classification** to view message on the screen

Parameter Codes

- Parameter codes either totally replace a result or appear next to the parameter result in question
- Possible codes and their causes are listed in the Operator's Guide
- Codes indicate that the operator needs to review the results of the affected parameter and handle the abnormality according to their laboratory's protocol

OBJECTIVES

When the subject is complete, you will be able to . . .

Module and Component Identification

- Name each module.
- Locate and name module components.

Sample Flow

- Find the aspiration hole in the Secondary mode probe.
- Explain the purpose of the venting pathway in the needle.
- Trace on the instrument the Primary mode aspiration pathway.
- Explain the purpose of the blood detectors.
- Tell whether the bubble placed between diluent and blood during aspiration is normal or abnormal.
- Trace on the instrument the drain pathway from the baths to the waste chamber.
- List the components used to make the RBC and WBC dilutions.
- List the components of the Diff sample.
- Trace the paths of diluent and CBC lytic reagent from the lyse pumps to the WBC bath.
- Tell how one knows that CBC lytic reagent was added to the WBC bath.
- Trace the paths of Erythrolyse II reagent and StabiLyse reagent from their pumps to the mixing chamber.
- Explain the two ways that CBC dilutions are mixed.
- Explain how the Diff sample is mixed.
- Trace on the instrument, the flow of CBC dilutions through their apertures to their vacuum isolator chambers.
- Find on the instrument, the internal and external electrodes.
- Explain basic sample flow for differential analysis from the mixing chamber through the flow cell.
- Explain crossrinse and its purpose.
- Explain the purpose of the burn circuit.
- Explain the purpose of backwash.

Basic Troubleshooting Techniques

- Recognize an instrument problem based on:
  - Abnormal sample results
  - Abnormal Startup or System Test results
  - Abnormal control results
  - Error messages
- Use what you know about normal sample flow and the derivation of parameter results to troubleshoot.
- Isolate the components involved, when given a symptom.

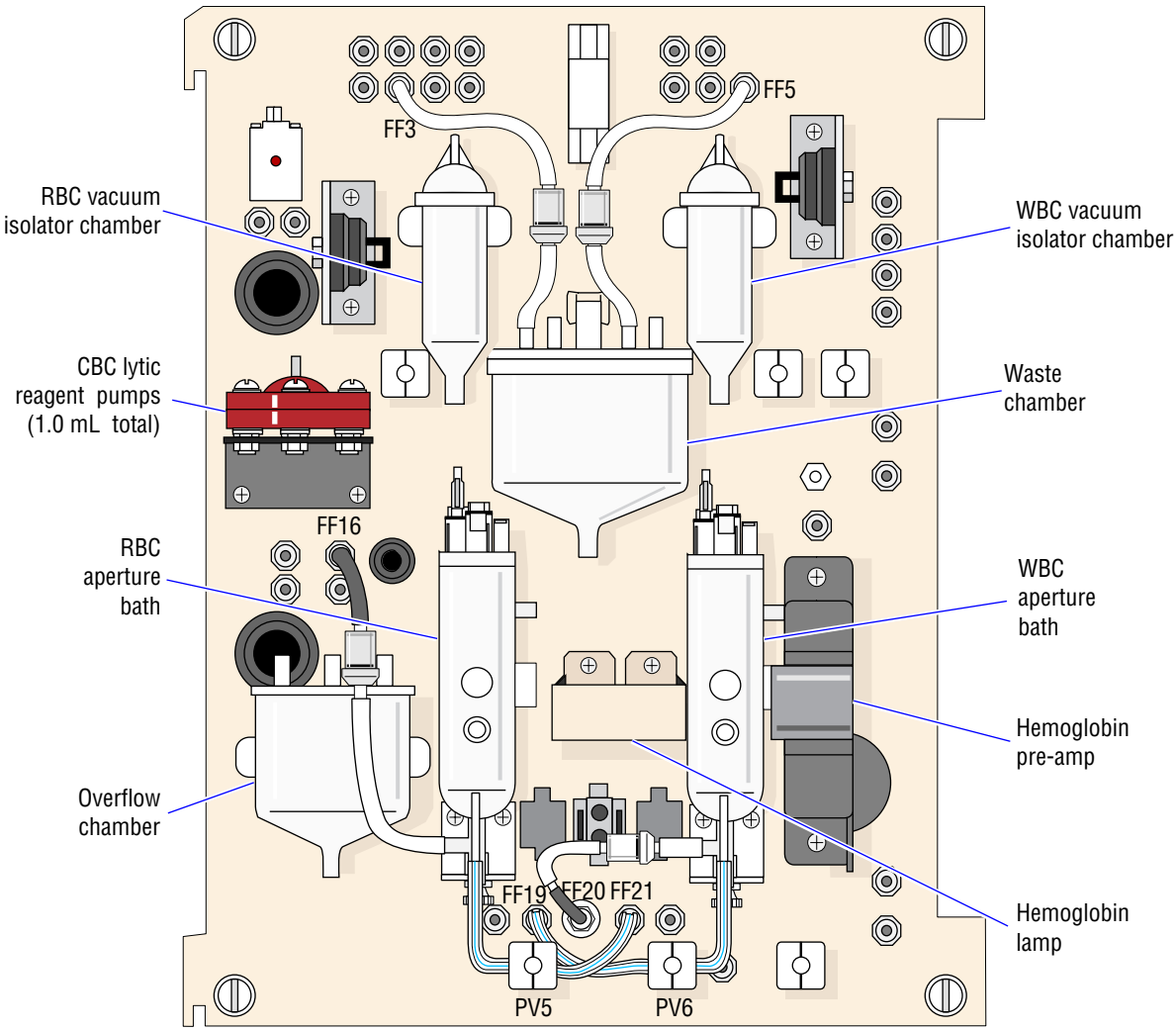
- Reproducibility check:
  - Explain the purpose of this check.
  - State at least one situation when performing a reproducibility check would be beneficial.
- Carryover check:
  - Explain the purpose of this check.
  - State at least one situation when performing a carryover check would be beneficial.

Replacement and Adjustment Procedures

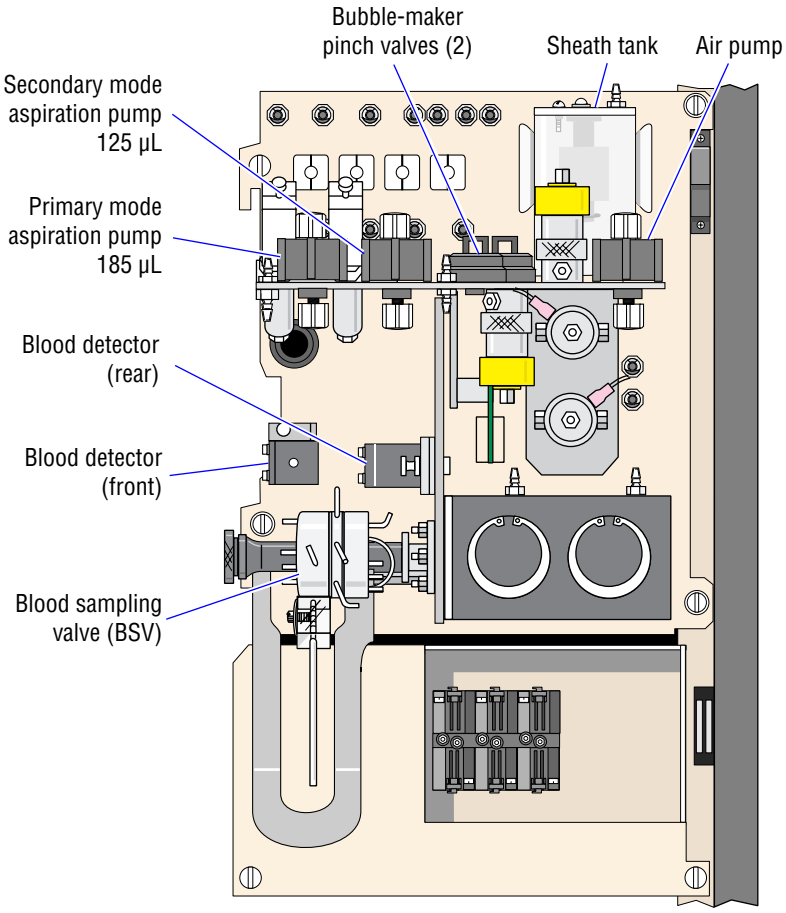
- Locate and perform selected replacement and adjustment procedures such as replacing the needle.

MODULE AND COMPONENT IDENTIFICATION

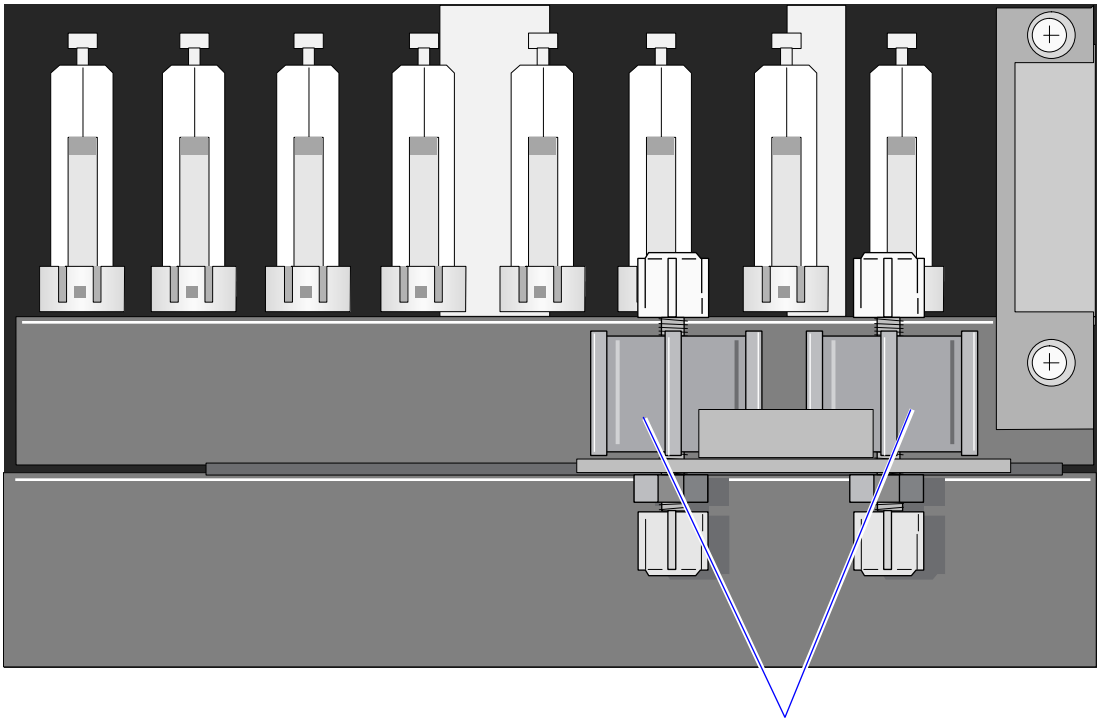
CBC Module



BSV Module

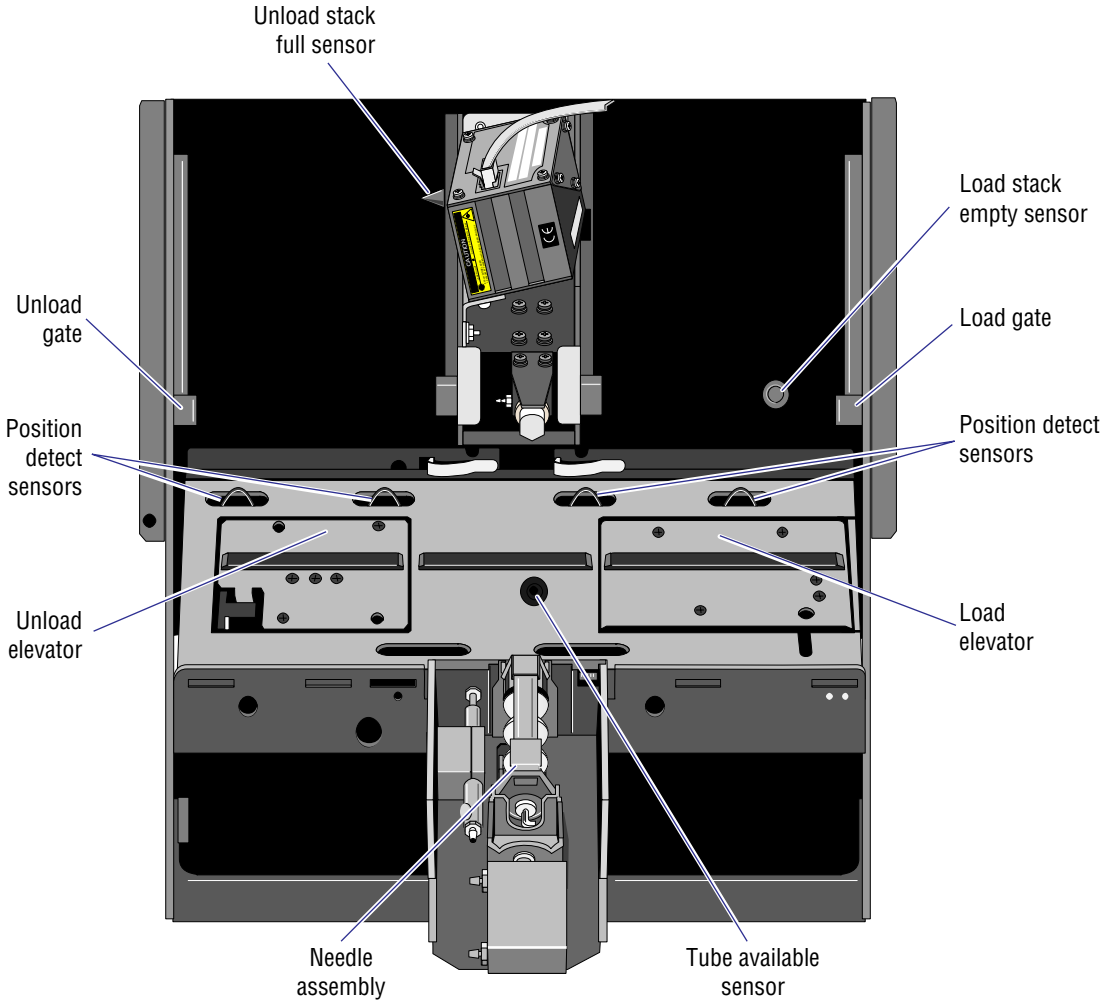

Bubble-maker
pinch valves (2)
Sheath tank
Air pump
Secondary mode
aspiration pump
125 µL
Primary mode
aspiration pump
185 µL
Blood detector
(rear)
Blood detector
(front)
Blood sampling
valve (BSV)

