Performing Complete Blood Count with WBC Differential on the DxH 520 Analyzer

Purpose

This procedure provides the instructions necessary for performing a complete blood count (CBC) and White Blood Cell (WBC) differential on the DxH 520. The complete blood count, the CBC, is the fundamental analytical test that evaluates the three main cellular components: White Blood Cells, Red Blood Cells and platelets. The WBC differential, also a fundamental test, identifies and enumerates the percent and absolute number (% & #) of the white blood cells present in whole blood. The complete blood count (CBC) assists with the identification of reactive, infectious or malignant processes. The DxH 520 CBC is based on the Coulter Principle. The WBC differential is determined using a combination of the impedance WBC data and the direct optical measurement data obtained using a blue LED focused through the WBC aperture.

This document is not intended to replace the information in the DxH 520 Instructions for Use Manual (IFU). Information in the IFU supersedes information in any other manual.

Principles of the Procedure

Parameter	Method	Description
WBC	Coulter Principle	**White Blood Cell Count or Leukocyte Count** • Measured directly, multiplied by the calibration factor • WBC = N x 10^3 cells/µL In the WBC bath, 1.25 mL of diluent is combined with an additional 0.5 mL of diluent and 14 µL of sample from the probe creating the initial WBC dilution of 1:125 (blood:diluent). The dilution is then mixed using mixing bubbles and 306 µL is aspirated into the aspiration probe. This aspirated sample is carried to the shear valve where 25 µL is segmented to be used for the RBC dilution. 0.66 mL of DxH 500 Series Lyse is dispensed into the WBC bath and mixed creating the final WBC dilution of 1:182. The system initially counts the CBC (RBC/PLT/WBC) parameters for 3 seconds followed by the second measurement of the CBC + DIFF for 7 seconds. The count goes to the computer for coincidence correction and voting. WBC data is displayed in the diff plot.

Principles of the Procedure (cont.)

Parameter	Method	Description
RBC	Coulter Principle	**Red Blood Cell Count or Erythrocyte Count** • Measured directly, multiplied by the calibration factor • RBC = N x 10^6 cells/µL In the RBC bath, 2.0 mL of diluent is combined with the 25 µL of segmented sample. This creates the final dilution of 1:10,125. The dilution is mixed and prepared for counting. The system initially counts the CBC (RBC/PLT/WBC) parameters for 3 seconds followed by the second measure of the CBC + DIFF for 7 seconds. The counts go to the computer for coincidence correction and voting. The RBC histogram shows a relative cell frequency (Y-axis) versus size (X-axis).
HGB	Photometric Measurement	**Hemoglobin or Hemoglobin Concentration** • Weight (mass) of hemoglobin is determined from the degree of absorbance found through the photo current transmittance. • HGB = g/dL In the WBC bath, human whole-blood hemoglobin is converted into stable oxyhemoglobin by DxH 500 Series Lyse. The absorbance of the pigment of the solution is proportional to the hemoglobin concentration of the sample. Hemoglobin is measured using an LED light source at 545 nm. The absorbency of the sample is compared to a blank reading, a calibration factor is applied, and the hemoglobin concentration result is reported.
HCT	Calculated	**Hematocrit** • Relative volume of packed erythrocytes to whole blood expressed in percent • HCT (%) = (RBC x MCV)/10
MCV	Derived from RBC Histogram	**Mean Corpuscular Volume** • Average volume of individual erythrocytes derived from the RBC histogram • MCV = femtoliters (fL)
MCH	Calculated	**Mean Corpuscular Hemoglobin** • Weight of hemoglobin in the average erythrocyte • MCH (pg) = (HGB/RBC) x 10

Principles of the Procedure (cont.)

Parameter	Method	Description
MCHC	Calculated	**Mean Corpuscular Hemoglobin Concentration** • Average weight of hemoglobin in a measured dilution • MCHC (g/dL) = (HGB/HCT) x 100
RDW	Derived from RBC Histogram	**Red Cell Distribution Width** • Size distribution spread of the erythrocyte population derived from the RBC histogram • Expressed as a coefficient of variation (%)
RDW-SD	Derived from RBC Histogram	**Red Cell Distribution Width - Standard Deviation** • Size distribution spread of the erythrocyte population derived from the RBC histogram • Expressed as a standard deviation in fL
PLT	Coulter Principle	**Platelet Count** • Derived from the PLT histogram, multiplied by a calibration factor • PLT = N x 10^3 cells/µL In the RBC bath, 2.0 mL of diluent is combined with the 25 µL of segmented sample. This creates the final dilution of 1:10,125. The dilution is mixed and prepared for counting. The system initially counts the CBC (RBC/PLT/WBC) parameters for 3 seconds followed by the second measure of the CBC + DIFF for 7 seconds. The counts go to the computer for coincidence correction and voting. The histogram shows a relative cell frequency (Y-axis) versus size (X-axis).
MPV	Derived from PLT Histogram	**Mean Platelet Volume** • Average volume of individual platelets derived from the PLT histogram, multiplied by a calibration factor • MPV = femtoliters (fL)

Principles of the Procedure (cont.)

