KAISER PERMANENTE®
COLORADO LABORATORY

# COMPLETE BLOOD COUNT ON THE SYSMEX pocH-100*i*

## PRINCIPLE

The Sysmex pocH-100*i* is a quantitative automated hematology analyzer for in vitro diagnostic use. It is used to determine 17 hematological parameters which include a complete blood count (CBC). Examination of the numerical and/or morphologic findings of the CBC is useful in diagnosis of such disease states as anemias, leukemias, allergic reactions, and viral, bacterial and parasitic infections. The Sysmex pocH-100*i* measures WBC, RBC, Hgb, HCT, MCV, MCH, MCHC, PLT, RDW-CV and RDW-SV, as well as absolute #s and % of neutrophils, lymphocytes and mixed cells.

## METHOD

The pocH-100*i* counts and sizes red blood cells (RBC) and platelets (PLT) using electronic resistance detection and hydrodynamically focused technology. Hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin (HGB) is a non-cyanide-based method and is converted to Oxyhemoglobin with self-check, and it is read photo metrically at 555nm.

White blood cells (WBC) are analyzed by direct current (DC Method) and discriminated into a three-part differential using Particle Distribution Analysis (PDA). The resulting WBC histogram is discriminated into lymphocyte, neutophil and mixed cell populations. The mixed cell population contains monocytes, basophils and eosinophils.

## SPECIMEN REQUIREMENTS and HANDLING

A. Required specimen:
   1. Whole blood anticoagulated with a salt of EDTA-K2 is the preferred anticoagulant.
   2. A well mixed lavender top tube is required. A mechanical mixer cannot be used to mix the specimen prior to placing it on the instrument.
   3. All specimens will be checked visually for obvious clots prior to sampling by the analyzer.

B. Specimen volumes required:
   1. Optimal draw is a tube drawn to capacity. The collection tube should be filled to a minimum of one-half full for acceptable results. EXCEPTION: a 2.5 ml EDTA tube filled less than one-half full is unacceptable.
   2. An EDTA micro-container filled above the 250 µl line is adequate for testing in the whole blood mode.

C. Unacceptable specimens including those listed below must be redrawn:
   1. Clotted samples or those containing clots or fibrin strands. All specimens will be checked visually for obvious clots prior to sampling by the analyzer.
   2. Grossly hemolyzed samples
   3. Samples drawn above an IV

D. Specimen characteristics that may affect test results are: lipemia, icterus, cold agglutinins, RBC fragments, giant platelets, clumped platelets and fibrin strands.

E. Do not place samples on a mechanical rocker. Constant rocking may cause PLT clumping and it may alter white cell membranes which may result in inappropriate flagging.

**STORAGE TEMPERATURES**

EDTA blood samples stored at 2-8°C with normal results may be analyzed up to 24 hours without significant loss of differential stability.

- Sample stability at room temperature is 8 hours. Samples stored at room temperature may exhibit an increase in MCV, an increase in HCT, and a decrease in MCHC after 16 hours.
- These changes may be minimized by refrigeration.
- Allow refrigerated samples to come to room temperature and mix well before analysis.

**EQUIPMENT and MATERIALS**

A. Supplies
   1. Clorox Ultra™ bleach at 6% solution (use when CELLCLEAN™ is indicated)
   2. Sysmex™ reagents
   3. Tri-level commercial controls, EIGHTCHECK-3WP X-TRA™
   4. Sysmex SCS-1000™ whole blood calibrator

| | |
|---|---|
| **Note:** | *EIGHTCHECK-3 WP X-TRA control material is not intended for calibration, however is made especially for the purpose of quality control.* |

B. Sysmex™ Reagents
   1. pocH-pack D is a ready-to-use diluent for DC detection and absorbance analysis. It is stored at room temperature (1° to 30° C) and directly supplied by Sysmex.

   Active Ingredients:

| | | | |
|---|---|---|---|
| Sodium Chloride | 6.38 g/L | Boric Acid | 1.0 g/L |
| Sodium Tetraborate | 0.2 g/L | EDTA 2K | 0.2 g/L |

   Storage and Stability:
   - The expiration date is shown on the outer packaging. If stored unopened at 1° to 30° C and it remains usable for 12 months.
   - Opened product stability is 60 days.

- If signs of instability are displayed, such as cloudiness or color change, the reagent should be replaced.