Pump Module

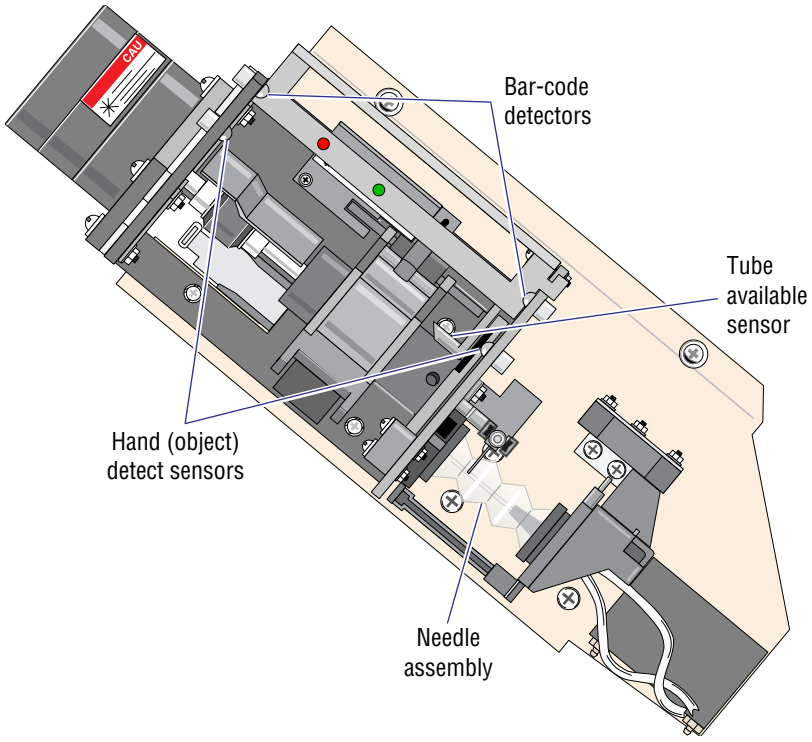


Erythrolyse II reagent pumps (2)
300 µL (total)

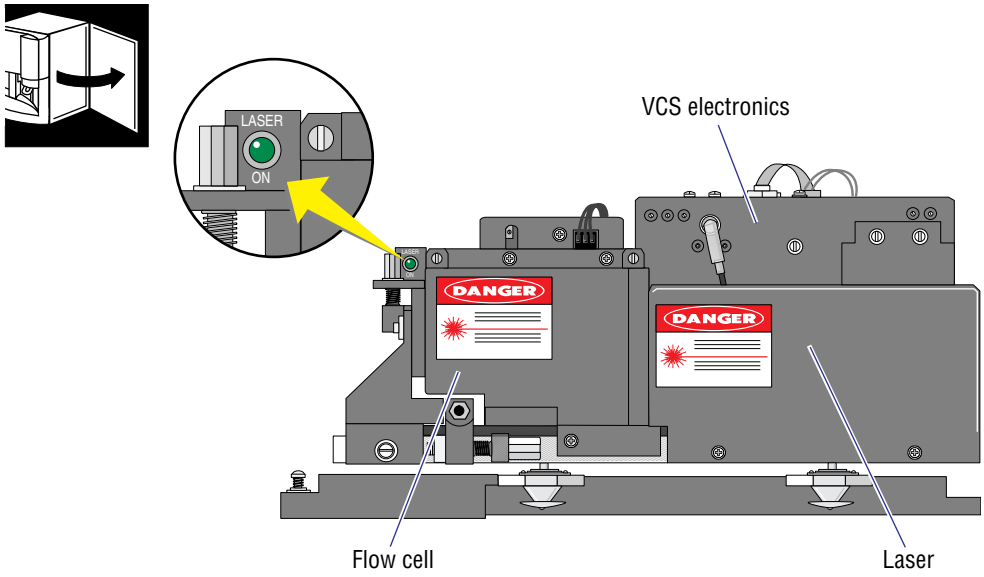
Autoloader Module



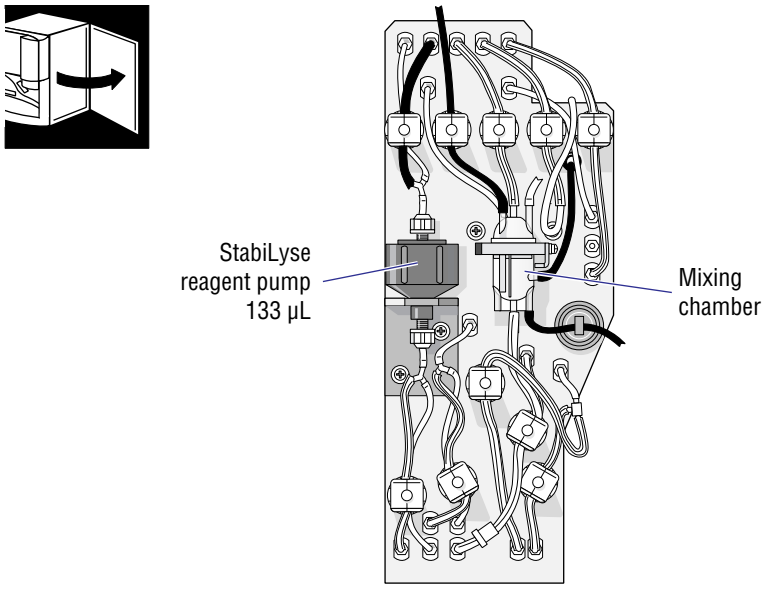
Rotary Cap-Pierce Module



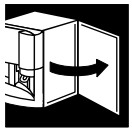
Flow Cell Module

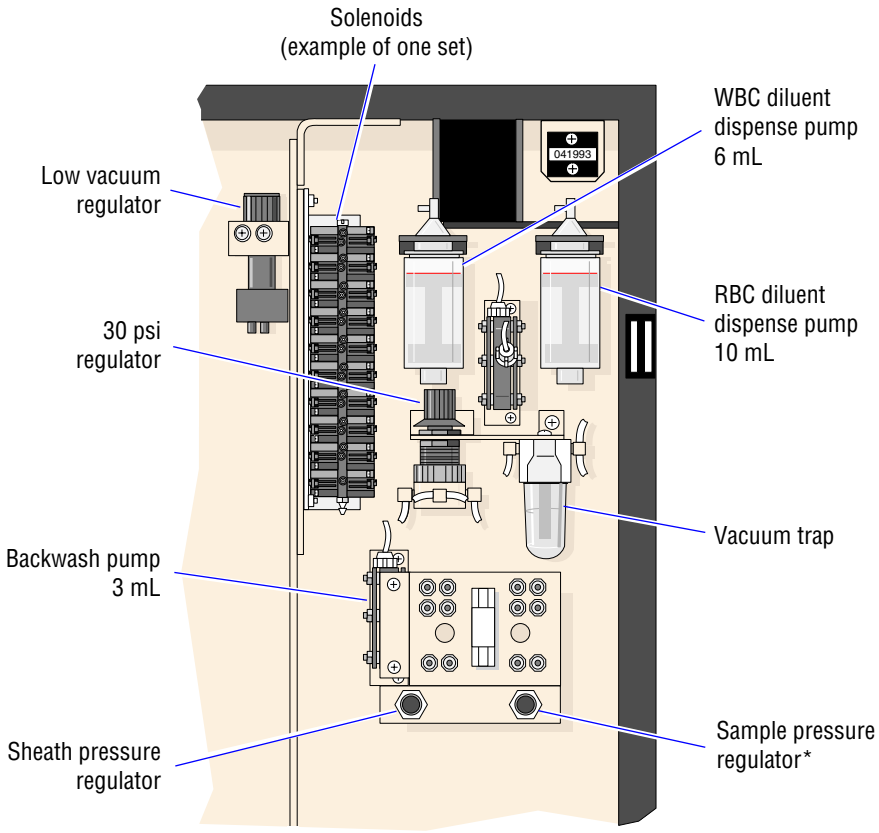


Mixing Module



Main Diluter Module



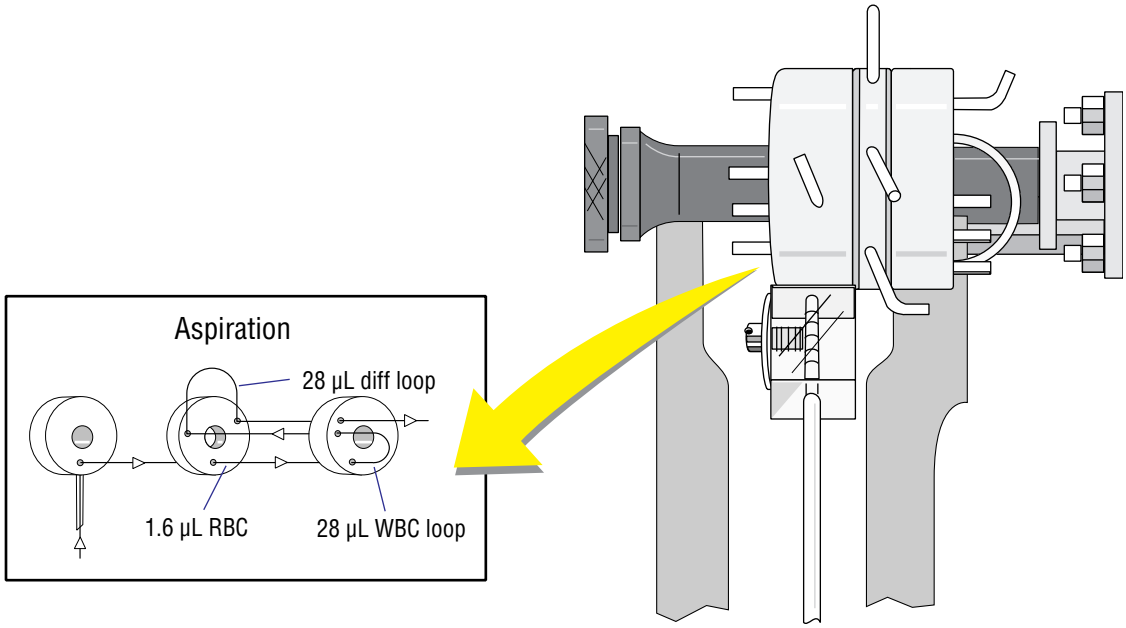


* Do not adjust the sample pressure unless directed to do so by your Beckman Coulter Technical Representative.

