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 with differential (CBC with diff). The Sysmex pocH-100*i* measures WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, RDW-SD and RDW-CV, as well as absolute (#) and percent (%) of neutrophils, lymphocytes and mixed cell populations.

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 absorbance method read photometrically at 555nm.

White blood cells (WBC) are analyzed by direct current (DC Method) and discriminated into a 3-part differential using Particle Distribution Analysis (PDA). The resulting WBC histogram is discriminated into lymphocyte, neutrophil and mixed cell populations. The mixed cell population contains monocytes, basophils and eosinophils.

Specimen

# Freshly drawn whole blood collected in dipotassium or tripotassium EDTA anticoagulant is the specimen of choice. Tubes must contain at least 2 mls of whole blood and must not contain clots or fibrin strands. Microtainer samples cannot be used as we do not have a suitable adapter. Perform whole blood analysis as soon as possible for maximum accuracy. Samples must be run within 4 hours of collection.

Equipment and Materials

1. Sysmex Reagents
2. pocH-pack D (Sysmex catalog # 024-0971-4) is a ready-to-use diluent for Direct Current (DC) detection and absorbance analysis. Storage and Stability: The expiration date is shown on the outer packaging. It is stored at room temperature and contains Sodium Chloride 6.38 g/L, Boric Acid 1.0g/L, Sodium Tetraborate 0.2 g/L, and EDTA-2K 0.2 g/L. Once opened, product stability is 60 days. If signs of instability, such as cloudiness or color change are displayed, the reagent should be replaced.
3. pocH-pack L (Sysmex catalog # 024-0991-9) Lysing reagent lyses RBCs for accurate WBC count, hemoglobin determination, and allows WBC count and volume distribution analysis by the DC detection method. Reagent is stored at 2° to 35° C. It contains Sodium Chloride 0.6 g/L, and Organic quarternary ammonium salt 8.5 g/L.
4. Storage and Stability: Use pocH-pack L at 15° to 30° C. The expiration date is shown on outer packaging. Once opened, product stability expires after 90 days. pocH-pack L reagent displaying any signs of contamination or instability, as indicated by cloudiness or color change, should be replaced. Do not use pocH-pack L once frozen.
5. 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 eye contact.

## Sysmex pocHi thermal paper (Sysmex catalog # C01-5050)

1. A 5% solution of Clorox® bleach is recommended for use in cleaning of the pocH-100i whenever CELLCLEAN is indicated as the cleaning agent. Scented or splashless varieties of Clorox bleach must not be used at any time.
2. Clorox Ingredients: Sodium Hypochlorite
3. Clorox Storage: Stable under normal use and storage conditions.
4. 5% Clorox should be made fresh prior to each use as follows:
5. 4.17mls of 6% hypochlorite solution and 0.83 mls of dionized water are mixed together in a 5 ml clear capped tube.
6. 3 mls of 8.25% hypochlorite solution and 2 mls of deionized water are mixed together in a 5 ml clear capped tube.
7. Reagent Replacement
8. When the reagent level is low, an error message is displayed on the pocH-100i LCD and the 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’.
9. Open the new reagent container. Document the lot number in use on the reagent log located in the QC book.
10. Using care to not contaminate the reagent line, remove the cap from the empty container. Pull the container spout kit straight out to remove it.
11. Insert the container spout kit immediately into the newly opened container and tighten the cap.
12. Record the open container expiration date on the new reagent container.
13. To enter the reagent replenish function, press **[EXECUTE]** followed by:
14. 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 lot number of reagent, a ‘Barcode error’ message is displayed.
15. Press **[EXECUTE]** to replace reagent. Main screen will display on completion.
16. If reagent replacement program is entered erroneously, press **[CANCEL]** to exit program.