Parameter	Method	Description
WBC Diff		**WBC Differential** The differential is determined using a combination of the impedance WBC data and the direct optical measurement data obtained using a blue LED focused through the WBC aperture. The digital information obtained from the WBC differential analysis is processed through the WBC differential algorithm. The DxH 520 uses simultaneous measurements of volume and axial light loss when passing cells interrupt the optical path. The amount of light falling on a sensor varies depending on cell structure. The algorithm generates the WBC differential, flagging, and messaging. This information is represented on a 2D scatter plot with volume on the Y-axis and axial light loss on the X-axis. The WBC subpopulations are identified by color and intensity (concentration) within the diff plot.
LY	Optical/ Impedance	**Lymphocyte Percent** • [LY events/(NE+LY+MO+EO+BA events)] x 100 • Expressed as a percentage (%)
MO		**Monocyte Percent** • [MO events/(NE+LY+MO+EO+BA events)] x 100 • Expressed as a percentage (%)
NE	↓	**Neutrophil Percent** • [NE events/(NE+LY+MO+EO+BA events)] x 100 • Expressed as a percentage (%)
EO		**Eosinophil Percent** • [EO events/NE+LY+MO+EO+BA events)] x 100 • Expressed as a percentage (%)
BA		**Basophil Percent** • [BA events/(NE+LY+MO+EO+BA events)] x 100 • Expressed as a percentage (%)
LY#	Calculated	**Lymphocyte Absolute Count** • LY# (10^3/µL) = (LY/100) x WBC
MO#		**Monocyte Absolute Count** • MO# (10^3/µL) = (MO/100) x WBC
NE#	↓	**Neutrophil Absolute Count** • NE# (10^3/µL) =(NE/100) x WBC
EO#		**Eosinophil Absolute Count** • EO# (10^3/µL) = (EO/100) x WBC
BA#		**Basophil Absolute Count** • BA# (10^3/µL) = (BA/100) x WBC

Clinical Utility

The DxH 520 analyzer is a quantitative, multi-parameter, automated hematology analyzer for in vitro diagnostic use in clinical laboratories and physician's office laboratories. It is used to identify the normal patient with normal system-generated parameters from patients with abnormal parameters and/or flags that require additional studies.

The DxH 520 identifies and enumerates the following parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, RDW-SD, PLT, MPV, LY%, LY#, MO%, MO#, NE%, NE#, EO%, EO#, BA%, BA# in whole blood samples (venous and capillary) collected in K_2 EDTA and K_3 EDTA anticoagulants, and prediluted whole blood.

This instrument is for use in adult and pediatric populations, including neonates.

Limitations: All numerical results reports for Basophil count and percent values must be reflexed for manual microscopy or followed up for additional testing based on the laboratory's Standard Operating Procedure (SOP).

Specimen Collection and Preparation

Whole Blood

Follow these requirements when handling samples:

- Collect and handle whole-blood samples according to the tube manufacturer's instructions and procedures in *CLSI GP41-A6* for venipuncture and *CLSI GP42-A6* for capillary.
- For recommendations for storage and mixing, review Sample Stability and Storage – Whole Blood in Chapter 1, System Overview.

The recommended anticoagulants are K_2 and K_3 EDTA.

Specimen Tubes

The DxH 520 can process a wide variety of specimen tubes. The DxH 520 can process a variety of specimen tubes. An optional tube holder is also available for the processing of specific tubes.

The minimum sample volume required for testing includes both the aspiration and dead volume in a tube and varies by tube manufacturer. For more information, refer to the Hematology Tube List (DxH 520) C12794 on the Beckman Coulter website www.beckmancoulter.com.

CAUTION: Risk of erroneous results. All tubes should contain a minimum volume of 1 mL for collection devices with greater than 1 mL fill volume or ¾ the fill volume for devices with less than 1 mL fill volume. Insufficient volume results in increased risk of erroneous results.

Using Barcode Labels

The supported specimen bar-code symbologies are Code 128, Codabar, NW7, Code 39, ISBT 128 (Donor ID Only), and Interleaved 2 of 5. The bar-code scanner can read up to 22 characters or the total number of characters that can be printed in the viewable height, whichever is less.

Sample Volume Aspirated

Approximately 16.7 µL of whole blood in closed-vial or open-vial whole blood analyzing mode

180 µL prediluted blood in predilute analyzing mode (prepared from 20 µL of whole blood and 300 µL of diluent)

Specimen Collection and Preparation (cont.)

Sample Stability and Storage

Beckman Coulter recommends analyzing refrigerated and non-refrigerated whole-blood samples within eight hours.

Samples stored at ≤ 19°C (66°F) for longer than two hours may exhibit increased cellular interference messages.

Sample stability may be evaluated as the change (drift) of the parameter during 24 hours at 18 to 26°C (64 to 79°F). For a refrigerated temperature of 2 to 8°C (35.6 to 46.4°F), drift may be evaluated at 8 hours for WBC and differential parameters, and at 24 hours for the remainder of the parameters. The drift should be within the difference or percent difference, whichever is greater.

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Special Instructions – Specimen Handling

Special Instructions – Specimen Rejection

Special Instructions- Storage and Stability

Reagents

The following are the recommended reagents:

DxH 500 Series Diluent PN B36845 (10 L)

DxH 500 Series Diluent is an enhanced low formaldehyde-producing isotonic-buffered solution. DxH 500 Series Diluent dilutes the specimen and is used for rinsing module components between sample analyses.