NOTES

SAMPLE FLOW

Aspiration

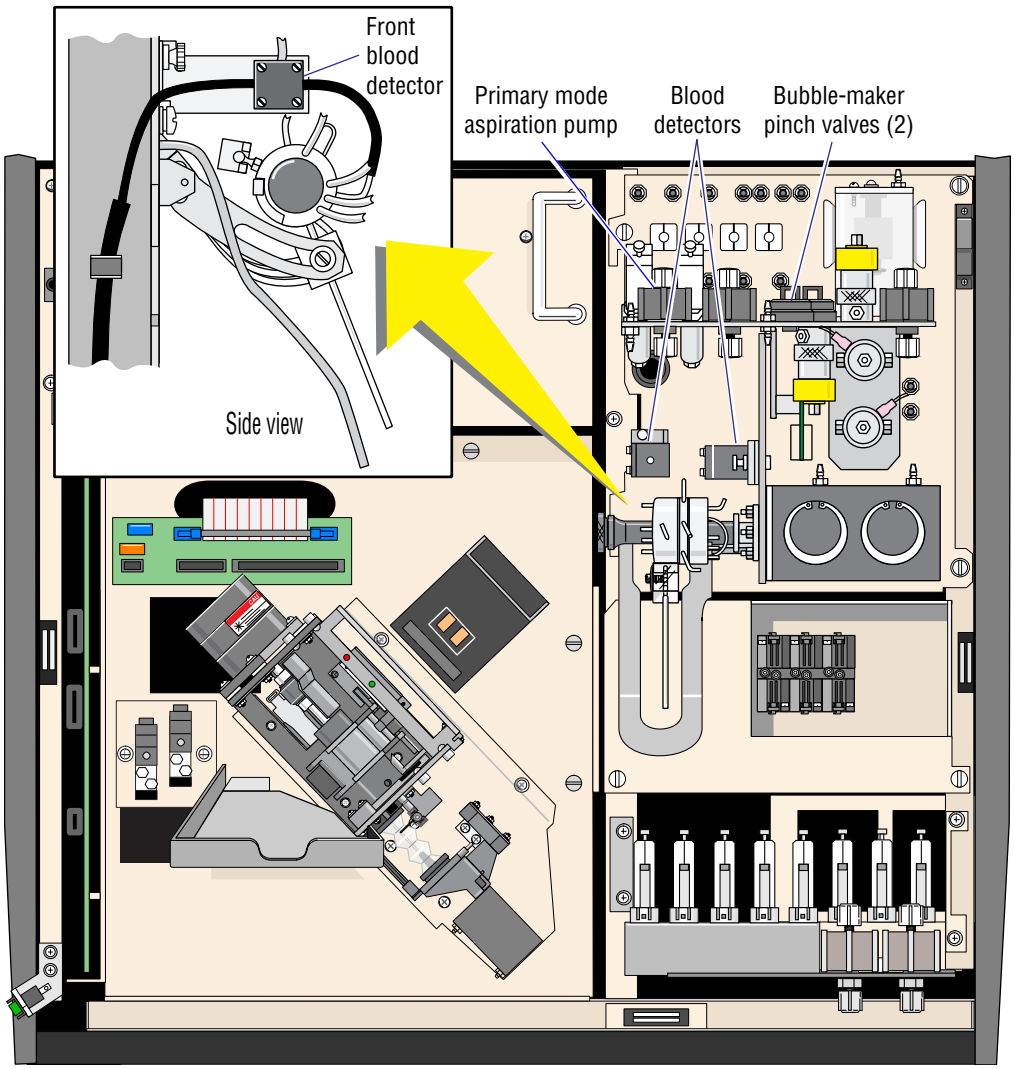


- Blood is aspirated into the Blood Sampling Valve (BSV)
  - 185 µL in the Primary mode (Closed-Vial mode)
  - 125 µL in the Secondary mode (Open-Vial mode)

To continue HmX Hematology Analyzer training, proceed to page 8-12.

To continue HmX Hematology Analyzer with Autoloader training, go to page 8-14.

Primary Mode Aspiration on a HmX Hematology Analyzer

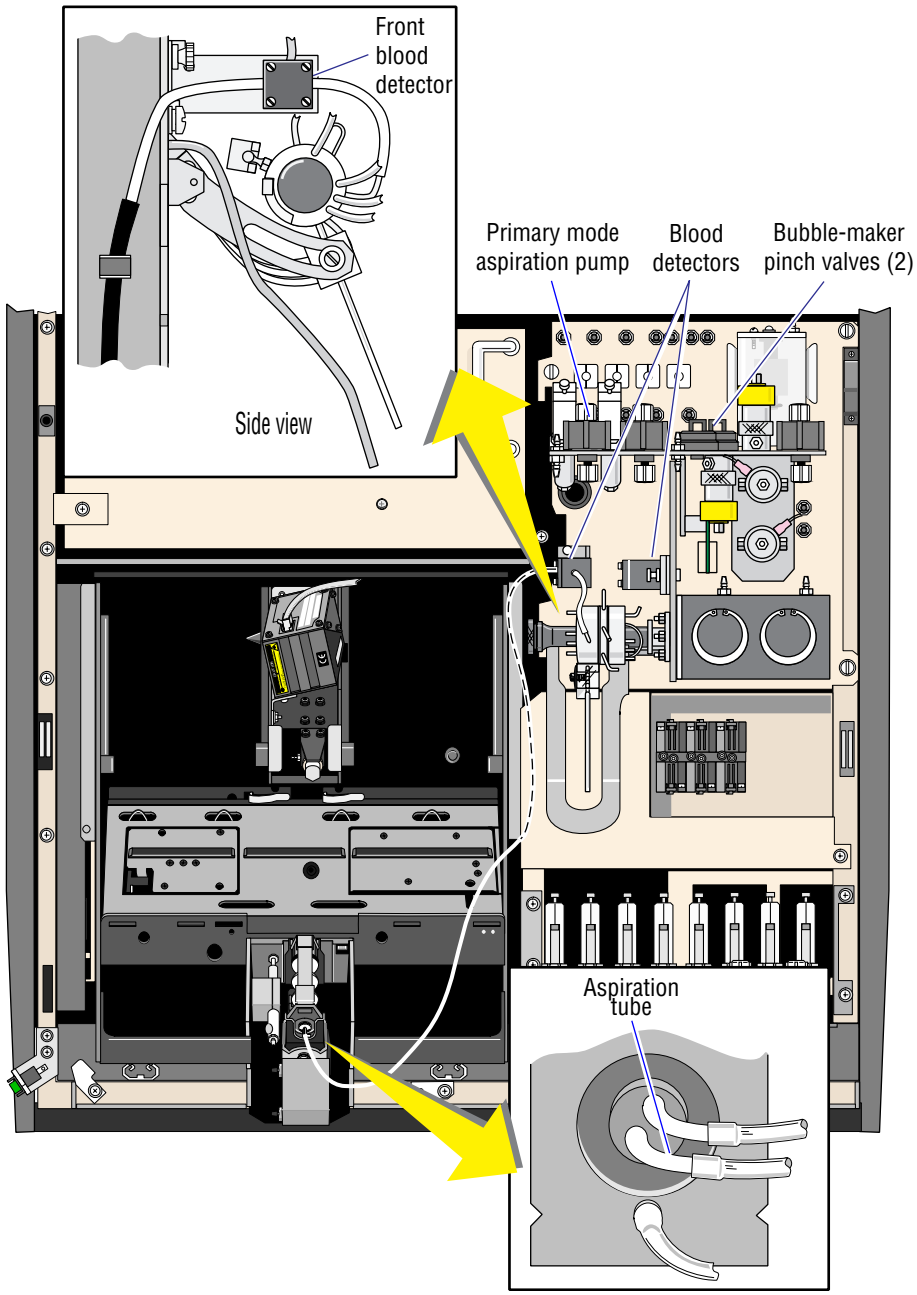


Primary Mode Aspiration on a HmX Hematology Analyzer - Step by Step

- The instrument puts the tube into the piercing position
- The bubble-maker pinch valves activate and pinch the tubing of the aspiration pathway, thus expelling a drop of diluent from the tip of the needle
- The bubble-maker pinch valves deactivate and an air bubble forms at the tip of the needle
- The needle pierces the tube's stopper
- The Primary mode aspiration pump aspirates 185 µL of sample from the tube
- The needle retracts from the tube
- Vacuum pulls the blood segment through the front blood detector, the BSV and the rear blood detector
- As soon as the rear blood detector senses the air bubble, the vacuum turns off
- The blood segment is now in the correct position for the blood detectors to assess the quality of the aspiration and determine if the aspirated blood is acceptable

To continue HmX Hematology Analyzer training, go to page 8-16.

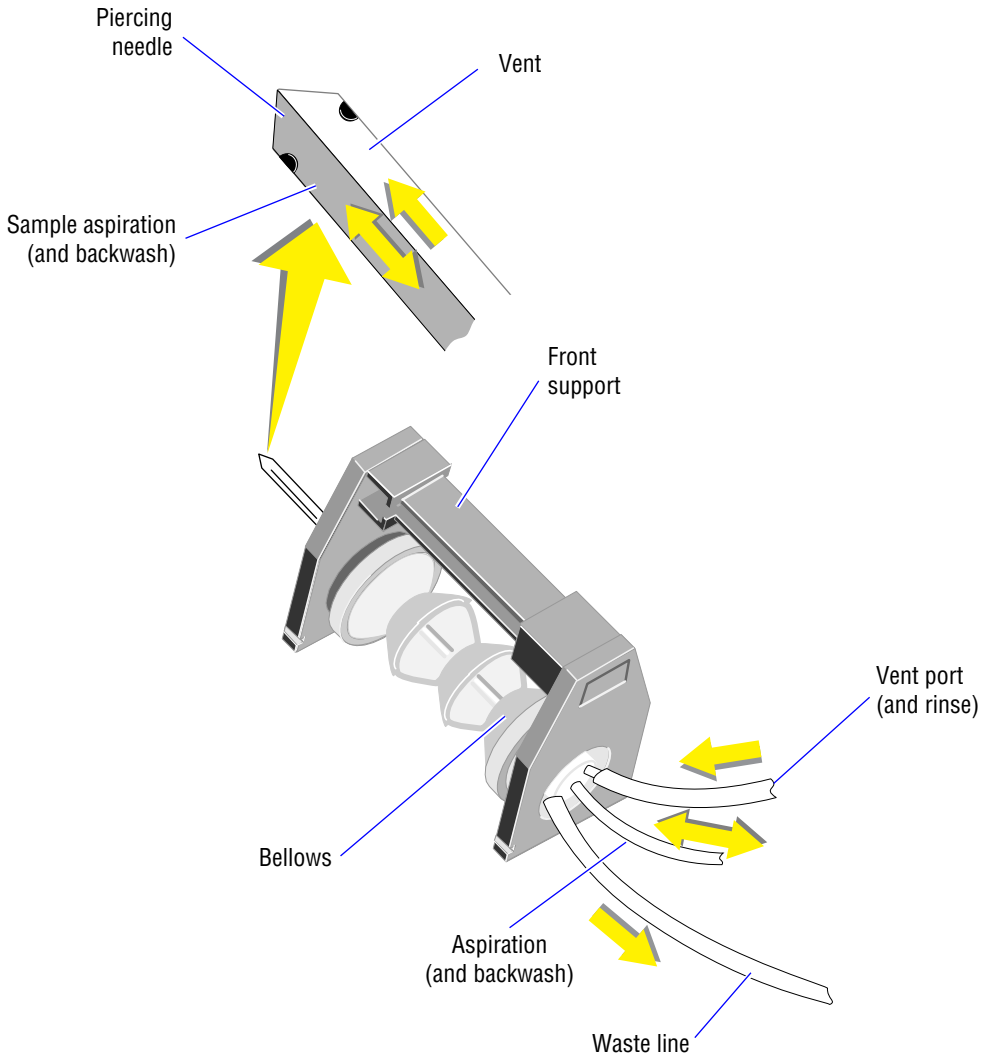
Primary Mode Aspiration on a HmX Hematology Analyzer with Autoloader



Primary Mode Aspiration on a HmX Hematology Analyzer with Autoloader - Step by Step

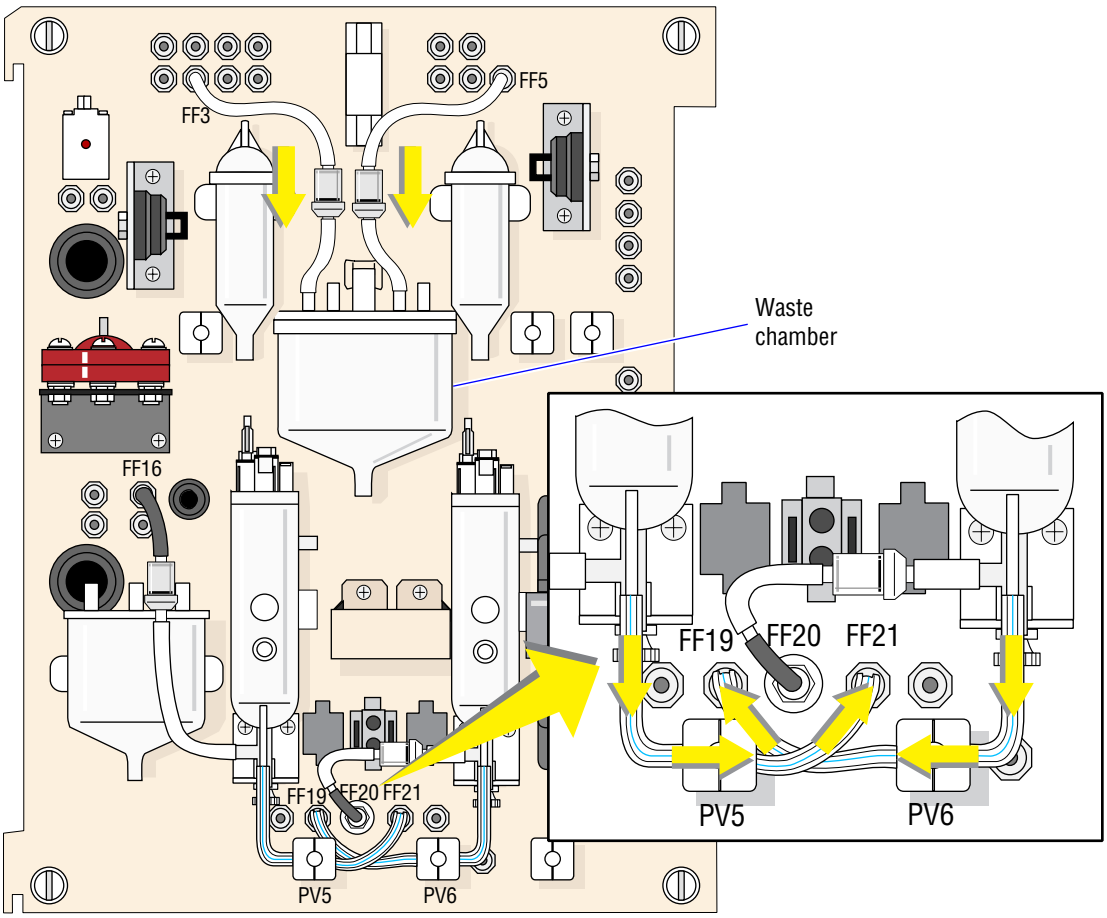
- The instrument puts the tube into the piercing position
- The bubble-maker pinch valves activate and pinch the tubing of the aspiration pathway, thus expelling a drop of diluent from the tip of the needle
- The bubble-maker pinch valves deactivate and an air bubble forms at the tip of the needle
- The needle pierces the tube's stopper
- The Primary mode aspiration pump aspirates 185 µL of sample from the tube
- The needle retracts from the tube
- Vacuum pulls the blood segment through the front blood detector, the BSV and the rear blood detector
- As soon as the rear blood detector senses the air bubble, the vacuum turns off
- The blood segment is now in the correct position for the blood detectors to assess the quality of the aspiration and determine if the aspirated blood is acceptable