2. pocH-pack L is a reagent that lyses RBC for accurate WBC count and hemoglobin determination. It is stored at 2° to 35° C and supplied by Sysmex.

Active Ingredients:

| | |
|---|---|
| Sodium Chloride | 0.5 g/dl |
| Org. quart. ammonium salt | 8.5 g/dl |

Storage and Stability:

- Use pocH-pack L at 15° to 30° C. If analysis is performed outside of these temperatures, correct analysis results may not be obtained. The expiration date is shown on outer packaging.
- If unopened and stored at 2° to 35° C reagents remain usable for 12 months. Once opened.
- Opened product stability is 90 days.
- If signs of contamination or instability, as indicated by cloudiness or color change, should be replaced.
- Do not use pocH-pack L once frozen.

**WARNING:** Avoid contact with skin and eyes. In case of contact with skin and eyes, flush with plenty of water immediately. Consult with a physician in case of ingestion and/or eyes contact.

3. 6% solution of CLOROX Ultra™ bleach is recommended for use in cleaning of the pocH-100*i* whenever CELLCLEAN is stated as the cleaning agent.

**WARNING**: Clorox Ultra contains a strong oxidizing agent. Causes substantial but temporary eye injury. May irritate skin. May cause nausea and vomiting if ingested. Exposure to vapor or mist may irritate nose, throat and lungs.

4. Commercial Control

**EIGHTCHECK-3WP X-TRA** is a tri-level whole blood commercial control for use with the Sysmex pocH-100*i* hematology analyzer. EIGHTCHECK-3WP X-TRA consists of stabilized human erythrocytes, human and simulated leukocytes and a platelet component in a plasma-like fluid. Each vial contains 2.0 mL of control material.

EIGHTCHECK-3WP X-TRA Storage:

a. Vials are stored at 2 $^{o}$ to 8$^{o}$ C.
b. **DO NOT** freeze or expose to excessive heat.

EIGHTCHECK-3WP X-TRA Stability:

a. Unopened and properly stored, EIGHTCHECK-3WP X-TRA is stable until the expiration date stated on the vial.
b. Open vial stability is 14 days when promptly refrigerated after each use.
c. Write the new 14 day expiration date on the vial after opening.

d. Heat or freezing can damage EIGHTCHECK-3WP X-TRA without gross visible changes. Moderate hemolysis can be normal. Deterioration is suspected when the mean of the control results is not within the assay expected ranges after appropriate troubleshooting.
e. If deterioration is suspected, call the Sysmex Technical Assistance Center at 1-866-879-7639 (1-866-8SYSMEX).

**REAGENT REPLACEMENT**

When the reagent level is low, an error message is displayed on the LCD and instrument operation stops. For poch-pack D the message will read: 'Replace pocH-pack D'. For poch-pack L the message will read: 'Replace poch-pack L'.

1. Press **[HELP]**. Either "Replenish Diluent container" (CELLPACK) or "Replenish Lyse container" (STROMATOLYSER-WH) appears.

| **WARNING**: Wear gloves, lab coat and safety glasses when replacing reagents. |
|---|

2. Open the new reagent container.
3. Using care to not contaminate the reagent line; remove the cap from the empty container.
4. Pull the container spout kit straight out to remove it.
5. Insert the container spout kit immediately into the newly opened container and tighten the cap.
6. Record the open date on the new reagent container.
7. Record the new reagent information on the Sysmex pocH-100i reagent log
8. Press **[MENU]** on the Main screen. To enter the reagent replenish function, press **[EXECUTE]** followed by **[CHG.REAG.]**.
9. Press **[POCHPACK D]** to replace the pocH-pack D reagent. Press **[POCHPACK L]** to replace the pocH pack L reagent.
10. To display the individual reagent replacement log, press **[REAG.LOG]**.
11. Scan the barcode label or enter the barcode affixed to the new reagent, by pressing **[MANUAL]**. The numerical key pad appears and the barcode can be entered using the key pad. Press **[OK]**. In case an error is made entering the numbers of the lot number of reagent, a 'Barcode error' message is displayed.
12. Press **[EXECUTE]** to replace reagent.
13. If reagent replacement program is entered erroneously, press **[CANCEL]** to exit program.
14. Run at least one level of QC to ensure that the system is working properly when the pochpack L or pochpack D reagents are replaced and document this on the reagent log.