DxH 500 Series Lyse PN B36846 (500 mL)

DxH 500 Series Lyse is a cyanide-free reagent that lyses red blood cells for:

- White blood cell count (WBC)
- Classification of WBC subpopulations
- Hemoglobin measurement (HGB)

DxH 500 Series Cleaner PN B36868 (500 mL)

DxH 500 Series Cleaner is an azide-free, formaldehyde-free, biodegradable cleaner that contains a proteolytic enzyme that aids in the removal of protein buildup.

Beckman Coulter documentation can be found at www.beckmancoulter.com

Reagent Storage and Stability

DxH 500 Series Diluent

Store at 2-30°C. Do not use product past expiration date. Use product at temperatures stated in the Instructions for Use for your instrument. Discard opened container after 60 days. Dispose of waste product, unused product, and contaminated packaging in compliance with federal, state, and local regulations.

IMPORTANT If product has been partially or completely frozen, allow product to warm to room temperature. Invert the container 16 times to ensure complete mixing prior to placement on the instrument. Install and prime the diluent as directed in the Instructions for Use for your instrument. Verify background counts are acceptable before analyzing samples.

DxH 500 Series Lyse

Store at 4- 25°C. Do not use product past expiration date. Use product at temperatures stated in the Instructions for Use for your instrument. Opened containers are stable for 60 days when stored as recommended. Dispose of waste product, unused product, and contaminated packaging in compliance with federal, state, and local regulations.

IMPORTANT If product has been partially or completely frozen, allow product to warm to room temperature. Mix product by gentle inversion prior to placement on the instrument. Install and prime the reagent as directed in the Instructions for Use for your instrument.

DxH 500 Series Cleaner

Store at 2-25°C. Do not use product past expiration date. Use product at temperatures stated in the Instructions for Use for your instrument. Opened containers are stable for 3 months when stored as recommended. Dispose of waste product, unused product, and contaminated packaging in compliance with federal, state, and local regulations.

IMPORTANT If product has been partially or completely frozen, allow product to warm to room temperature. Mix product by gentle inversion prior to placement on the instrument. Install and prime the reagent as directed in your Instructions for Use for your instrument.

Special Instructions – Reagent Handling and Storage

Warnings and Precautions

DxH 500 Series Diluent

- Do not inhale and/or ingest.
- Avoid eye contact. In case of contact with eyes, flush eyes with copious amounts of water for several minutes.
- Avoid skin contact. In case of skin contact, wash area with soap and water. Rinse well with water.
- DO NOT REUSE CONTAINER.

DxH 500 Series Lyse

- Product contains Glutaraldehyde < 0.1%/L.
- May cause skin and eyes irritation.
- Do not inhale and/or ingest.
- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- DO NOT REUSE CONTAINER.

Warnings and Precautions (Cont.)

DxH 500 Series Cleaner

- Do not inhale and/or ingest.
- Avoid eye and skin contact. In case of eye or skin contact, flush affected area with copious amounts of water for at least 15 minutes.
- DO NOT REUSE CONTAINER.

To obtain an SDS for Beckman Coulter reagents used on the instrument, go to: www.beckmancoulter.com

- Select **Safety Data Sheets (SDS/MSDS)** from the *Support* menu.
- Follow the instructions on the screen.

Reagent SDS location

Materials Required But Not Provided

The following controls and calibrator are recommended:

DxH 500 Series Control PN B36872

DxH 500 Series Control is a tri-level control that enables monitoring of the system's performance and calibration status for all directly measured and calculated CBC and Diff parameters.

Assigned values are presented as a mean and range. The laboratory's recovered mean should be within the assay range. For greater control sensitivity each laboratory should establish its own mean and acceptable range and periodically reevaluate the mean.

DxH 500 Series Calibrator PN B36880

DxH 500 Series Calibrator is traceable to reference methods and recommended for determining the adjustment of the directly measured CBC parameters. Calibration status should be monitored with Beckman Coulter controls.

Beckman Coulter documentation can be found at: www.beckmancoulter.com

Special Instructions for Control Handling and Storage

Calibration

The calibration procedure consists of comparing instrument measurements to known values for WBC, RBC, HGB, MCV, PLT, and MPV. The procedure may indicate that the instrument requires standardization, by first determining the deviation from calibrator reference, and then applying recommended correction factors (CAL factors).

The instrument comes calibrated from the factory. You should verify the calibration status. If verification fails, proceed with calibration.

Beckman Coulter recommends verifying the calibration on the instrument as dictated by your laboratory procedures and local or national regulations. Beckman Coulter recommends DxH 500 Series Calibrator, or an exact equivalent, as an acceptable alternative to whole blood calibration. In the normal process of tracking data for an extended period, your laboratory can make a specific decision to recalibrate a given parameter. The differential parameters do not require calibration in the laboratory.

Detailed information for Calibration is in Chapter 11, Quality Assurance of the IFU.