**Quality Control**

1. EIGHTCHECK-3WP X-TRA™ is a tri-level whole blood commercial control for use with the Sysmex pocH-100i hematology analyzer.
2. EIGHTCHECK-3WP X-TRA Ingredients (formulation): 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.
3. EIGHTCHECK-3WP X-TRA Storage: After opening, vials should be stored in the upright position at 2o to 8o C.
4. DO NOT freeze or expose to excessive heat.
5. EIGHTCHECK-3WP X-TRA Stability: Unopened and properly stored, EIGHTCHECK-3WP X-TRA is stable until the expiration date stated on the vial.
6. Open vial stability is 14 days when promptly refrigerated after each use. If controls are run weekly, three control runs should be usable from each vial.
7. Record the expiration date on each vial upon opening.
8. Setup of Quality Control files for new lot number
9. Each new lot of control will have at least one parallel point run to verify acceptability of new lot prior to expiration of existing lot as we are using manufacturer’s determined means and ranges.
10. Select the corresponding file.
11. Clear previous data from file.
12. Press **[Menu]** or **[Top].**
13. Press **[QC Charts].**
14. Press **[Delete].**
15. Press **[Setup].**
16. Scan lot and expiration date from the assay sheet using barcode reader or enter manually using keypad.
17. Enter TARGET and LIMIT values for each parameter by barcode or by hand switching between the six QC screens as needed.
18. Press **[SAVE].**
19. At Quit QC Setting? Press **[OK].**
20. Frequency of Control Use
21. Three levels of controls shall be run every Monday regardless of analyzer use while analyzer is stored in Hematology Dept.
22. Three levels of control shall be run each day of patient use prior to patient samples while stored in Hood in Biocontainment Lab.
23. Test results are considered "in control" when the controls exhibit the expected results. If controls do not give expected results, patient testing must not be initiated until the problem is identified and solved.
24. Instructions for Use
25. Remove EIGHTCHECK-3WP X-TRA vials from refrigerator and allow them to come to room temperature (18o to 25o C) for approximately 15 minutes.
26. Mix vials 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.
27. Return vials to the refrigerator promptly after use.
28. Use the appropriate green adapter to analyze the control blood sample in the same manner as patient whole blood.
29. Wait until analyzer displays the ‘Ready’ message and press **[QC]** on Main screen.
30. Press the correct lot number for QC sample to be analyzed. The analyzing start screen will be displayed.
31. Press the top of the sample position to open it automatically. Do not force it to open or the analyzer may be damaged. Set the control blood in the sample position. Close the sample holder.
32. Press **[RUN].** The analysis starts.
    1. The status display reads ‘Aspirating’.
    2. When sample aspiration is completed, status display ‘Aspirating’ changes to ‘Running’.
    3. When ‘Running’ is displayed, the sample position door can be opened and the control blood can be removed safely.
    4. 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. The analyzing screen will appear.
    5. 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.
    6. Press **[QUIT]** to accept the analysis results and output the results to the built-in thermal printer. 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.
33. Transfer results of tape to offline QC files on shared drive. S/APS/Clinlab/Hemo/Instruments and Test Validations/Sysmex pocHi/QC

**Calibrator and Calibration**

1. SCS-1000™ Calibrator
2. SCS-1000 is a secondary whole blood calibrator for use with the Sysmex pocH-100i hematology analyzer. Assay values for primary parameters are traceable to reference methods. SCS-1000 consists of human red and white blood cells with a platelet component suspended in fluid medium. Each vial contains 2.0 mL of calibrator material.
3. Store vials as packaged, at 2o to 8o C. DO NOT freeze or expose to excessive heat.
4. Unopened and properly stored, SCS-1000 is stable until the expiration date stated on the vial.
5. Open vial stability is 4 hours.
6. Storage outside of 2o to 8o C can damage SCS-1000 causing deterioration that risks inaccurate calibration.
7. Use of the product at environmental temperatures that exceed 30oC (86oF) can reduce calibration accuracy.
8. Calibration compensates for any bias inherent to the pneumatic, hydraulic, and electrical system that may affect the accuracy of results.
9. Calibration verification is also required if one or more of the following occur:
10. Critical parts, such as manometers, apertures or detector circuit boards are replaced.
11. Controls show an unusual trend or are outside of acceptable limits and cannot be corrected by maintenance or troubleshooting.
12. When advised by pocH-100i Technical Assistance Center (1-866-8SYSMEX).
13. Calibration verification may be accomplished by review and documentation of at least two (2) levels of commercial control each day patient specimens are run, proficiency testing results and patient control testing results.
14. The operator may calibrate HGB and HCT with normal fresh whole blood, or use SCS-1000 calibrator to calibrate WBC, RBC, HGB, HCT, and PLT. Before calibration, ensure that the maintenance procedures and precision run have been performed.
15. Establish the reference values on 5 fresh and normal samples. Calculate the mean values for the calibrated parameters (WBC, RBC, HGB, HCT, PLT).
16. Analyze the samples in Whole Blood mode and determine mean values for each of the calibrated parameters on the pocHi.
17. Calculate the new calibration value for each parameter using the formula:
18. New cal value = old cal value x (mean of reference/mean of pochi)
19. Press **[Menu].** Then press **[Calib].** Finally press **[Manual(WB)].**
20. Enter the new calibration values and press **[Ent]** and confirm with **[Quit].**