Piercing Needle Assembly



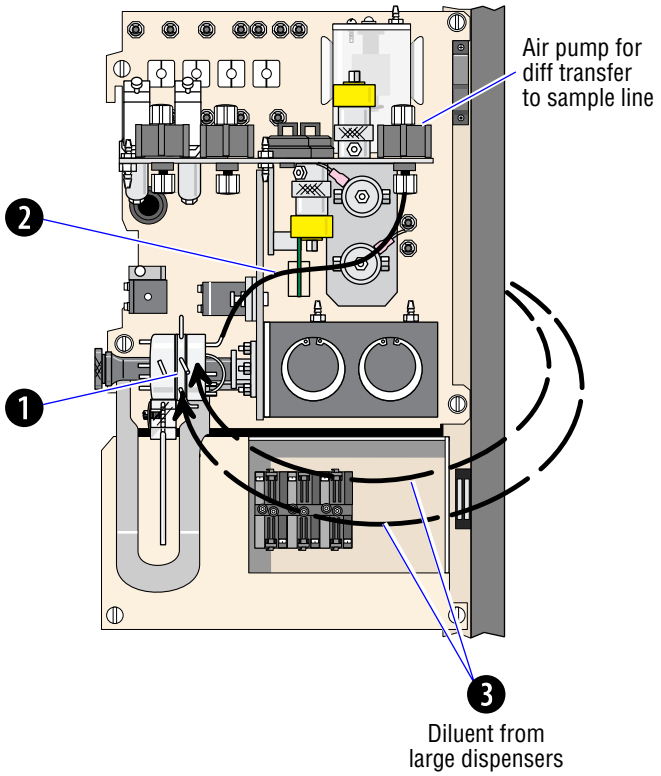
- Used for closed-vial sampling in the Primary mode
- Piercing needle has two ports: one for aspiration and a second for venting
- Vent port allows air to pass into (or out of) the stoppered specimen tube to equilibrate any residual vacuum or pressure inside the tube
- Aspiration port opens a pathway so that vacuum applied by the Primary mode aspiration pump can pull a sample of whole blood from the specimen tube through the Blood Sampling Valve (BSV)
- Piercing should consistently occur near the center of the specimen tube's stopper

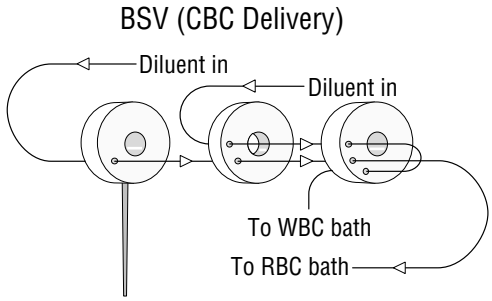
Draining the Baths



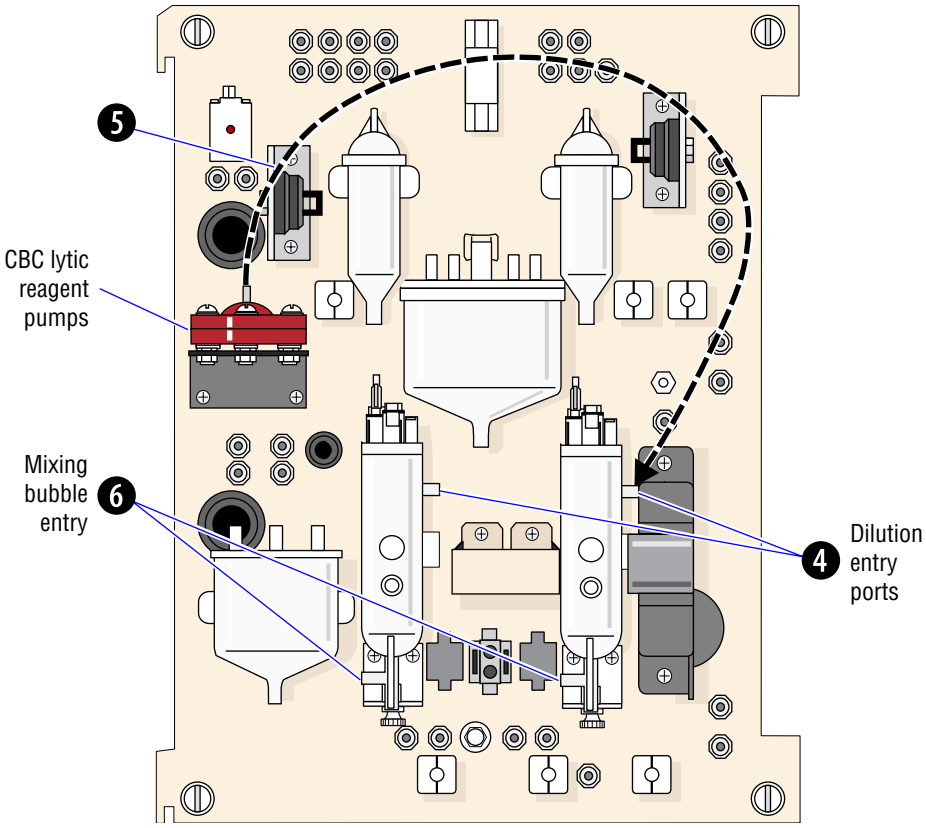
- While a sample is aspirating, diluent from the last cycle's rinse drains to the waste chamber
  - Locate the drain line at the bottom of each bath
  - Trace this line through pinch valves 5 and 6 to the fittings on the back panel
  - Pathway continues in back of CBC Module and up to the top where the lines come back out through fittings FF3 and FF5 which are located above the waste chamber
  - Note the check valve in each tubing and trace the line to the top of the waste chamber

Preparing the CBC Sample





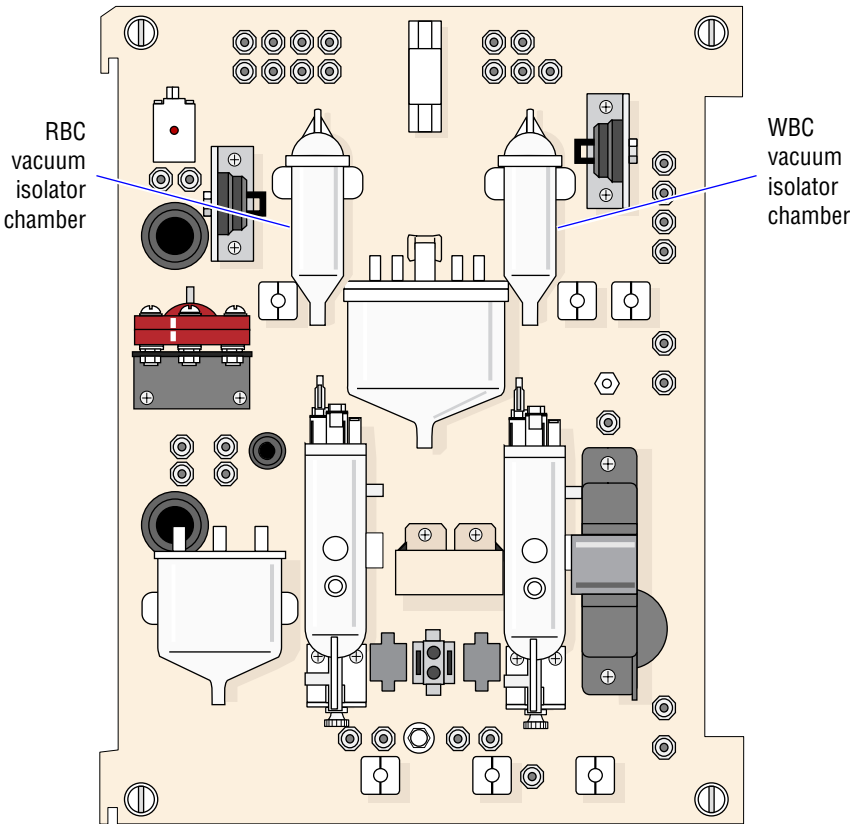
1. The center section of the BSV rotates to segment three portions of the sample:
   - 1.6 µL for the RBC bath dilution
   - 28.0 µL for the WBC bath dilution
   - 28.0 µL for the Diff dilution
2. The air pump transfers the Diff portion to a sample line just outside (and behind) the BSV
3. The RBC and WBC diluent dispensers send ISOTON III diluent through the BSV to pick up and dilute the RBC and WBC segments of blood



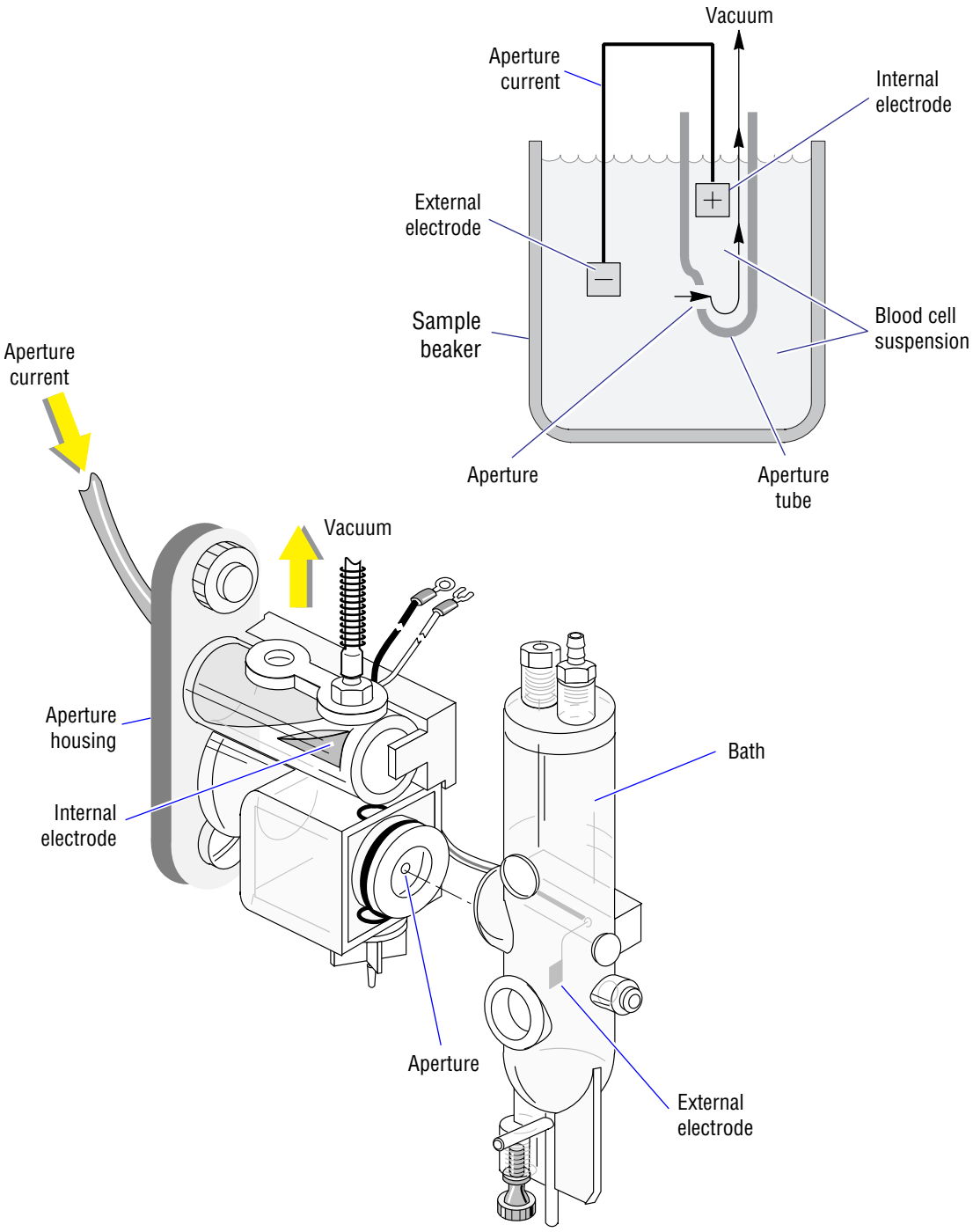
4. The dilutions are delivered to the aperture baths through the inlet ports on the upper right
5. The CBC lytic reagent pumps send LYSE S III diff lytic reagent to join the WBC dilution on its way to the WBC bath

   Note: This lytic reagent lyses the RBCs and converts hemoglobin into a stable compound.
6. Mixing bubbles enter both baths as several bursts of bubbles