Calibration Overview

- Ensure the instrument is properly functioning, maintained, and the aspiration probe and apertures are clean prior to calibration
- The instrument has sufficient volume(s) of reagents to complete calibration
- Daily Checks passed
- Prepare/set up instrument for Calibration
- Prepare and mix the DxH 500 Series Calibrator according to the instructions for use
- Run Calibration
- Review results
- Adjust as needed
- Verify Calibration using DxH 500 Series Calibrator as indicated in the instructions for use

When to Calibrate:

Calibrate your instrument if:

- Calibration verification fails
- Any component involved in dilution or primary measurement was replaced. This includes the aspiration line or probe, and the apertures
- When advised to do so by your Beckman Coulter Representative

When to Verify Calibration:

Verify the calibration of your instrument:

- As dictated by your laboratory procedures and local or national regulations
- When controls show evidence of unusual trends (all levels demonstrate similar parameter recovery)
- When controls exceed the manufacturer’s defined acceptable limits
- If the average ambient room temperature changes more than 10°F or 6°C from the calibrating temperature

Quality Control

Quality Control is the routine monitoring of performance using commercial controls. Controls have known characteristics when run on a given system and are analyzed periodically in the same manner that patient specimens are analyzed. The results of analyzed controls are then compared to the known characteristics using statistical methods. This comparison allows changes in the system performance to be detected. If the changes detected are significant, you can then take action to improve system performance.

The purpose of Quality Control is to monitor various aspects of the instrument's performance. Quality Assurance includes service and maintenance as required with the use of controls and calibrators. The combination of these methods provides the assurance of complete quality control and should be applied separately or in combination, according to your laboratory and accreditation requirements.

Beckman Coulter recommends that Quality Control checks be performed using commercial controls at intervals established by your laboratory. When using a commercial control, refer to the instructions for use. Failure to recover control values within your laboratory's expected limits or the presence of unexplained shifts or trends in any method of presentation should be investigated. If control problems cannot be resolved, call your Beckman Coulter Representative.

Patient results obtained between the last acceptable run and an unacceptable control run should be reevaluated to determine if patient test results have been adversely affected. Take corrective action, if necessary.

Quality Control monitoring includes notification and recovery within the system on an ongoing basis. Instrument sensor and hardware status tracking by event notifications is completed via the icons, alarms, and the History Event Log. Events can be addressed as they occur.

Procedure Setting Up Controls

SETTING UP CONTROLS

Step	Action
1	Select  >  .
2	Perform one of the following: • To add a control file, highlight a blank line, select  , and use the bar-code scanner to scan the 2D bar codes on the Table of Expected Results. To add the information manually, select  and use the on-screen keyboard (do not use hyphens). • To edit a control file, highlight the existing control file, select  , and use the on-screen keyboard to edit the information manually.

Procedure Setting Up Controls (cont.)

3	Select **Auto Transmit** to automatically transmit control results to your LIS or **Auto Print** to automatically print control results.
4	Select  when prompted to confirm your selection.

Procedure Analyzing Commercial Controls

Analyzing Commercial Controls

The controls for the DxH 520 are the DxH 500 Series Controls.

Assay values on a new lot of control should be confirmed before the new lot is put into routine use. Test the new lot when the instrument is in good working order and quality control results on the old lot are acceptable. The laboratory's recovered mean should be within the assay range.

For greater control sensitivity, each laboratory should establish its own mean and acceptable range and periodically reevaluate the mean. The laboratory range may include values outside of the assay range. The user may establish assay values not listed on the Assay Sheet if the control is suitable for the method.

The default mode for the DxH 520 is to process samples in closed-vial mode. Quality Control can also be processed in open-vial mode.

Step	Action
1	Prepare the control according to the instructions for use.
2	Select  to display the Sample Analysis - Patient Results screen. The door automatically opens. **NOTE** The door does not open automatically if the Worklist contains orders. Acknowledge the message displayed to open the door.
3	Use the bar-code scanner to enter the specimen information from the control bar code OR Select  , use the on-screen keyboard to manually enter the control lot number in the *Specimen ID* field, and select  when prompted to confirm the information entered.

Procedure Analyzing Commercial Controls (cont.)

4	Mix the control according to the control's instructions for use.
5	Fully insert the control into the tube holder and select  . The door automatically closes to initiate the analysis. **IMPORTANT** If you manually close the door, a cycle is not initiated. To re-open the door, select  .
6	Remove the control when the door opens and the vial has finished processing.
7	Store the control according to the instructions for use.

If a control is not within the range configured for the test, the control is considered out and is indicated by  . The values that are out are flagged as *L* (low) or *H* (high) on the Display/Run screen. On the Quality Control (Data View) Screen, out-of-range values are highlighted in red and flagged as *L* or *H*.

Before rerunning the control:

1. Ensure the material is properly mixed according to the instructions for use
2. Ensure the identification information is entered correctly. If using a bar-code scanner, ensure the bar-code labels are clean and positioned correctly
3. Ensure the setup information (assigned values and expected ranges) matches either the Table of Expected Results for the control or your laboratory's established values. If they do not, change the control information to match
4. Ensure there are no errors during the cycle
5. Rerun the control. If the control fails again, try running a new tube on that same control level (or another level of control, if desired).
6. If the control recovery failure continues, contact your Beckman Coulter Representative.
7. To accept the out-of-range results and remove the error indicator, select  and  to accept all of the QC/EQC out conditions. Accepted results are displayed in blue.
8. To remove the OUT runs from data analysis, select the **Exclude** checkbox.