**Maintenance**

1. Routine Maintenance
2. During start-up of the pocH-100i, any maintenance that is due based on cycle count is displayed on the LCD.
3. Perform the maintenance when prompted on the LCD, and the cycle counter is reset, OR press **[3]** to Cancel and bypass performing the maintenance temporarily. When bypassed the counter does not reset and the maintenance reminder continues to reappear on Start-up.
4. Biweekly:
5. Clean Transducer. If either the counter value exceeds 150 or if 2 weeks have passed since the last maintenance, a message will appear to prompt the operator to perform periodic maintenance (transducer cleaning) when the main power is switched on.
   1. Perform the transducer cleaning according to the following procedure:
   2. Prepare an empty sample tube of 5% Clorox sodium hypochlorite (bleach) as outlined above.
   3. Press the top of the sample position to open it.
   4. Set the sample adapter (tan color) in the sample position.
   5. Place the prepared tube of bleach solution in adapter and close the door.
   6. Press **[EXECUTE].** The cleaning procedure of the transducer will be executed and the cleaning transducer screen will appear. An auto rinse will be performed following the cleaning procedure.
   7. After the cleaning procedure is completed, the Main screen will appear.
   8. Remove the bleach solution from the analysis position.
6. Even when the above mentioned message is not displayed, the transducer cleaning procedure can be executed by pressing **[MAINT.]** on the Menu screen, then pressing **[CLEAN TRANSDUCER]** on the maintenance screen.
7. Quarterly or every 1500 Cycles Maintenance: Clean Waste Chamber. If either the counter value exceeds 1500, or if 3 months have passed since the last maintenance, a message will appear prompting the operator to perform periodic maintenance (Waste Chamber Cleaning), when the main switch is turned on.
8. When the message is displayed, perform the procedure as follows:
9. Prepare 5% bleach as above in an empty 13mm diameter sample tube.
10. Press the top of the sample position to open it. Do not use force, because the analyzer may be damaged.
11. Set the sample adapter (cream color) in the sample position.
12. Place the prepared tube with the bleach solution in adapter and close the sample position.
13. Press **[EXECUTE].** Cleaning the waste chamber will be executed and the cleaning waste chamber screen will appear. An auto rinse procedure will be performed after the completion of the cleaning procedure.
14. After completion of the waste chamber cleaning procedure, the Main screen will appear.
15. Remove the sample tube with the bleach solution from the sample position.
16. Even when the message ‘Scheduled maintenance’ does not appear, waste chamber cleaning can be executed by pressing **[MAINT.]** on the Menu screen then pressing **[CLEAN W. CHAMBER]** on the maintenance screen.
17. As needed maintenance. Refer to the Sysmex pocH-100i Instructions for Use for any “As needed” Procedures including:
18. Auto Rinse procedure
19. Disposal of waste fluid
20. Cleaning the sample tube adapter
21. Removing a clog from the aperture
22. Replacing of reagent
23. Draining of reagent
24. Calibrating the LCD panel
25. Settings sequence
26. System fuse replacement
27. Replacing thermal printer paper

**Procedure**

1. Pavilion Start-Up
2. Check built-in thermal printer paper supply and check that paper is properly aligned.
3. Check and empty the waste if necessary.
4. Assure proper diluent and lyse levels to complete the run.
5. Power on the pocH-100i Main Unit.
6. The LCD screen will be illuminated and the Sysmex logo will be displayed. 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.
7. If values from electronic and hydraulic checks exceed specifications upon start-up, an error message is displayed on the LCD screen. Turn the analyzer’s main switch off, wait 15 seconds, and then turn it back on again. Auto-rinse cycles are identified by the sample ID# of zero.
8. A background check is performed on the third rinse. If the background check is outside of specifications, two more background checks will be done automatically. It takes approximately one (1) minute for the automatic rinse cycle and approximately 2 - 7 minutes for the background check. If after the second repeat background check, the values are still outside specifications, a continuous alarm will sound and the message ‘Blank Error’ is displayed on LCD screen. If Background count is acceptable, the Main Menu will be displayed. Press the ‘Result’ button and the Background Counts will appear.
9. Record the background counts on the maintenance log.
10. If the results are not displayed they can be accessed from the stored data screen.
11. Press **[Menu]** then **[Str. Data].** Using the up and down arrows move to the appropriate date and background check. Use the right and left arrows to visualize all the necessary parameters.