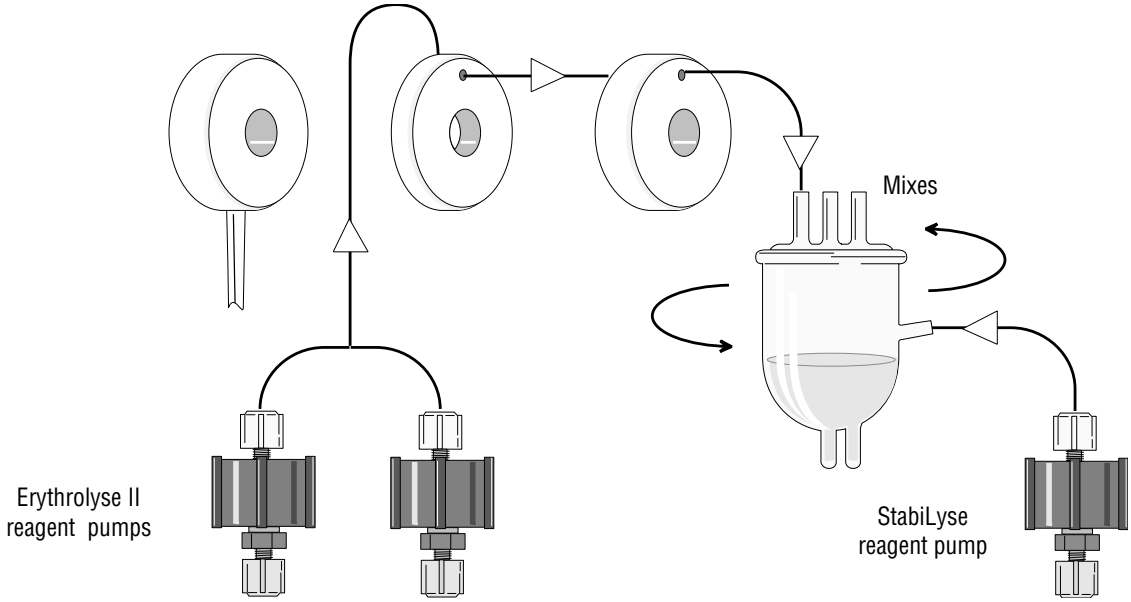
CBC Counting and Sensing



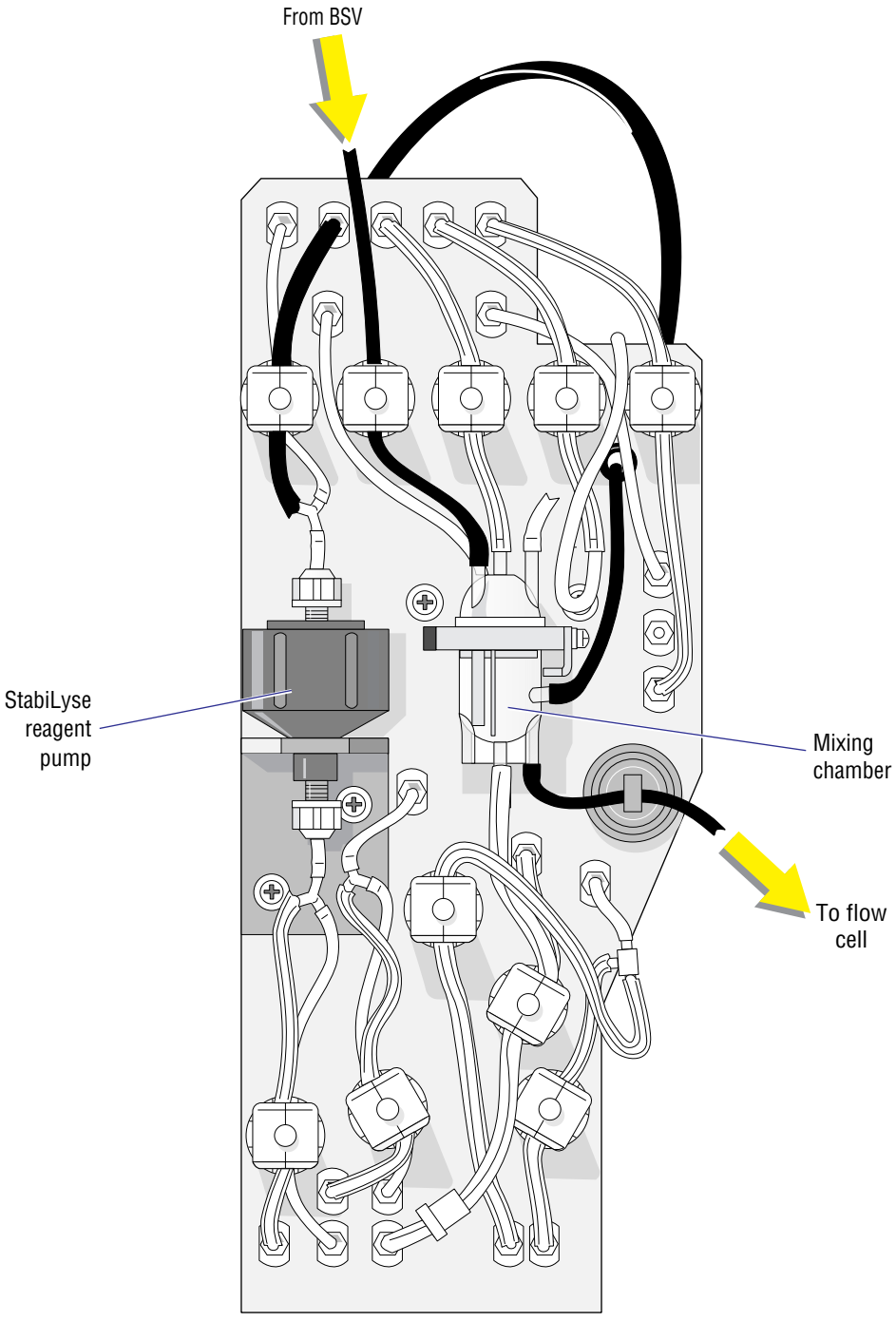
- The low vacuum supplied to the aperture baths to pull the diluted sample through the apertures is housed in the vacuum isolator chambers
- The RBC aperture senses red blood cells and platelets
- The WBC aperture senses white blood cells
- Hemoglobin is measured photometrically through the WBC bath


Vacuum
Aperture current
Internal electrode
External electrode
Sample beaker
Blood cell suspension
Aperture
Aperture tube
Aperture current
Vacuum
Aperture housing
Internal electrode
Bath
Aperture
External electrode

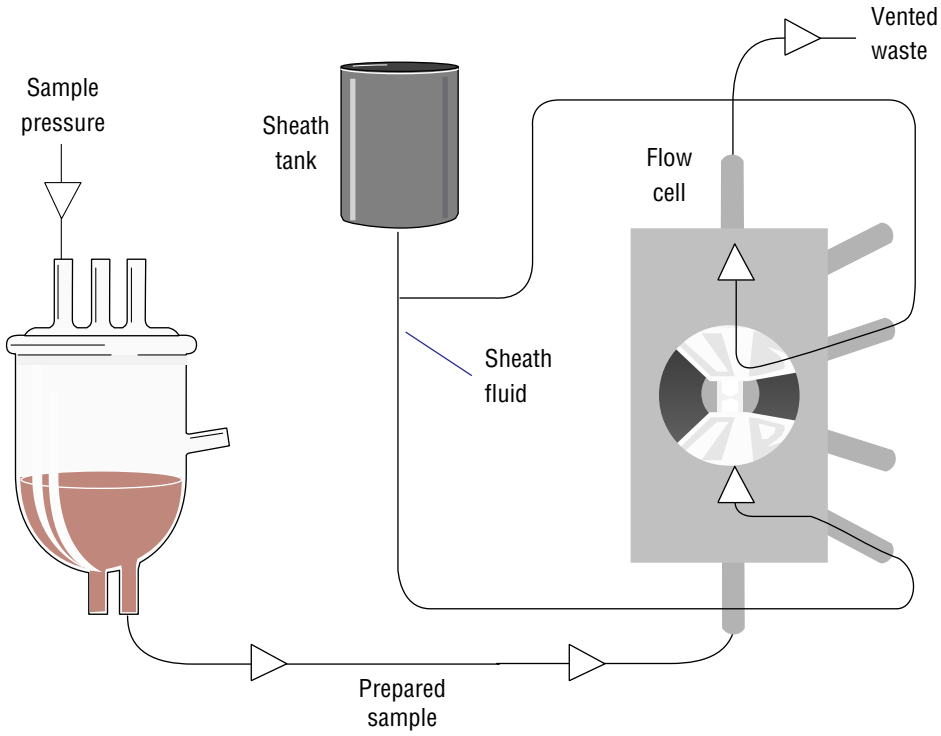
Preparing the Diff Sample



- The BSV rotates back to its original position
- The Diff lytic reagent pumps send Erythrolyse II reagent to pick up the diff blood segment from the line just behind the BSV and deliver it to the mixing chamber
- The first rotation of the mixing chamber occurs and the red blood cell membranes are ruptured and dissolved
- StabiLyse reagent is added to the mixing chamber during the second mixing process to stabilize the white blood cells and ensure that they remain in a near-native state


From BSV
StabiLyse reagent pump
Mixing chamber
To flow cell

Diff Analysis



- Sample pressure applied to the mixing chamber pushes the Diff sample to the flow cell
- As the sample stream is pushed into the flow cell, alignment of the sample stream with the sheath fluid (hydrodynamic focusing) allows the white blood cells to pass one at a time through the flow cell aperture
- As each white blood cell passes through the aperture, the three independent VCS measurements (Volume, Conductivity, and Light Scatter) are taken simultaneously

The Cycle Completes

- The RBC and WBC aperture baths drain into their waste chamber and a rinse of ISOTON III diluent enters the baths
  - From the RBC diluent dispenser, 10.0 mL of diluent is dispensed via the BSV to the WBC bath
  - From the WBC diluent dispenser, 6.0 mL of diluent is dispensed via the BSV to the RBC bath
  - This process, referred to as crossrinse, is needed to minimize the potential for carryover in the WBC bath
- High current is applied to the apertures (the burn circuit) to clean them
- The aspiration system is backwashed and the needle vent is rinsed and dried
- The mixing chamber and the sample pathway through the flow cell are rinsed
- Waste chambers are drained

NOTES

BASIC TROUBLESHOOTING TECHNIQUES

How to Recognize an Instrument Problem

- By the data that is generated
  - Start Up
  - Latex primer and control
  - Commercial cell controls
  - Diff Comparison procedure
  - $\overline{X}_B$ Analysis
  - Patient samples
  - System Test
  - IQAP
- By the way the instrument functions . . . abnormalities in the sample flow
- By the error messages generated
  - Error messages with corrective actions are listed in the Special Procedures and Troubleshooting (SPT) Manual

Three Steps to More Efficient Troubleshooting

1 Be completely familiar with normal operation

- Start Up, Sample Analysis, Shut Down
- Normal Sample Flow
  - Know what should be happening during each part of the cycle
  - Become familiar with the normal sounds of operation

2 Use a logical approach to obtain a clear symptom and isolate the components involved

- Use the error message information as a starting point
- Use your knowledge of sample flow

3 Acquire the knowledge and skills necessary to locate and correct problems

- Use the instrument
- Read the manuals and become familiar with the kind of information they contain
- Call your Beckman Coulter Representative

Troubleshooting by Instrument Subsystems

- Three instrument subsystems:
  - Electronic
  - Reagent
  - Fluidic
    - Pneumatics (pressures and vacuums)
    - Hydraulics (liquids)

Electronic Subsystem

- If there's no power,
  - Check to see if the Standby indicator light is on or off
  - Check power switches
  - Check plug connections
  - Check fuses (refer to the SPT Manual)
- If the Hgb lamp is burned out, see the SPT Manual

Reagent Subsystem

Level Sensing

- Level sensing is accomplished using two different types of sensors
  - Reagent level sensing uses optical sensors
  - Sensing waste container level uses a float-level sensor

Replacement Procedures

- To replace a reagent, see the summary
- To replace a waste container, see the summary
- To replace pickup tubes, see the SPT Manual

Reagent Related Problems

- May be contaminated
- May be expired
- May have been frozen causing the ingredients to separate
- May be the source of electrical interference due to electrolytic characteristics

Fluidic Subsystem

Key Components

- Tubing
- Pinch Valves
- Pumps
- Solenoids

Hydraulics

- Check tubing connections and routing
  - At the reagent container
  - At the input manifold on the back of the Main Unit
  - Throughout the Diluter part of the Main Unit
- Check associated pumps, pinch valves, and solenoids

Pneumatics

Vacuums

- High vacuum
  - Equivalent of 17 to 28 inches of mercury
  - Generated in the pneumatic power supply
  - Used in aspiration, refilling diaphragm pumps and draining the aperture baths
  - Not operator adjustable
  - Appears on the **System Test**
  - If out of range on the low side, check the vacuum trap and clean as needed using the procedure in the SPT Manual
  - If the vacuum trap is not the problem, call your Beckman Coulter Representative for assistance
- Low vacuum
  - Equivalent to 6 inches of mercury
  - Comes from high vacuum
  - Also called aperture vacuum
  - Pulls the dilutions through the RBC and WBC apertures and sweep flow diluent behind the RBC aperture
  - Can be adjusted by the operator using the **System Test** option and the low vacuum regulator knob, see the SPT Manual

Pressures

- 60 psi
  - Generated in the pneumatic power supply
  - Is the source of 6 psi and 30 psi
  - If out of range, call your Beckman Coulter Representative
- 30 psi
  - Used for dispensing reagents from pumps, opening pinch valves, and moving air cylinder shafts
  - Can be adjusted by the operator using the **System Test** option and the 30 psi regulator knob, see the SPT Manual
- 6 psi
  - Used for sheath pressure and mixing bubbles
  - Sheath pressure can be adjusted by the operator using the **System Test** option and the sheath pressure regulator knob, see the SPT Manual