Viewing Control Files

Viewing Control Files

Step	Action
1	Select  to display the Quality Control (Data View) screen.
2	Select the **Lot #** from the drop-down list.
3	Review the information on the screen. Out-of-range values are highlighted in red and flagged as *L* or *H*.
4	Select the applicable icon to review that information or complete an action.

Viewing Control File Graphs

Viewing Control File Graphs

Step	Action
1	Select  .
2	Select the **Lot#** from the drop-down list.
3	Select  .
4	Select **CBC** or **Diff** and use the arrows to view the applicable graphs.
5	Verify that the correct lot number is selected and review the graphs.
6	Select  to return to the Quality Control (Data View) Screen.

Viewing Expanded Levey-Jennings Graphs

Viewing Expanded Levey-Jennings Graphs

Step	Action
1	Select  .
2	Select the **Lot#** from the drop-down list.
3	Select  .
4	Select the applicable parameter graph from the multiple graphs on the Quality Control (Graph View) screen to display the expanded graph view and summary.
5	Select the applicable icon to review that information or complete an action.

Printing Control Files

Printing Control Files

Step	Action
1	Select  .
2	Select the **Lot #** from the drop-down list.
3	Select  >  .
4	From the warning window, select one of the following: - **Selected Result** - **All Results with Graphs** - **All Results**

Printing Control Files (Cont.)

5	Select  when prompted to confirm your selection.

Exporting Control Files

Exporting Control Files

Step	Action
1	Insert a USB flash drive into the USB port in front of the instrument.
2	Select  .
3	Select the **Lot #** from the drop-down list.
4	Select  .
5	From the warning window, select **Selected Result** or **All Results in File**.
6	Select  when prompted to confirm your selection.
7	Remove the USB flash drive from the USB port.

Procedure Downloading Controls for IQAP

Downloading to IQAP

The Interlaboratory Quality Assurance Program (IQAP) is a Beckman Coulter program available to you through enrollment that complements and enhances your laboratory in-house quality control. Essentially, IQAP lets you submit your control recovery data to Beckman Coulter. In return, you receive a personalized report that summarizes your results and compares them to the results from your peer group (pool).

Step	Action
1	Ensure that your IQAP participant number has been entered correctly.

Procedure Downloading Controls for IQAP (Cont.)

2	Ensure that you have reviewed the control files, the number of runs, the mean and that the correct control was analyzed in the correct file.
3	Print the control file, if necessary.
4	Insert the USB flash drive into the USB port on the instrument.
5	Select  >  .
6	Select the files to download and select  .
7	Select  to download the files.
8	When download is complete, remove the USB flash drive.

Procedure Deleting Control Files

Deleting Control Files

Step	Action
1	Select  .
2	Select the **Lot #** from the drop-down list.
3	Select  .
4	From the warning window, select  to delete all of the results for the selected file.

Procedure Deleting Control Files (Cont.)

5	Select  >  , highlight the existing control file line, and select  >  to delete the control file.

Running Blood Samples

RUNNING BLOOD SAMPLES

You are ready to run samples when you have set the instrument to the correct analysis mode and verified the sample identification.

All specimens require a valid specimen ID:

- A specimen ID can be manually entered using the on-screen keyboard, scanning the bar-code label of a specimen, or by using the Auto-Incrementing feature. If the specimen ID is not entered and the auto-incrementing feature is not enabled, the system automatically assigns an instrument-generated auto-sequence number (AutoSID).
- A specimen ID must be 1 to 16 characters in length, consist of ASCII-printable characters, and must not have two or more consecutive spaces between characters.

Patient information and specimen IDs can be added to a worklist. The information is either downloaded from the Host LIS system or you can manually enter the information into the worklist.

Setting Up a Test Order

Setting Up a Test Order

Step	Action
1	Select 
2	Highlight a blank line and select 
3	On the Worklist – New Order screen, use the on-screen keyboard to enter the information requested and use the drop-down list to select information including Specimen ID, Patient ID, Last Name, First Name, etc.
4	Select  to accept the information.
5	Select  to return to the Worklist screen and view orders.

Editing a Test Order

Editing a Test Order

Step	Action
1	Select 
2	Highlight a test order and select 
3	On the Worklist screen, use the on-screen keyboard to enter information including Specimen ID, Patient ID, Last Name, First Name, etc.
4	Select  when prompted to accept the information.

Deleting a Test Order

Deleting a Test Order

Step	Action
1	Select 
2	Highlight a test order and select 
3	From the warning window, select **Selected Order** or **All Orders**. **NOTE** If **All Orders** is selected, all entries on the Worklist are deleted.
4	Select  when prompted to confirm your selection.

Running Whole-Blood Samples in Closed-Vial Mode

Sample Analysis: Running Whole-Blood Samples in Closed-Vial Mode

Step	Action
1	Select  .
2	Note the following: • If the worklist does not have any entries, the door automatically opens. • If the worklist contains entries, the system displays a warning message. Verify that the *Next Specimen ID* in the bottom right corner of the screen is correct and select  . The door automatically opens.
3	Identify a sample using one of the following methods: • If the *Next Specimen ID* is not correct or entered, scan the specimen tube's bar code and the default test is assigned (see Setting Up Next Specimen in Chapter 9, Setup). • Select  , use the on-screen keyboard to enter the Specimen ID and Test (CD or CBC), and verify that the Specimen is WB and CP is selected. Select  when prompted to confirm your selection. The door automatically opens.
4	Mix the whole-blood sample.
5	Fully insert the sample into the tube holder.
6	Select  and the door automatically closes. The status LED flashes red during aspiration and turns to solid red throughout processing. A message in the bottom left corner of the screen indicates the Specimen ID being analyzed. When the process is complete, the door automatically opens. The status LED turns green and the results are displayed.
7	Remove the tube from the tube holder.
8	Transmit or print the patient results, if applicable.