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| pocH 100i Acceptable Background Counts | |
| Parameter | Count |
| WBC | 0.3 x 103/ μL or less |
| RBC | 0.02 x 106/ μL or less |
| HGB | 0.1 g/dL or less |
| PLT | 10 x 103/ μL or less |

1. Analyze commercial controls and document control results.
2. Shutdown analyzer if in Pavilion.
3. Biocontainment Startup.
   1. If instrument is to be moved to the Biocontainment lab for patient testing reagents must be drained prior to moving analyzer.
4. Power on analyzer.
5. Once startup is complete, select [Top]. Select [Maint.] then select [deprime seq.].
6. Remove sip tubes from reagents (diluent and lyse) and place on clean paper toweling.
7. Select [Execute] to deprime reagents.
8. Once deprime is complete, return sip tubes to bottles and power off instrument.
   1. Assure you are bringing enough supplies to complete patient testing for the duration of testing.
   2. Bring the currently in use control vials to the biocontainment lab with the analyzer to be run once system has been powered up.
   3. Place analyzer and reagent tray in biocontainment hood and check liquid levels prior to powering analyzer on.
   4. Run three levels of controls and remove control vials from contaminated area prior to suspected sample being brought in the lab.
9. Sample Analysis

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1. The default mode is the WB (whole blood) mode and the aspirated sample volume is ~15μL.
2. Be sure the display indicates ‘Ready’.
3. When **[WB]** is pressed, the ‘WB’ button turns red and appears shadeless.
4. Enter sample ID by either numeric keypad or hand-held barcode reader.
5. Numeric key pad
6. The space for the sample ID can accept 15 characters in combination of alpha/numeric characters, hyphens and spaces.
7. Press **[SAMPLE ID].** The numerical key pad will appear. Enter sample ID using barcode reader or keypad to enter.
8. Press **[ENTER],** the sample ID is displayed and the status turns to ‘Ready’.
9. Hand held barcode reader
10. Press **[SAMPLE ID].** The barcode can be scanned, instead of the numerical entry. If the displayed sample ID is not correct, scan the barcode again.
11. Press **[ENTER].** When the manual input of sample number is selected, the barcode cannot be read. In this case, press **[ENTER]** once, then press **[SAMPLE ID]** again to read barcode.
12. Patient Sample Processing
13. Press the top of the sample position to open.
14. Set the tan adapter into the sample position.
15. Mix the sample by gently inverting end to end ten times.
16. Set the sample into the adapter and close the door.
17. Make sure that ‘Ready’ is displayed on the instrument status indicator at the top of the display area.
18. Check the analysis mode; then press **[RUN].**
19. The analysis starts and the status display indicates ‘Aspirating’.
20. When sample aspiration is completed, status display ‘Aspirating’ changes to ‘Running’.
21. When ‘Running’ is displayed, the sample position door can be opened and the sample can be removed safely.
22. WBC and HGB results are displayed on the LCD screen in about 90 seconds after starting the analysis. The analysis for all parameters is displayed after 125 seconds. The results are displayed on 4 screen pages. Switch the pages by pressing [→] button. Press [TOP] to return to the Main screen. When the status display indicates ‘Ready’, another sample can be analyzed.
23. Printing and output of results
24. If the results do not print automatically, press [PRINT], and the thermal printer starts to print the results.
25. Flags on the Blood Count portion of the histogram include WL, RL, PL, WU, RU, PU, DW, MP which are indicative of incomplete lysis, fragments, clumping or interference on the parameters for which the flag is printed next to.
26. Repeat those samples with flags one time and then report with a comment about possible interferences affecting results.
27. Flags on the differential include T1, T2, F1, F2, F3, and AG and are indicative of poor population separation and incomplete lysis or immature cells. Report with a comment that results may be affected
28. Some differentials may vote out and not give results. Some may give a lymph % only. Repeat one time and then report what numbers are given with a comment.
29. Decontamination prior to removal from biocontainment hood.
    1. Prepare 5% bleach as above in an empty 13mm diameter sample tube.
    2. Press the top of the sample position to open it. Do not use force, because the analyzer may be damaged.
    3. Set the sample adapter (tan color) in the sample position.
    4. Place the prepared tube with the bleach solution in adapter and close the sample position.
    5. Waste chamber cleaning is executed by pressing **[MAINT.]** on the Menu screen then pressing **[CLEAN W. CHAMBER]** on the maintenance screen.
    6. The cleaning waste chamber screen will appear. An auto rinse procedure will be performed after the completion of the cleaning procedure.
    7. After completion of the waste chamber cleaning procedure, the Main screen will appear.
    8. Remove the sample tube with the bleach solution from the sample position.
    9. Discard waste bottle in biocontainment contaminated trash.
    10. Shutdown analyzer power. See steps below.
    11. Thoroughly wipe the entire analyzer and remaining reagents with a gauze wet with isopropyl alcohol.
    12. Remove the sample adapter and thoroughly clean with isopropyl alcohol.
30. Daily Shutdown – approximately 2 minutes to perform
31. The Shutdown program cleans the transducer chambers and the diluted sample line.
32. Perform Shutdown at the end of daily operation or at least once every 24 hours.
33. 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.
34. Press **[EXECUTE].**
35. Check that the shutdown sequence was completed. The display of the Shutdown Completion screen will be displayed.
36. Turn off the main switch on the right side of the analyzer.
37. If **[RESTART]** is pressed, the Main Menu screen will appear.
38. Record the daily shutdown on your lab’s maintenance log.
39. Reporting Results
40. Results are hand entered into the LIS under the Ebola CBC protocol which includes a 9 part Blood count (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, and PLT) and a 3 part diff (Lymph % and #, Neut % and #, and MXD % and #). The MXD or mixed population includes monocytes, eosinophils and basophils.
41. Tape the printout to a copy of the LIS report and store in results binder in biocontainment lab.