Troubleshooting through Sample Flow

- To successfully troubleshoot this instrument,
  - Become completely familiar with normal sample flow
  - Know what should be happening during each part of the cycle
  - Be familiar with the normal sounds of operation
- This type of knowledge is gained mostly through experience
- To increase your familiarity and your comfort level with the normal sample flow of this instrument, periodically review the Sample Flow section of this training guide or the Sample Flow descriptions in Chapter 3 of the **Reference** Manual
- The Key Operator should know normal sample flow well enough to verbally describe the normal operation sequence without the aid of a document

Troubleshooting using Special Procedures

Reproducibility Check

- Defining reproducibility
  - Checks how close several results from the same specimen are to each other; repeatability
  - Also known as precision
  - Measurement is expressed as a percentage of deviation from the mean which is called a Coefficient of Variation or CV
  - Reproducibility is not a measure of accuracy, but true accuracy is not possible unless an instrument is precise

- Procedure is located in the Special Procedures and Troubleshooting (SPT) Manual, Chapter 2
  - Two procedures are available, one using the normal level of 5C cell control (Procedure A) and a second using normal whole blood (Procedure B)
  - Verify **PR↓** on the Status line to avoid printing the results of every cycle

Carryover Check

- Defining carryover
  - Carryover is the interaction of the previous sample with the current sample
  - High to low carryover check to verify the high results of one sample do not affect the low results of the next sample
- Procedure is located in the Special Procedures and Troubleshooting (SPT) Manual, Chapter 2
  - DMS program directs you to run two blood samples followed by three diluent samples then the program calculates the carryover between the second sample and the first diluent, at the same time subtracting the background count
  - The formula is:

$$\%\ Carryover\ =\ \frac{1st\ diluent\ result - 3rd\ diluent\ result}{2nd\ sample\ result}$$

  - For a statistically valid result, the second sample should be aspirated from a tube containing a specimen with a WBC count of 10,000 ± 1,000 (all other parameters should be within normal ranges)
  - Ignore count period (CP) voteouts on diluent runs

NOTES

OVERVIEW

What are Summary Pages?

Each summary is an abbreviated reference of essential operating instructions detailed in the various instrument manuals. These abbreviated instructions are referred to as either summary pages or operational summaries.

Purpose

Each summary page is an individual, stand-alone document that is designed for use in your laboratory as a training aid and/or as a reference tool for a trained operator.

This chapter contains a master copy of each summary page. These pages may be copied for use during training or as quick reference guides for trained operators while operating the instrument.

Design

These summary pages are designed for ease of use by a newly trained operator as well as an experienced operator. As you examine these summary pages, notice that each summary consists of two levels of detail. The first level states the actual task (usually in bold) and the second level contains abbreviated instructions (usually in a bulleted sequence) that an operator may need to complete that task.

A novice operator is likely to need all this information, but as an operator's skills develop, the bulleted instructions will be needed less and less because the operator already knows how to complete the stated task. An experienced operator is likely to use a summary page more as a quick reference to make sure all required steps are completed in the proper sequence.

Presentation in This Training Guide

Since these summary pages will be helpful in training other operators in your laboratory and since they are designed to be used as reference tools after training is complete, it is important for you, as the Key Operator, to become proficient in the use of this tool during this training. To eliminate the need to flip back and forth between the topic and the summary masters, the appropriate operational summaries are inserted within each topic. When an operational summary is inserted in the training material, a rotated chevron (➤) appears to the left of the summary page title. For example, at the point of insertion, the Startup Summary would begin with

➤ Startup Summary

Since this is a stand-alone summary for use after training is complete, some of the instructions discussed in the training topic may be repeated in the summary. Two rotated chevrons (➤ ➤) mark the end of the inserted summary.

CONTENTS

Since these summary pages are designed to be used as stand-alone documents, consecutive page numbering was not used on these pages.

Most operational summaries consist of either a single-side or a double-sided page. However, to accommodate the few summaries that are three or four pages long, the footer for each summary shows the total number of pages — for example, 3 of 4 appears on page 3 of a 4-page summary and 1 of 1 appears on a single page summary.

To assist you in locating a desired summary (since the page numbering is not sequential), the summary pages are arranged in the following order.

Routine Procedures

- Clean Cycle (includes automatic Startup)
- Running LATRON Primer and Control
- Running 5C Cell Control
- Running Whole-Blood Patient Samples (includes Primary and Secondary modes) (Using a HmX Hematology Analyzer with Autoloader)
- Running Whole Blood Patient Samples in the Primary Mode (Using a HmX Hematology Analyzer)
- Running Whole Blood Patient Samples in the Secondary Mode (Using a HmX Hematology Analyzer)
- Running Patient Samples in the Predilute Mode
- Data Base Query Quick Reference Guide
- Startup
- Shutdown

Retic Analysis

- Running Retic-C Cell Control
- Running Samples in the Retic Mode

QC File Management

- Making Changes to a Control File Currently In Use
- Control File Management (Using the Default Limits/Expected Range from the Package Insert)
- Control File Management (Using Your Laboratory's Custom Ranges)

Replacement Procedures

- Changing Reagents
- Replacing a Waste Container

CLEAN CYCLE SUMMARY

1 Perform the Clean Cycle

- From the Access screen, press F3 **CLEAN** then press Enter

2 Let the Instrument Stand

- Cleaning agent remains in the instrument
- Leave instrument power on
- After 30 minutes, a Startup automatically begins

3 Review Results

- Once the startup cycles are complete, review the Summary screen and, if necessary, the System Status Values screen
  - If the Background Check fails, press F2 to view the System Status Values screen and then press F3 **Repeat Background** to redo the background counts
  - If Fail appears for any other check, press F2 to view the System Status Values screen then perform a **System Test**

    Special Functions ▸▸ Diagnostics ▸▸ Operator Options ▸▸ System Test
    Press Enter then F3 **Run** to initiate the test then follow the prompts
- Startup results are not saved, but do print automatically
- Save the Summary and System Status Values screen printouts for documentation purposes

4 Perform Quality Control Checks

- Perform quality control checks before running patient specimens

RUNNING LATRON PRIMER AND CONTROL SUMMARY

1 Status for Proceeding

- Ensure Startup is complete
- LATRON Primer and Control must be at room temperature

2 Run LATRON Primer

At the DMS

- From the Main Menu, select **Controls ▸▸ Control Run**
- If the LATRON file does not appear, press F2 **File** and select your current Latex file
- Press F3 **Run** then F4 **PRIMER**

At the Diluter

- Cycle LATRON Primer in the Secondary mode
  - Immerse the aspirator tip completely in the Primer
  - Do not place the tip against the side of the bottle
  - Press and release the sample bar
  - Listen for the beep or verify the status line message changes from *Aspirating* to *Diluting* **before** removing the bottle

Reviewing Primer Results at the DMS

- Check the count for both DIFF and Retic when the analysis is complete
- If both counts are ≤500,
  - Press Esc to remove the Primer Run window
  - Go to step 3 and run LATRON Control
- If either or both counts are ≥500,
  - Rerun the LATRON Primer
  - Evaluate the new count and troubleshoot if needed

3 Run LATRON Control

At the DMS

- If necessary, press Esc to close the Primer Run window
- Press F3 **Run** then press F3 **CONTROL (SECONDARY)**

At the Diluter

- Gently invert the LATRON Control bottle 5 to 8 times
- Cycle LATRON Control in the Secondary mode
  - Immerse the aspirator tip completely in the control
  - Do not place the tip against the side of the bottle
  - Press and release the sample bar
  - Listen for the beep or verify the status line message changes from *Aspirating* to *Diluting* **before** removing the bottle

Reviewing Control Results at the DMS

- Review results once the run appears on the Control Run screen
- If a result is out-of-limits, repeat the entire process beginning with LATRON Primer
- If the results of two control runs are unacceptable,
  - Follow your laboratory's protocol
    or
  - Refer to the When LATRON is Out of Limits table in the Operator's Guide
- Compare the results of this run with previous runs, as desired
  - To evaluate stored numerical data, select **Controls ▸▸ Review or Report**

    Note: Use the → and ← keys to move between DIFF and Retic data.
  - To evaluate Levey-Jennings graphs, select **Controls ▸▸ Graphs**

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RUNNING 5C CELL CONTROL SUMMARY

1 Getting the DMS Ready

If *SELECT FUNCTION* is displayed

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

  Note: Preparation for cycling in the Primary mode with the DIFF mode ON begins automatically.

If *SELECT FUNCTION* is <u>not</u> displayed

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**
- Press F3 **Run**
- If DIFF mode is OFF, press F9 **STOP** then press F6 **DIFF ON/OFF**
- Is the *PRIMARY: SAMPLE ANALYSIS* prompt at the top of the F3-Run window?
  - If yes, press Esc
  - If no, press F2 **START PRIMARY**

2 Prepare Controls according to the Package Insert

- Warm controls at room temperature 10 to 15 minutes
- Mix each control tube using the 8 x 8 x 8 method twice
  - Do not use a mechanical mixer!
  - Do not write an open tube date or your initials in the white space between the bar code and the tube's cap

3 Run Controls in the same manner as Patient Samples

If using a HmX Hematology Analyzer with Autoloader

- Load the control tubes into a cassette
  - Load in any order
  - Verify the bar-code label is facing upward
- Place the cassette in the loading bay

If using a HmX Hematology Analyzer

- Scan the bar-code label of the control tube you wish to run

  Note: Control levels may be processed in any order.
- After a successful read, insert the control tube through the entry port into the carousel
- Repeat for each control tube

4 Review Results

If using a HmX Hematology Analyzer with Autoloader

- To verify control results are stored in the control file, select **Controls ▸▸ Review or Report**
- If the bar code was not facing up properly or if the bar code was not read because it is damaged, control results are stored in the patient data base
  - If the bar code was not facing up, position it correctly and run the control again
  - If the bar code is damaged, see the Cycling Commercial Cell Controls without Bar-Code Labels heading in the Operator's Guide Startup and Controls chapter

For both the HmX Hematology Analyzer with Autoloader and the HmX Hematology Analyzer

- If a result is out-of-limits,
  - Follow your laboratory's protocol
    or
  - Refer to the When CBC/DIFF Control is Out of Limits table in the Operator's Guide
- Compare the results of this run with previous runs, as desired
  - To evaluate stored numerical data, select **Controls ▸▸ Review or Report**
  - To evaluate Levey-Jennings graphs for the presence of shifts or trends, select **Controls ▸▸ Graphs**
- Print results from the Control Run screen, as desired

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RUNNING WHOLE BLOOD PATIENT SAMPLES SUMMARY

Using a HmX Hematology Analyzer with Autoloader

Running Whole Blood Samples in the Primary Mode

1 Getting the DMS Ready

If not using the Worklist

- If *SELECT FUNCTION* is displayed,
  - From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

    Note: Preparation for cycling in the Primary mode with the DIFF mode ON begins automatically.
  - To continue operation with the DIFF mode ON, go to step 2
  - To operate with DIFF mode OFF, go to Finish DMS Preparation
- If *SELECT FUNCTION* is **not** displayed,
  - From the Main Menu, select **Sample Analysis ▸▸ Run Samples**
  - Go to Finish DMS Preparation

If using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Worklist**
- Enter appropriate identifiers
- Press F3 **Comments** to enter additional demographics then press F10 **Save/Esc**
- If *SELECT FUNCTION* is displayed,
  - Press Ctrl + W

    Note: Preparation for cycling in the Primary mode with the DIFF mode ON begins automatically.
  - To continue operation with the DIFF mode ON, go to step 2
  - To operate with DIFF mode OFF, go to Finish DMS Preparation
- If *SELECT FUNCTION* is **not** displayed,
  - Press Ctrl + W
  - Go to Finish DMS Preparation

Finish DMS Preparation

- Press F3 **Run**
- If the DIFF mode needs to be changed, press F9 **STOP** then F6 **DIFF ON/OFF**
- If the *PRIMARY: SAMPLE ANALYSIS* prompt is at the top of the F3-Run window, press Esc; if not, press F2 **START PRIMARY**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

2 Load Cassettes

- Load the specimen tubes into the cassette making sure any bar-code labels are visible through the top of the cassette
- Place up to 5 cassettes in the loading bay

3 When the Run is Complete

- Verify all sample ID entries before reporting results
- Remove cassettes from the unloading bay
- If using the Worklist, periodically check for accumulation of Status messages

Interrupting Primary Mode Operation to Run a STAT

- Press F3 **Run** then F3 **SECONDARY** (the bed continues to rock and the current cycle continues to completion)
- When the Enter ID field appears, type the sample ID then press Enter
- Run the STAT specimen in the Secondary mode
- Press F3 **Run** then F2 **START PRIMARY** to resume operation in the Primary mode (Autoloader picks up where it left off)

Running Whole Blood Samples in the Secondary Mode

1 Getting the DMS Ready

If not using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

If using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Worklist**
- Press F2 **Sec**
- Enter appropriate identifiers
- Press F3 **Comments** to enter additional demographics then press F10 **Save/Esc**
- Press Ctrl + W

- Press F3 **Run**
- If the DIFF mode needs to be changed, press F9 **STOP** then F6 **DIFF ON/OFF**
- Press F3 **SECONDARY**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

2 Process the Sample

- Type the sample ID at the keyboard then press Enter
- Mix the specimen gently but thoroughly
- Aspirate the sample into the system

3 When the Cycle is Complete

- Verify the sample ID entry and results before reporting
- If using the Worklist, periodically check for accumulation of Status messages