Running Whole-Blood Samples in Open-Vial Mode

Sample Analysis: Running Whole-Blood Samples in Open-Vial Mode

The open-vial mode on the DxH 520 offers an alternative method for processing samples with lower volumes or samples with tube caps that cannot be pierced.

All test orders entered in the worklist are defaulted to closed-vial mode. To process open-vial samples, the correct mode needs to be selected.

Step	Action
1	Select  .
2	Note the following: • If the worklist does not have any entries, the door automatically opens. • If the worklist contains entries, the system displays a warning message. Verify that the *Next Specimen ID* in the bottom right corner of the screen is correct and select  . The door automatically opens.
3	Identify a sample using one of the following methods: • If the *Next Specimen ID* is correct, select  >  to accept the specimen to be processed in open-vial mode (the probe is extended). • If the *Next Specimen ID* is not correct, scan the specimen tube's bar code, select  >  to accept the specimen to be processed in open-vial mode (the probe is extended). • Select  and use the on-screen keyboard to enter the Specimen ID, verify and Test (CD or CBC), and verify that the Specimen is WB and OV is selected. Select  , when prompted to confirm your selection (the probe is extended).
4	Mix the whole-blood sample.

Running Whole-Blood Samples in Open-Vial Mode (cont.)

5	Carefully remove the cap from the tube.
6	Clean any residual blood from the rim of the tube before presenting the sample.
7	Fully immerse the probe into the tube and select  to analyze the sample. The status LED flashes red during aspiration indicating that the sample aspiration is in progress.
8	Remove the tube from the probe when the status LED turns solid red and the probe is fully retracted. A message is displayed on the bottom left of the screen indicating that the Specimen ID is being analyzed.
9	Recap the tube.
10	Wait for the instrument to process the sample and display the results. The status LED turns green.
11	Transmit or print the patient results, if applicable.

Running Prediluted Blood Samples

Sample Analysis: Running Prediluted Blood Samples

The predilute panel on the DxH 520 offers an alternative sample preparation method for samples that cannot be directly aspirated in a whole-blood mode. The predilute panel is not intended for obtaining results that exceed the upper limit of the analytical measuring range.

The DxH 520 predilute panel accepts a 1:16 dilution, prepared by mixing 20 µL of whole blood with 300 µL of diluent. The diluted sample is analyzed using the predilute mode. The instrument reports the final results and no correction is required.

Step	Action
1	Ensure that the sample volume exceeds 300 µL to allow enough room for mixing the blood/diluent solution.
2	Dispense the diluent as described in Diluent Dispense in Chapter 10, Troubleshooting.

Running Prediluted Blood Samples (cont.)

3	Dispense 20 µL of blood into the same tube where the diluent was dispensed.
4	Mix the blood/diluent solution.
5	Select  .
6	Note the following: • If the worklist does not have any entries, the door automatically opens. • If the worklist contains entries, the system displays a warning message. Verify that the *Next Specimen ID* on the bottom right corner of the screen is correct and select  .
7	Identify a sample using one of the following methods: • If the *Next Specimen ID* is correct, select  to accept the specimen to be processed in pre-dilute mode. • If the *Next Specimen ID* is not correct, select  , if applicable, and scan the tube's bar code, select  >  to accept the specimen to be processed in pre-dilute mode, (the probe is extended). • Select  , use the on-screen keyboard to enter the Specimen ID and Test (CD or CBC), verify that the Specimen is PD. Select  when prompted to confirm your selection (the probe is extended).
8	Fully immerse the probe into the tube and select  to analyze a sample. A message is displayed at the bottom left of the screen indicating that the Specimen ID is being analyzed.
9	Remove the tube from the probe when the status LED turns solid red and the probe is fully retracted.

Running Prediluted Blood Samples (cont.)

10	Wait for the instrument to process the sample and display the results. The LED status turns green.
11	Transmit or print the patient results, if applicable.

Running Whole-Blood Samples Above Measuring Range

Sample Analysis: Running Whole-Blood Samples Above Measuring Range

If samples are above the measuring range of the DxH 520, it is the laboratory's responsibility to create and validate a protocol for diluting samples. For example, a WBC result of 150.00 x 10^3 cells/µL was received with a + flag. In order to accept the results, a dilution of the whole-blood sample would be needed to obtain results within the measuring range of the instrument.

Example:

- A dilution is created by using one part whole blood and 1 part diluent.
- If a 1:2 dilution is desired, equal amounts of whole blood and diluent should be added together. For example, 25 µL of whole blood is mixed with 25 µL of diluent for a final dilution of 1:2.
- Analyze dilution sample as soon as possible within 15 minutes of preparation.

Results

CBC, Diff, parameter results are reported customer selectable units of measure. If any flags or alarms are present, or for additional information, refer to Flags, Codes, and Messages Displayed in Chapter 6, Data Review in the Instructions for Use.