Limitations and Interferences

1. Linearities for the measured parameters are as follows:

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| **Parameter** | **Range** |
| WBC | 1.0 - 99.9 x103/ μL |
| RBC | 0.30 - 7.00 x106/ μL |
| HGB | 0.1 - 25.0 g/dL |
| HCT | 10.0 - 60.0 % |
| PLT | 10 - 999 x103/μL |

* 1. Parameters that exceed these limits are flagged with an exclamation point (!) beside the result. The sample must be diluted with pocH pack D, rerun and multiplied by the dilution factor.

1. Note the use of dilution for linearity on the patient report.
2. Bleaching Sample:
   1. 5 samples were split and tested with the bleach decontamination of the outside of one tube and the other with no treatment. There was no difference in samples treated with bleach or not. All testing performed was performed without removal of the cap to allow fumes into the tube. Recommendation is to run the CBC first on the Sysmex prior to removal of the cap for any additional testing to assure that bleach fumes do not enter the sample and damage the cells.

1. Known Interfering Substances
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.
5. Extremely elevated WBC's (>100,000/μL) may cause turbidity and falsely 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.
8. Platelet clumping and/or "platelet satellitism" can occur in specimens collected in EDTA.  This may falsely elevate the WBC count and falsely decrease the platelet count.
9. Abnormal paraproteins found in Multiple Myeloma patients can falsely increase the HGB and may falsely increase the WBC count.
10. Lipemia falsely elevates the HGB & MCHC.
11. Severely icteric samples may falsely elevate the HGB value and related indices. Make a 1:5 dilution with pocH-pack D.
12. Rocking a specimen excessively may affect the WBC differential.
13. The hemoglobin method on this analyzer cannot detect sulfhemoglobin, verdohemoglobin, choleglobin or other unusual degradation products of hemoglobin.

Reference Range

Reference ranges remain the same on the Sysmex pocHi as the Beckman Coulter LH780 and can be found in the LIS.

References

1. Sysmex pocH-100*i* Instructions for Use, Sysmex Corporation, Kobe, Japan. January 2009.
2. Clinical and Laboratory Standards Institute (CLSI), Laboratory Documents Development and Control; Approved Guideline; Fifth Edition (GP2-A5, 2006).
3. Sysmex America Inc., Mundelein, IL., SCS-1000™ Hematology Calibrator: A Whole Blood Calibrator for Sysmex Hematology Analyzers, Package Insert, Rev. 13, 06-May-08.
4. Sysmex Reagents of America, Inc. Mundelein, IL. MSDS sheets and reagent product inserts.
5. Sysmex America Inc., Mundelein, IL. EIGHTCHECK-3WP X-TRA Hematology Control Assay Sheet, Revision 14, 17-Dec-08.Sysmex America, Inc., Mundelein, IL.

Procedure History

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| Date | Written by | Revision | Approved by | Approval date |
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