**QUALITY CONTROL**

A. Setup of Quality Control Files for the first time.

1. Before performing QC analysis for the first time or at change of lot number set up the 3 QC files.
2. Go to the main "Ready Screen" of the analyzer
3. Click on the QC icon on the lower left corner of the screen
4. Click on the file you want to use. If you are using an old file, you must delete the file first
5. Click on **[SETTINGS].**
6. Using the assay sheet, scan the barcode for the LOT ID information. The information can also be typed in.
7. Enter the expiration date of the QC.
8. Press [→] to access the next screen. Using the barcode from the assay sheet, scan in the targets for each parameter. The Limits are automatically entered.
9. Press **[SAVE]** and then **[OK]** to exit the screen.n the entering of control values is completed, press **[SAVE]**.

**NOTE**:

- LIS setup of new Lot is performed regionally for all Medical Office Laboratories prior to the start of the Lot. This is coordinated between the designee and the LIS team.

B. Frequency of Control Use and QC Data Review and Submission for peer review

- All three levels will be run on the morning after startup. The supervisor or designee reviews commercial and patient control charts every 30 days in the LIS and as needed.
- QC data is sent into Sysmex insight program for peer review during the 2nd and 3rd submission dates. See Insight submission calendar. The supervisor or designee will review the Insight report and take appropriate actions as needed.

D. QC Analysis

Use the appropriate GREEN QC adaptor to analyze the control blood sample in the same manner as patient whole blood. Failure to use the correct adapter will result in permanent instrument damage.

1. Remove the QC vials from the refrigerator and allow the vials to warm to room temperature (15-25$^0$C) for at least 15 minutes.
2. Verify that the cap is secure and mix the vial by gentle end to end inversion until the cell button in the bottom of the vial is completely suspended. **DO NOT USE A MECHANICAL ROCKER**.
3. Remove the cap.
4. Return the vials to the refrigerator promptly after use.
5. Wait until analyzer displays the 'Ready' message and press **[QC]** on Main screen.
6. Press the correct lot number for QC sample to be analyzed. The analyzing start screen will be displayed.
7. Press the top of the sample position to open it automatically. Do not force it to open or the analyzer may be damaged. Make sure no object, such as a pencil, obstructs the opening.
8. Set the control blood adapter in the sample position.

9. Press **[RUN]**. The analysis starts; the status display reads 'Aspirating'. When sample aspiration is completed, status display 'Aspirating' changes to 'Running'. When 'Running' is displayed, the sample position door can be opened and the control blood can be removed safely. Do not open the sample position while 'Aspirating' is displayed. If the sample position is opened while displaying 'Aspirating' an incorrect analysis result will be displayed.
10. The analyzing screen will appear.
11. After completion of a single analysis, these analysis results will be displayed on the LCD screen. Use [↓] or [↑] to scroll the screen page. The analysis values are compared to the target range.
12. When **[TOP]** is pressed, the quality control analysis 'Quit QC analysis?' message will be displayed.
13. Press **[OK]** to discard all analysis results and return to Main screen.

14. Press **[CANCEL]** to return to previous screen.
15. Press **[QUIT]** to accept the analysis results and output the results to the built-in thermal printer AND host computer. If the results of the analysis are not to be accepted, press **[BACK]** to perform a new analysis. When performing re-analysis, remove the control blood from the adapter and mix well. Place into the adapter in the sample position and press **[RUN]**. The analysis is saved to stored data, i.e. file No. 3 is saved as QC03. From the stored data screen for output, the result data can be printed out or transferred to the host computer. For details about actions to resolve QC refer to the pocH-100*i* Instructions for Use.
16. Accept the QC results in the LIS. If results are out of limits, enter the appropriate action taken to resolve the problem. The QC accession numbers used are:
    - Low = 0-QC-XX-2001 (XX = 2 digit location, ie. EN = 14)
    - Norm = 0-QC-XX-2002
    - High = 0-QC-XX-2003

E. Starting a New Lot of Controls

- Parallel test new controls with the current lot by analyzing the three levels twice a day for 3 days, prior to expiration of the current lot.