As with any analysis method in which a specimen of suspect quality is used, pay particular attention to the results. Review and verify the accuracy of all flagged results that exceed your laboratory's action limit.

Flag and Positions

Flag and Position			**Description**
1	**2**	**3**	
E			Manual edit of a primary parameter
e			Automatic edit of a calculated parameter
+			Result is above the analytical measuring range high limit
-			Result is below the analytical measuring range low limit
	R		Review Results

Results (Cont.)

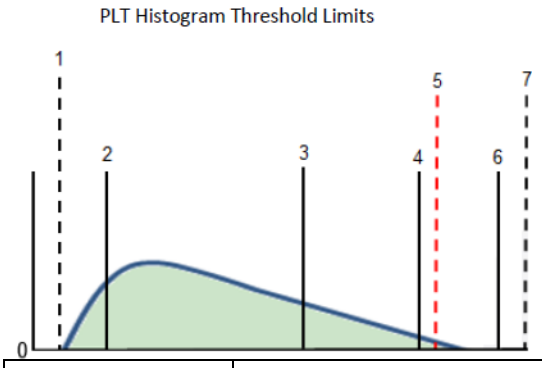
	*		Hemoglobin and Hematocrit (H&H) check failure (HCT – 3) < (HGB*3) < (HCT + 3)
		H	• Patient results above the action limit • Control results above the expected range
		L	• Patient results below the action limit • Control results below the expected range
		h	Patient results above the reference interval, but less than the action limit (H)
		L	Patient results below the reference interval, but less than the action limit (L)

Codes

Code	Description
-----	Total vote out (dashes). Inconsistent data between count periods.
…..	Incomplete computation (dots). Data cannot be derived.
+++++	Above operating range (plus signs)
?????	Result is outside the range of values that can be formatted for display (question marks)

The PLT histogram has four fixed thresholds (CP1, CP2, CP3, and CP3-2) and one variable threshold (P) that moves based on the presence of interference. When threshold limits are surpassed, specific messages are displayed.

Results (cont.)

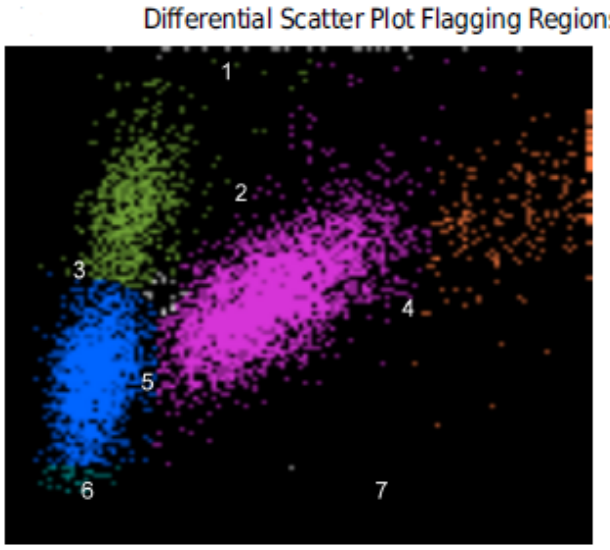


Number	Threshold	Approximate Volume (fl)
1	Minimum PLT	2.0
2	CP1	5.0
3	CP2	18.0
4	P	27.0*
5	Minimum RBC	28.0
6	CP3	32.0
7	Maximum PLT/CP3-2	34.0

*Variable Threshold

Populations that are normally separated generate flags or messages when internal criteria for separation is exceeded. The following figure is a normal population with good separation. Depending on the regains of the scatter plot, the presence of too many particles or an unclear separation between populations will trigger a message that informs you of the need to review the differential.

Results (Cont.)



Number	Flagging Region	Message
1	Large Immature Cell	Large Cells
2	MN (Monocyte/Neutrophil)	MO/NE Overlap
3	LM (Lymphocyte/Monocyte)	LY/MO Overlap
4	NE (Neutrophil/Eosinophil)	NE/EO Overlap
5	NL (Neutrophil/Lymphocyte)	NE/LY Overlap
6	LLYM (Lower Lymphocyte)	Cellular Interference
7	Debris	Debris

Interpretation of Results

TEST	EXAMPLES OF CAUSES OF A LOW COUNT (AACC)	EXAMPLES OF CAUSES OF A HIGH COUNT

Limitations of the Procedure

CBC and Diff parameters may be affected by interferences. Confirmatory testing within the laboratory should be performed to determine the source of unexpected abnormal results. Refer to Limitations in Chapter 1, System Overview in the IFU for more information.

Analytical Measuring Range

Measuring range is the set of values of quantities of the same kind that can be measured by a given measuring instrument or system with specified instrument measurement uncertainty under defined conditions. Values that are between the measuring range and operating range are flagged and should not be reported. These values require additional testing based on the laboratory's Standard Operating Procedure (SOP).

Current laboratory reference range for _____ using reagent _____:

Range	Mean	S.D.	Date Performed/Initials

Operating Ranges

The operating range is the range over which the system, inclusive of the predilute functionality, reports, displays, prints, exports, and/or transmits results. Values that are between the measuring range and operating range are flagged and should not be reported. These values require additional testing based on the laboratory's Standard Operating Procedure (SOP). The operating range is shown under Measuring and Operating Ranges, and Linearity in the Performance section in Chapter 1, System Overview in the IFU.