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RUNNING WHOLE BLOOD PATIENT SAMPLES IN THE PRIMARY MODE SUMMARY

Using a HmX Hematology Analyzer

1 Getting the DMS Ready

If not using the Worklist

- If *SELECT FUNCTION* is displayed,
  - From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

    Note: Preparation for cycling in the Primary mode with the DIFF mode ON begins automatically.
  - To continue operation with the DIFF mode ON, go to step 2
  - To operate with DIFF mode OFF, go to Finish DMS Preparation
- If *SELECT FUNCTION* is **not** displayed,
  - From the Main Menu, select **Sample Analysis ▸▸ Run Samples**
  - Go to Finish DMS Preparation

If using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Worklist**
- Enter appropriate identifiers
- Press F3 **Comments** to enter additional demographics then press F10 **Save/Esc**
- If *SELECT FUNCTION* is displayed,
  - Press Ctrl + W

    Note: Preparation for cycling in the Primary mode with the DIFF mode ON begins automatically.
  - To continue operation with the DIFF mode ON, go to step 2
  - To operate with DIFF mode OFF, go to Finish DMS Preparation
- If *SELECT FUNCTION* is **not** displayed,
  - Press Ctrl + W
  - Go to Finish DMS Preparation

Finish DMS Preparation

- Press F3 **Run**
- If the DIFF mode needs to be changed, press F9 **STOP** then F6 **DIFF ON/OFF**
- If the *PRIMARY: SAMPLE ANALYSIS* prompt is at the top of the F3-Run window, press Esc; if not, press F2 **START PRIMARY**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

2 Process the Sample

With Bar-Code Labels

- Mix the specimen gently but thoroughly
- Hold the tube's bar-code label in front of the reader
- Listen for the beep and verify the green LED is glowing
- Confirm the ID appears in the Enter ID field
- Properly insert the specimen tube into the carousel

Without Bar-Code Labels

- Type the sample ID at the keyboard then press Enter
- Mix the specimen gently but thoroughly
- Verify the green LED is glowing
- Properly insert the specimen tube into the carousel

3 When the Cycle is Complete

- Verify the sample ID entry and results before reporting
- Remove the specimen tube from the exit tray
- If using the Worklist, periodically check for accumulation of Status messages

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RUNNING WHOLE BLOOD PATIENT SAMPLES IN THE SECONDARY MODE SUMMARY

Using a HmX Hematology Analyzer

1 Getting the DMS Ready

If not using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

If using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Worklist**
- Press F2 **Sec**
- Enter appropriate identifiers
- Press F3 **Comments** to enter additional demographics then press F10 **Save/Esc**
- Press Ctrl + W

- Press F3 **Run**
- If the DIFF mode needs to be changed, press F9 **STOP** then F6 **DIFF ON/OFF**
- Press F3 **SECONDARY**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

2 Process the Sample

With Bar-Code Labels

- Mix the specimen gently but thoroughly
- Hold the tube's bar-code label in front of the reader
- Listen for the beep and verify the green LED is glowing
- Confirm the ID appears in the Enter ID field
- Aspirate the sample into the system

Without Bar-Code Labels

- Type the sample ID at the keyboard then press Enter
- Mix the specimen gently but thoroughly
- Aspirate the sample into the system

3 When the Cycle is Complete

- Verify the sample ID entry and results before reporting
- If using the Worklist, periodically check for accumulation of Status messages

RUNNING PATIENT SAMPLES IN THE PREDILUTE MODE SUMMARY

For HmX Hematology Analyzer with Autoloader and HmX Hematology Analyzer

1 Getting the DMS Ready

If not using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**

If using the Worklist

- From the Main Menu, select **Sample Analysis ▸▸ Worklist**
- Press F2 **Sec**
- Enter appropriate identifiers
- Press F3 **Comments** to enter additional demographics then press F10 **Save/Esc**
- Press Ctrl + W

- Press F3 **Run**
- Press F4 **PREDILUTE CBC**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

2 Make the Dilution

- Make an accurate 1:3 dilution of the specimen (Use a minimum of 50 µL of well-mixed, fresh whole blood and 100 µL of diluent)
- Mix the dilution gently but thoroughly

3 Process the Dilution

- Type the sample ID at the keyboard then press Enter
- Mix the dilution gently again
- Aspirate the dilution into the system using the Secondary mode's aspirator tip and sample bar

4 When the Cycle is Complete

- The instrument automatically multiplies the diluted results by 3
- Verify the sample ID entry and results before reporting
- If using the Worklist, periodically check for accumulation of Status messages

DATA BASE QUERY QUICK REFERENCE GUIDE

If the *SELECT FUNCTION* prompt isn't displayed, press F9 **STOP**

From the Main Menu, select **Sample Analysis ▸▸ Data Base Query**

To Sort Sample Data

1. Press F6 **Sort** if the Sort window is not displayed
2. Select the group of samples you want to review or work on
   - To find samples run during a certain time period, enter the DATE and TIME in the appropriate FROM and TO fields (enter "00" for seconds)
   - To locate a specific sample,
     - DATE and TIME field entries may be cleared, if desired (use F6 **Clear**)
     - Use ↓ or Enter to go to ID# 1 or ID# 2 (if the sample was identified using the Worklist)
     - Type the sample identification **exactly** as it was entered when the sample was run
   - Sort Rules for setting up a successful sort:
     - You can sort by ID# 1 or ID# 2, but not both
     - Pay close attention to case, commas and spaces when sorting by name
     - Time requires entry of hour, minute, and seconds
     - If you select a time period, that time period applies for all days you included
     - You can sort samples based on whether they are flagged (**F**), not flagged (**N**), or both (**B**)
3. Press F8 **Execute** to activate the sort
   - Selected samples, including date and time run, should appear almost immediately
   - If the samples you selected do not appear, check the sort criteria you entered against the requirements stated in the Sort Rules above

To View Sample Results

1. Execute a sort
2. Use ↑ or ↓ to highlight the desired sample
3. Press F12 **DB** → the Run Samples display of this sample's results, scatterplot, and histograms appears

To Save Sample Data

1. Execute a sort
2. Use ↑ or ↓ to highlight the sample to be saved
3. Press F2 **Save** → an **S** appears in the Output field to indicate the sample is saved from overwriting

   Note: Use this same routine to unsave a sample.

To Edit Sample Data

1. Execute a sort
2. Use ↑ or ↓ to highlight the sample to be edited
3. Press F3 **Edit** to view all the identifiers that were entered from the Worklist and/or read from a bar-code label as well as the individual parameter results
   - Press End or ↑ to view any suspect and/or definitive messages, or an overrange result estimate

     Note: Overrange result estimate may be used to determine the dilution ratio needed to bring the overrange result within the linear or operating range.
   - Press Home or ↓ to return to the parameter result screen
4. Edit results according to the following guidelines and press Enter to save each individual input then press F10 **Save/Esc** when completely finished
   - Any field except the primary identifier, sequence number or the date and time a sample is processed may be edited provided a *NO READ, NO MATCH*, or *PART ASP* message **does not** appear in the Status field
     - *NO READ* error messages only occur on a HmX Analyzer with Autoloader
   - You may edit parameter results that are replaced with (+ + + + +), (- - - - -), or (: : : : :) codes
   - Editing a principle parameter requires changing all dependent (computed) parameters
     - Edit the WBC and the diff absolute numbers must also be changed
     - Edit the RBC and the Hct, MCH, MCHC, and Retic # must also be changed
     - Edit the Hgb and the MCH and MCHC must also be changed
     - Edit the MCV and the Hct and MCHC must also be changed
     - Edit the Retic % and the Retic number must also be changed
   - **E** overrides an **H** or **L** flag and appears in the same position
   - When an operator edits any parameter field, an *Edited Data* message appears in the Population Condition field on the following:
     - Sample analysis display
     - Sample analysis data base displays
     - With RBC and Retic # if transmitted to a host computer
     - Printouts
   - When an operator edits any parameter, all Suspect and Definitive messages for that sample are deleted from the data base and all displays

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To Output Sample Data: Print and Reprint / Transmit and Retransmit

To Print a Single Sample Data Set

1. Execute a sort
2. Use ↑ or ↓ to highlight the desired sample
3. Press F12 **DB** → the Run Samples display of this sample's results, scatterplot, and histograms appears
   - For a ticket format printout, press F2 **Ticket**
   - For a graphics format printout, press F4 **Print**

To Batch Print or Transmit a Group of Samples

1. Execute a sort
2. Tag the desired samples using F7 **Tag** or F8 **Tag All**
3. Press F5 **Batch**
4. Use ↑ or ↓ to highlight Print or Host and Spacebar to toggle to *Yes*

   Note: Batch print, transmit, and archive functions must be done independently. When one of these three options is selected, the other two are automatically disabled.
5. Verify the needed peripheral (printer and/or host computer) is online
6. Press F8 **Execute** to start the process
   - As needed, use F7 **Abort** to stop the process and F6 **Resume** to begin where it left off when stopped
7. Press Esc to return to the data base

To Print a Condensed Line List

1. Execute a sort
2. Press F4 **Print** to produce a condensed line list printout of all sorted samples

To Archive a Group of Stored Sample Results

1. Execute a sort
2. Tag the desired samples using F7 **Tag** or F8 **Tag All**
3. Press F5 **Batch**
4. Move the cursor to the Archive field then press F2 **Choice List**
5. Use the Spacebar to select either New (process all tagged samples not yet archived) or All (process all tagged samples, archived or not) then press Enter
6. In the Filename: field, type A:\ then up to eight characters (of your choice)
7. Insert a formatted diskette into the DMS diskette drive and press F8 **Execute**
8. When the *Batch is Inactive* message appears, remove the diskette from the drive

To Review Archived Files

1. Insert the diskette into a computer with a spreadsheet program that is compatible with the WKS format
2. Retrieve the desired file
   - If a column label is condensed either widen the column or move the active cell cursor to the label to see the complete label
   - One character codes include:

Fields	Key for One Character Codes
C/P Edit	0 = Not edited 1 = Edited
ID1	0 = Not edited 1 = Edited
Status	0 = Blank/Not used/Matched 1 = Partial Aspiration 2 = No Match 3 = No Read
WBC / RBC / Plt Population	0 = Normal 1 = Abnormal 2 = Edited
All Suspect and Definitive flags **except** Imm Grans / Bands	blank = No flag 1 = Flag
Imm Grans / Bands Suspect Flag	blank = No flag 1 = IMM GRANS/BANDS 1 2 = IMM GRANS/BANDS 2
Mode of Aspiration	1 = Primary (Closed-Vial) mode 2 = Secondary (Open-Vial) mode

3. Close the file and remove the diskette from the diskette drive

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1 Check Current Instrument Status

- If the power is off, turn it on
- If the Main unit is in Standby, switch it to Ready
- If the DMS monitor is dark, press Spacebar
- If Startup results are already displayed as the result of a Clean Cycle, go to step 3; if not, enter your three character Operator ID (optional)
  - From the Main Menu, press F5 **Options**
  - Type your three character Operator ID
  - Press Esc to return to the Main Menu

2 Perform Start Up

- From the Main Menu, select **Diluter Functions ▸▸ Start Up**
- Press Enter

3 Review Results

- Once the startup cycles are complete, review the Summary screen and, if necessary, the System Status Values screen
  - If the Background Check fails, press F2 to view the System Status Values screen and then press F3 **Repeat Background** to redo the background counts
  - If Fail appears for any other check, press F2 to view the System Status Values screen then perform a **System Test**

    Special Functions ▸▸ Diagnostics ▸▸ Operator Options ▸▸ System Test
    Press Enter then F3 **Run** to initiate the test then follow the prompts
- Startup results are not saved, but do print automatically
- Save the Summary and System Status Values screen printouts for documentation purposes

4 Perform Quality Control Checks

- Perform quality control checks before running patient specimens

1 Perform Shut Down

- Verify *SELECT FUNCTION* is displayed on the status line
- From the Main Menu, select **Diluter Functions ▸▸ Shut Down**
- Press Enter

2 Let the Instrument Stand

- Allow cleaning agent to remain in the instrument a minimum of 30 minutes
- Leave instrument power on

  Note: If the power is turned off and the instrument is going to be idle for more than 48 hours, do the Prolonged Shutdown Procedure in the Operator's Guide, Chapter 5.
- Perform the Startup procedures before running patient specimens or controls

RUNNING RETIC-C CELL CONTROL SUMMARY

1 Prepare Controls according to the Package Insert

- Warm controls at room temperature 10 to 15 minutes
- Mix each control tube using the 8 x 8 x 8 method once
- Do not use a mechanical mixer

2 Preparing the Control

- For each control tested, label two 12 x 75 mm test tubes "A" and "B"
- Place 4 drops of Reagent A into tube "A"
- Dispense 50 µL of the well mixed control into tube "A"
- Gently mix tube "A"
  - Let the tube stand for a minimum of 5 minutes and a maximum of 60 minutes

3 Getting the DMS Ready

- From the Main Menu, select **Controls ▸▸ Control Run**
- Press F2 **File** and select the desired file
- Press F3 **Run**, F3 **CONTROL (SECONDARY)**

4 Run Controls in the Same Manner as Patient Samples

- Gently mix the "A" tube
- Pipette 2 µL of the control/stain mixture from tube "A" into the bottom of tube "B"
- Place tube "B" with the control/stain aliquot at an angle under the tip of the Reagent B dispenser
- Dispense 2 mL of Reagent B into tube "B"
- **Do not mix**
- Wait 30 seconds then analyze the retic preparation you just made
  - Immerse the aspirator tip into the retic preparation
  - Press and release the sample bar
  - Listen for the beep or verify the status line message changes from *Aspirating* to *Diluting* **before** removing the tube