**PROCEDURE**

A. Start-Up

1. Allow the instrument to perform its automatic microprocessor tests, motor check, auto-rinses and a background count. It will take 2 minutes to complete the self-check functions. It will take approximately 6-11 minutes before the Main Screen will appear.
2. An auto rinse is performed. To manually perform an auto rinse Press: [Menu], [Maint], [Auto Rinse], [Execute]. After completion, the Main screen will appear. Verify results for acceptable limits.

| pocH 100*i* Acceptable Background Counts | |
|---|---|
| **Parameter** | **Count** |
| WBC | 0.3 x $10^3$/ µL or less |
| RBC | 0.02 x $10^6$/ µL or less |
| HGB | 0.1 g/dl or less |
| PLT | 10 x $10^3$/ µL or less |

3. This instrument needs maintenance performed every 2 weeks or at 150 cycles – See the Sysmex poch-100i maintenance procedure for detailed instructions.
4. Analyze commercial controls and document control results as described above.

B. Sample Analysis - Whole blood(WB) mode

1. This mode is used to analyze two types of samples: Closed tube or Open tube.
2. Required volume is a minimum of 1 mL or more for WB for tubes with a diameter of 13mm, and 500 µL or more of WB for the microtainer tubes. Be sure to use the appropriate size adaptor for the tube or microtainer in use to assure that adequate sample is aspirated.
3. Specimen must be adequately mixed prior to placing on the instrument for analysis. Mix by gently inverting the tube 10-12 times. **DO NOT USE A MECHANICAL MIXER**. Check the specimen for obvious clots.
4. Aspirated sample volume is ~15 µL.
5. Ensure that the analyzer is in WB mode setting. The default mode is the WB mode.
6. Be sure the display indicates 'Ready'
7. When **[WB]** is pressed, the 'WB' button turns red and appears shade less.
8. Enter the sample ID by scanning the barcode label (large or small) on the vacutainer tube with hand-held barcode reader. The accession number can also be manually entered using the numeric key pad.
    a. Directions for using the barcode scanner
        i. Press **[SAMPLE ID]**.
        ii. If the displayed sample ID is not correct, wand the barcode again. Press **[ENTER]**.
    b. Directions for using the numeric key pad
        i. Press **[SAMPLE ID]**. The numerical key pad will appear.
        ii. Enter the LIS accession number.
        iii. Press **[ENTER]**, the sample. ID is displayed and the status turns to 'Ready'

C. Daily Shutdown - approximately 2 minutes to perform

The Shutdown program cleans the transducer chambers and the diluted sample line. Perform Shutdown at the end of daily operation - **DO NOT** turn off the power as this will disable the LIS connection. Select the "RESTART" option.

1. Press **[SHUTDOWN]** when the 'Ready' status appears. The shutdown confirmation message appears. The Shutdown procedure may be aborted by pressing **[CANCEL]**. The analyzer returns to the Main Menu screen.
2. Press **[EXECUTE]**.

3. Check that the shutdown sequence was completed. The display of the Shutdown completion screen will be displayed.
4. Press **[RESTART]** and the Main Menu screen will appear.
5. Record the daily shutdown on Sysmex pocH-100i maintenance log.

**REPORTING of RESULTS**

It is necessary to review all flagging generated by the analyzer prior to reporting the patient results. Per laboratory protocol, either perform a scan or manual differential to verify the flagging before reporting the result. Maintain the instrument printout.

1. "H" and "L" flags in the LIS are used for reference ranges and are NOT the criteria for performing manual or scanning differential. Refer to the manual diff or scanning protocol.

2. If a sodium citrate tube is used as an alternative because of EDTA induced platelet clumping, multiply the citrate platelet count by 1.11 to correct for anticoagulant dilution.

3. Dilution patient sample. (Performed when results are greater than linearity)

**Note:** If you are working at an MOL without graduated pipettes, and are unable to perform the dilution, notify provider of the delay in testing and send to your normal STAT reference site.

When using a diluted patient specimen multiply the directly measured parameters x dilution factor.

a. Normal Saline or packpoch-L diluent can be used to make the dilution.
   i. Weekly Dilution check is performed on the Saline or stock poch-L diluent.
   ii. See separate protocol
   iii. See separate log
b. Make the smallest dilution possible
   Examples:
   1:2 Dilution = 500 µL sample + 500 µL poch-L or Saline
   1:5 dilution = 500 µL sample + 2000 µL poch-L or Saline
c. Once the dilution is made, it must be analyzed within 15 minutes. Do not run the diluted sample if it has been more than 15 minutes.
d. **HGB Correction for High WBC.**

Multiply the hemoglobin reading of the diluted sample by the dilution factor.

Record all calculations on the diluted sample report.