Linearity

Linearity can be assessed by testing levels of an analyte known by formulation, by using commercially available materials qualified for use on the DxH 520, or according to *CLSI EP06-A*. The measuring and operating ranges apply to both whole blood and prediluted samples. Linearity limits apply to whole blood only.

Whole-Blood Parameter	Units	Measuring Range	Linearity Limits (r^2)	Operating Range
WBC	$x10^3$ cells/µL	0.20 to 100.0	$r^2 > 0.95$	0.00 to 150.00
RBC	$x10^6$ cells/µL	0.20 to 8.00	$r^2 > 0.95$	0.00 to 12.00
HGB	g/dL	0.20 to 25.00	$r^2 > 0.95$	0.00 to 25.00
HCT	%	0.0 to 85.0	N/A	0.0 to 85.0
MCV	fL	50.0 to 150.0	N/A	50.0 to 150.0
RDW	%	10.0 to 40.0	N/A	0.0 to 70.0
RDW-SD	fL	15.0 to 150.0	N/A	0.0 to 220.0
PLT	$x10^3$ cells/µL	7.0 to 2000.0	$r^2 > 0.95$	0.0 to 4000.0
MPV	fL	5.00 to 25.00	N/A	0.00 to 25.00
MCH	pg	0.0 to 99.9	N/A	0.0 to 99.9
MCHC	g/dL	0.0 to 99.9	N/A	0.0 to 99.9
NE	%	0.00 to 100.00	N/A	0.00 to 100.00
LY	%	0.00 to 100.00	N/A	0.00 to 100.00
MO	%	0.00 to 100.00	N/A	0.00 to 100.00
EO	%	0.00 to 100.00	N/A	0.00 to 100.00
BA	%	0.00 to 100.00	N/A	0.00 to 100.00
NE #	$x10^3$ cells/µL	0.00 to 100.00	N/A	0.00 to 150.00
LY #	$x10^3$ cells/µL	0.00 to 100.00	N/A	0.00 to 150.00
MO #	$x10^3$ cells/µL	0.00 to 100.00	N/A	0.00 to 150.00
EO #	$x10^3$ cells/µL	0.00 to 100.00	N/A	0.00 to 150.00
BA #	$x10^3$ cells/µL	0.00 to 100.00	N/A	0.00 to 150.00

Specific Performance Characteristics Specifications

Additional performance characteristics and specifications are shown in Chapter 1, System Overview- Performance in the Instructions for Use.

Reference Range(s)

An adult reference interval study was conducted to assess the reference intervals for the DxH 520. Whole-blood samples were collected from at least 240 healthy adult donors aged 22 to 65 years (males and females). The selection of donors was consistent with guidelines in *CLSI EP28-A3c*. These intervals are used as default adult reference interval flags. Your patient population intervals may be different. These reference intervals are referred to as reference ranges on the instrument screens.

Current laboratory reference range for _____ using reagent _____:

Range	Mean	S.D.	Date Performed/Initials

Reporting Results

CBC and Diff parameter results are reports in the units of measure JAPAN, SI-1, SI-2, SI-3, SI-4, SI-5, SI-6, and US-1. If any flags or alarms are present, or for additional information, refer to the IFU, Chapter 6, Data Review.

Flags, codes, and messages are evaluated when the sample is analyzed. Review the results and pay close attention to any flags, codes, or messages that are intended to alert you to issues with results or with the instrument. Look for data patterns when examining flags, codes, and messages. Determine if individual or sets of results (for example, WBC and differential results) exhibit flags, codes, and messages. Some flagging occurs as a result of the flagging or editing of other parameters. In all cases, follow your laboratory's policy for reviewing results.

Related Procedures

DxH 520
Instructions for Use PN C41838AA
Software Version 2.0

CHAPTER 1: System Overview
CHAPTER 2: Operation Principles
CHAPTER 3: Startup and Daily Checks
CHAPTER 4: Quality Control
CHAPTER 5: Sample Analysis
CHAPTER 6: Data Review
CHAPTER 7: Worklist
CHAPTER 8: Shutdown
CHAPTER 9: Setup
CHAPTER 10: Troubleshooting
CHAPTER 11: Quality Assurance
CHAPTER 12: Cleaning Procedures
CHAPTER 13: Replacement/Adjustment Procedures
APPENDIX A: Access Levels and Reports
APPENDIX B: Bar Codes
APPENDIX C: Training Checklist
APPENDIX D: Implementation Checklist

Revision History

Revision AA
March 2019

Beckman Coulter documentation can be found at: www.beckmancoulter.com

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Additional Information

Beckman Coulter DxH 520

DxH 520 with Instructions for Use PN C41838AA
● System Overview • Operation Principles • Specifications/Characteristics • Precautions/Hazards • References • Glossary • Daily Checks • Quality Control ● Quality Assurance • Sample Analysis • Data Review • Shutdown • Setup • Troubleshooting ● Appendices ● References ● Warranty ● Glossary

For additional system documentation, please refer to www.beckmancoulter.com > Support > Technical Documents.

Trademarks

Appendices/ Attachments

REVIEWS/APPROVALS

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date