5 Review Results

- If a result is out-of-limits,
  - Follow your laboratory's protocol
    or
  - Refer to the When Retic Control is Out of Limits table in the Operator's Guide
- Compare the results of this run with previous runs, as desired
  - To evaluate stored numerical data, select **Controls ▸▸ Review or Report**
  - To evaluate Levey-Jennings graphs for the presence of shifts or trends, select **Controls ▸▸ Graphs**

RUNNING SAMPLES IN THE RETIC MODE SUMMARY

For HmX Hematology Analyzer with Autoloader and HmX Hematology Analyzer

1 Preparing the Sample

- For each patient tested, label two 12 x 75 mm test tubes with the patient's ID then label one tube "A" and the other "B"
- Place 4 drops of Reagent A into each of the tubes labeled "A"
- Dispense 50 µL of the well-mixed, whole-blood specimen into the tube labeled "A"
- Gently mix tube "A"
- Let the tube stand for a minimum of 5 minutes and a maximum of 60 minutes

2 Getting the DMS Ready

- From the Main Menu, select **Sample Analysis ▸▸ Run Samples**
- Press F3 **Run**
- Press F5 **RETIC**
- If necessary, press F5 **Optns** to set up run options then press Esc to exit

3 Process the Sample

- Gently mix tube "A"
- Pipette 2 µL of the patient/stain mixture from tube "A" into the bottom of tube "B"
- Place tube "B" with the patient/stain aliquot at an angle under the top of the Reagent B dispenser
- Dispense 2 mL of Reagent B into tube "B"
  - Do not mix
- Wait 30 seconds
- While waiting, type the Sample ID# at the keyboard and press Enter
- At **exactly** 30 seconds, analyze the specimen
  - Immerse the aspirator tip into the retic preparation
  - Press and release the sample bar
  - Listen for the beep or verify the status line message changes from *Aspirating* to *Diluting* **before** removing the tube

4 Reporting Retic Absolute Number

- Locate your Retic result in **Data Base Query**
- Press F3 **Edit** and enter the patient's RBC value
- The system automatically calculates the Retic #
- An **E** appears beside the RBC value and an **e** beside the Retic # value

MAKING CHANGES TO A CONTROL FILE CURRENTLY IN USE

Use only if you want to make a change in a control file that is currently in use. This summary should be used when changing individual items in a previously set up control file, such as entering a running mean or correcting a typographical error. If you want to make a set up change (such as changing the lot number), use the appropriate Control File Management Summary.

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up**
- Select the type of file and then select the existing file you want to change

2 Answer the Questions

- *Recompute Existing Runs ?*
  - Press Spacebar to answer *Yes* then press Enter
  - *Yes* automatically adjusts the statistics and graphs to the change
  - Operator must toggle to *Yes* to look at the screen even if no changes are made
- *Use Default Limits / Expected Range ?*
  - To maintain laboratory custom ranges, press Enter to answer *No*
  - To use Expected Ranges (from the package insert), press Spacebar to answer *Yes* then press Enter

3 Make the Changes

- Enter the new information

4 Verify All Changes

- Check all entries are correct then press F10 to save and exit the screen

Using the Default Limits/Expected Range from the Package Insert

Note: To maintain customized expected ranges in a control file, use the Control File Management Using Your Laboratory's Custom Ranges Summary.

Review the Numeric Control Data and Graphs, if applicable

1 Locate the Control File

- From the Main Menu, select **Controls ▸▸ Graphs**
- Press F2 **File** and select the file for review then press Enter

2 Review the Graphs

- Using F6 **Additional Graphs** review all graphs noting any trends, shifts or plot points inside the red area
- Press Esc then R to switch to the **Review or Report** option

3 Review Numeric Data

- Review % CV of all parameters (press → to see additional parameters)
- If a CV is high, use Page Down and examine each run for results mistakenly run into the file (the graph review should correlate)
- Delete any mistakes from the file using F6 **Remove/Restore**

Print / Download the Control File Data

- From the Review or Report screen, press F4 to automatically print all parameters and all runs
- If desired, press Esc then G then F4 to print all graphs
- Download IQAP data, as needed

Delete the Control File Data

1 Go to Control Review Screen

- From the Main Menu, select **Controls ▸▸ Review or Report**
- Press F2 **File** and select the file you want to delete then press Enter

2 Delete the Control File Data

- Press F8 **Delete File**
- Press Spacebar to toggle to *Yes*
- Press Enter to confirm and delete the control file data

Set Up the New Lot Number of Control

Any Control File can be set up as a:

- 5C cell control file (CBC/DIFF file)
- LATRON control file (Latex file)
- Retic-C cell control file (RETIC file)

Setting Up a 5C Cell Control File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ CBC/DIFF file**
- Move the cursor to a **NOT SETUP** file or an inactive file where the control run data is deleted then press Enter

2 Upload the Assay Values

- Insert the 5C cell control diskette into the A: drive of the computer
- Press F5 **Upload Assay Values**
- Press the function key for the desired level of control

3 Type Manual Entries

- Select the Shift and manually type the Operator ID

4 Verify All Information

- Make sure the Host transmit setting correlates with your laboratory's protocol
- Check all entries are correct then press F10 to save and exit the screen

5 Set Up Another Level of Control, if applicable

- Move the cursor to a **NOT SETUP** file or an inactive file where the control run data is deleted then press Enter
- Repeat steps 2 through 5

6 Remove the Diskette from Drive A: (after all needed set ups are complete)

Setting Up a LATRON Control File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ Latex file**
- Move the cursor to a **NOT SETUP** file or an inactive file where the control run data is deleted then press Enter

2 Type Manual Entries

- Enter the name of the file and your Operator ID
- Enter the Lot # and expiration date from the package insert

3 Verify All Information

- Make sure the Host transmit setting correlates with your laboratory's protocol
- Check all entries are correct then press F10 to save and exit the screen

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Setting Up a RETIC File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ RETIC file**
- Move the cursor to a **NOT SETUP** file or an inactive file where the control run data is deleted then press Enter

2 Type Entries from the Table of Expected Results

- Enter the Lot #, Expiration Date, Shift, and your Operator ID
- Enter the RBC and Retic % values from the assay sheet

  Note: If you enter the wrong RBC value and then correct it after running controls, the DMS does not recalculate the incorrect RET # control results stored in the RETIC file.

3 If the MRV and IRF Parameters Are Enabled

- Enter 99.9 for MRV and IRF assigned values and expected ranges if customized values are not established

4 Verify All Information

- Make sure the Host transmit setting correlates with your laboratory's protocol
- Check all entries are correct then press F10 to save and exit the screen

Using Your Laboratory's Custom Ranges

Use this summary only if you want to maintain the customized expected ranges previously entered in an existing control file (typically a 5C control file).

Review the Numeric Control Data and Graphs, if applicable

1 Locate the Control File

- From the Main Menu, select **Controls ▸▸ Graphs**
- Press F2 **File** and select the file for review then press Enter

2 Review the Graphs

- Using F6 **Additional Graphs** review all 20 graphs noting any trends, shifts or plot points inside the red area
- Press Esc then R to switch to the **Review or Report** option

3 Review Numeric Data

- Review % CV of all parameters (press → to see additional parameters)
- If a CV is high, use Page Down and examine each run for results mistakenly run into the file (the graph review should correlate)
- Delete any mistakes from the file using F6 **Remove/Restore**

Print / Download the Control File Data

- From the Review or Report screen, press F4 to automatically print all parameters and all runs
- If desired, press Esc then G then F4 to print all 20 graphs
- Download IQAP data, as needed

Changing the Existing File

1 Select the File

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ Control set up ▸▸ CBC/DIFF file**
- Select the existing file you want to change

2 Answer the Questions

- *Recompute Existing Runs ?*
  - Press Spacebar to answer *Yes* then press Enter
  - *Yes* automatically adjusts the statistics and graphs to the change (operator must toggle to *Yes* to look at the screen even if no changes are made)
- *Use Default Limits / Expected Range ?*
  - Press Enter to answer *No* (maintains your laboratory's custom ranges)

3 Upload the 5C Control Assay Values

- Insert the 5C cell control diskette into the A: drive of the computer
- Press F5 **Upload Assay Values**
- Press the function key for the desired level of control

4 Type Manual Entries

- Select the Shift and manually type the Operator ID

5 Verify All Information

- Make sure the Host transmit setting correlates with your laboratory's protocol
- Check all entries are correct then press F10 to save and exit the screen

6 Set Up Another Level of Control, if applicable

- Select another **CBC/DIFF file** you want to change
- Repeat steps 2 through 6

7 Remove the Diskette from Drive A: (after all needed set ups are complete)

IMPORTANT Risk of misleading control results. Old control runs remaining in a newly set up control file are included in the statistical data for the new file. Delete all controls runs before starting to run the new control.

Delete the Control File Data

1 Go to Control Review Screen

- From the Main Menu, select **Controls ▸▸ Review or Report**
- Press F2 **File** and select the file you want to delete then press Enter

2 Delete the Control File Data

- Press F8 **Delete File**
- Press Spacebar to toggle to *Yes*
- Press Enter to confirm and delete the control file data

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CHANGING REAGENTS SUMMARY

1 Record the New Reagent Information

- From the Main Menu, select **Special Functions ▸▸ Set Up ▸▸ System Set Up ▸▸ Reagents**
- Enter the lot number and the expiration date or open container stability date, as applicable
  - The date opened automatically changes to today's date when the lot number is entered (this is the reason the lot number should always be typed even if it is the same as the last container)
  - Six digits must be entered for the expiration date or open container stability date

  Note: Open container stability is stated on the reagent container, when applicable.
- Press F10 to save the information then F9 to return to the Main Menu

2 Replace the Reagent Container

- Turn the compressor off

  From the Main Menu, select **Special Functions ▸▸ Diagnostics ▸▸ Operator Options ▸▸ Fluidics Tests ▸▸ Compressor On/Off** then press Enter; press the Spacebar to select **Off** and press Enter again
- Open the new reagent container
- Remove the pickup tube assembly from the old container then transfer it directly to the new container and tighten

  Note: If the lower part of the assembly touches you or anything outside the container, flood that lower part of the assembly with distilled water then wipe it with a lint-free tissue.

3 Prime the Lines

- From the Main Menu, select **Diluter Functions ▸▸ Prime Reagents**
- Select the reagent that was changed then press Enter, the compressor turns on and the Prime Reagents program activates
- Once priming is complete, press F9 to return to the Main Menu and continue normal operation

REPLACING A WASTE CONTAINER SUMMARY

1 Put the Instrument into Standby

- Move the Standby/Reset rocker switch to Standby, position O

2 Turn OFF the Main Power

- Use the Main POWER On/Off rocker switch located on the back of the Main Unit

3 Replace the Waste Container

- Using biohazardous precautions,
  - Remove the waste assembly from the full container
  - Transfer the waste assembly directly to an empty waste container and tighten
- Dispose of the biohazardous waste according to you laboratory's protocol

4 Turn ON the Main Power

- Use the Main POWER On/Off rocker switch located on the back of the Main Unit

5 Put the Instrument into Ready

- Move the Standby/Reset rocker switch to Ready, position I

OFFICIAL TRAINING DOCUMENTATION

(for COULTER HmX Hematology Analyzer with Autoloader or HmX Hematology Analyzer Training)

Laboratory _____

Address _____

_____

Phone _____

HmX Serial Number _____

Key Operator _____

Training Dates _____

Instructions

The overall objectives for COULTER HmX Hematology Analyzer with Autoloader or COULTER HmX Hematology Analyzer Key Operator training are listed below. Each objective is a comprehensive statement of what an operator should be able to do when training is complete. Please review these objectives and place a check mark (✓)□ next to any objectives that both the Trainer and Trainee agree are completed either during this training or through previous knowledge. Write N/A (not applicable) inside the block if the objective does not apply to this trainee.

Checklist

Your signature at the end of this checklist indicates agreement that the Trainee is able to . . .

_____ Locate important procedures in the appropriate manual including:

- _____ Startup and Shutdown procedures
- _____ QC and Calibration procedures
- _____ Sample Analysis procedures
- _____ Reagent replacement procedures
- _____ Printer paper and ribbon replacement procedures
- _____ Troubleshooting procedures

_____ Identify basic instrument modules / components and describe their functions

_____ Correctly perform Startup, Clean Cycle, and Shutdown procedures

_____ Explain the importance of the Startup, Clean Cycle, and Shutdown procedures and the frequency with which they should be performed

_____ Perform appropriate Quality Control checks

_____ Review, delete, and set up control files

_____ Download IQAP data, if applicable

_____ Perform all preliminary procedures required for calibration

_____ Perform the CBC Calibration with S-CAL Calibrator and document the results according to your laboratory's protocol

_____ Perform Sample Analysis in the Primary, Secondary, Predilute, and Retic modes using the designated Workflow

_____ Look up samples in the data base

_____ Understand the basics principles of parameter derivation and state if a parameter result is directly measured, determined using VCS measurements, derived from a histogram, or computed

_____ Review data and recognize if a scatterplot is normal or abnormal

_____ Review data and recognize if an RBC and/or Plt histogram is typical or atypical

_____ Explain what a particular flag indicates when provided with an example

_____ Recognize an instrument problem based on abnormal sample results, abnormal Startup or System Test results, abnormal control results, and/or error messages

Comments

_____

_____

_____

_____

_____

_____

_____

_____ _____

Trainee Signature Trainer Signature

TRADEMARKS

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