**Example:**

1:2 dilution hemoglobin = 6.1 g/dl
6.1 x 2 = 12.2 g/dl

- If the corrected Hemoglobin is within 0.3 gm/dl of the original hemoglobin, no correction is necessary
- If the corrected hemoglobin is greater than 0.3 gm/dl different than the original hemoglobin then the indices calculated by the hematology analyzer (MCH and MCHC) must be manually recalculated using the corrected Hemoglobin. Refer to calculations.(See below)

- Report the corrected Hgb, MCH, and MCHC in Cerner.

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4. When correcting the Hgb or Hct for interfering substances recalculate and correct the affected indices:

MCHC = HGB/Hct x 100
MCH = HGB/RBC x 10
MCV = Hct/RBC x 10

**PROCEDURE NOTES**

A. If the ANE is 0.0 and WBCs are seen on the differential add the following comment to the differential – "rare neutrophil seen on scan" or "rare neutrophil seen on diff"
B. For troubleshooting specifics refer to the Sysmex poch-100*i* Instructions for Use.
C. When megakaryocytes are present, perform a WBC and PLT estimate.
D. Do not place samples on a mechanical rocker. Excessive mixing may induce platelet clumping and alter white cell membranes resulting in false interpretive messages.
E. Clorox Ultra, filtered bleach, is recommended for use in cleaning. If Clorox Ultra is not available generic bleach must be 6% Sodium Hypochlorite concentration and be free of particles that may cause background contamination when used on the analyzer.

**LIMITATIONS of the PROCEDURE**

poch-100i Manufacturer's Stated Linearity (Whole Blood Mode)

| Parameter | Range | Units |
|---|---|---|
| WBC | 0-99.9 | $x10^3/\mu L$ |
| RBC | 0.3-7.00 | $x10^6/\mu L$ |
| HGB | 0.1-25.0 | g/dL |
| HCT | 10-60.0 | % |
| PLT | 10-999 | $x10^3/\mu L$ |

- ***Verification of Linearity was performed by each laboratory upon implementation of the new analyzer – See pocH-100i startup notebook at each Medical Office Laboratory.***

1. Parameters that exceed these limits are flagged with an exclamation point (!) beside the result. The sample must be diluted, rerun and multiplied by the dilution factor, or repeated using the pre-dilute mode. Note the use of dilution for linearity on the patient report.

pocH-100*i* Manufacturer's stated display range

| Parameter | Range | Units |
|---|---|---|
| WBC | 0.0 – 299.9 | $x10^3/\mu L$ |

| | | |
|---|---|---|
| RBC | 0.00-19.99 | $x10^6/\mu L$ |
| HGB | 0-25.0 | g/dL |
| HCT | 10-60 | % |
| PLT | 0-1999 | $x10^3/\mu L$ |

## INTERFERRING SUBSTANCES

1. Specimens must be free of clots and fibrin strands.
2. Marked changes in plasma constituents (e.g., low sodium, extremely elevated glucose) may cause cells to swell or shrink. The blood to anticoagulant ratio is important.
3. Red cell fragments, microcytic RBC's or white cell cytoplasmic fragments may interfere with automated platelet counts.
4. Cold agglutinins produce spurious macrocytosis, elevated MCH's MCHC's, falsely decreased RBC counts and HCT's. Rare Warm agglutinins produce the same spurious results as a cold agglutinin – See MCHC protocol
5. Extremely elevated WBC's (>100,000/μL) may cause turbidity and increase the hemoglobin.
6. Severely hemolyzed samples (in vitro) falsely decrease RBC and hematocrit. Recollect hemolyzed specimens.
7. Giant platelets and clumped platelets may falsely elevate the WBC count and falsely decrease the platelet count. Platelet clumping and/or "platelet satellitism" can occur in specimens collected in EDTA – See spurious result protocol on how to resolve this.
8. Abnormal paraproteins found in Multiple Myeloma patients can falsely increase the HGB – See MCHC protocol.
9. Lipemic or Icteric samples falsely elevate the HGB & MCHC – See MCHC protocol
10. Using a mechanical rocker will affect the WBC differential and give false flags.
11. Fragmented RBC may cause a falsely low HCT value.
12. The hemoglobin method on this analyzer cannot detect sulfhemoglobin, verdohemoglobin, choleglobin or other unusual degradation products of hemoglobin.

## REFERENCES


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