Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer

Clinical Significance

- The accurate assessment of fetal lung maturity can be extremely important in the treatment of complicated pregnancies.
- Respiratory distress syndrome (RDS) is a dire consequence of premature delivery.
- Laboratory analysis of amniotic fluid can predict susceptibility to RDS.
- Lamellar bodies are concentrically layered, phospholipids-filled vesicles which represent the storage form of surfactant.
- They are released by mature fetal respiratory epithelium into the amniotic fluid that is respired *in utero*.

Principle

- Lamellar body diameter (1 – 5 µm) is similar to that of small platelets. Lamellar body counts (LBCs) can be obtained rapidly with the use of the platelet channel of Sysmex *pocH-100i* analyzer.
- The Sysmex *pocH-100i* is a quantitative automated hematology analyzer for in vitro diagnostic use.
- The *pocH-100i* counts the platelets (PLT) using electronic resistance detection and hydrodynamically focused technology.
- Sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, then fed into each transducer.
- The transducer chamber has a minute hole called the aperture.
- Inside the detector, the sample nozzle is positioned in front of the aperture and in line with the center.
- After diluted sample is forced from the sample nozzle into the conical chamber, it is surrounded by front sheath reagent and passes through the aperture center.
- On both side of the aperture, there are the electrodes between which flows direct current.
- PLT suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes.
- As direct current resistance changes, PLT volume is detected as electrical pulses.
- PLT count is calculated by counting the pulses, and a histogram is plotted by determining the pulse heights.

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Work place safety

All laboratory employees are expected to maintain a safe working environment and an injury-free workplace. Laboratory employees are responsible for their own safety, the safety of others and adhering to all departmental and medical center safety policies and procedures.

- For standard precautions and safety practices in the laboratory; see LGM 8000, specifically, but not limited to, equipment safety, proper body mechanics, sharps exposure and proper use of personal protective equipment (PPE).
- For Universal Body Substance precautions, see LGM 8005, specifically, but not limited to, exposure to body fluids.
- For proper hand-washing, see LGM 8010, specifically, but not limited to, proper hand-washing.
- For proper infection control, see LGM 8004, specifically, but not limited to, proper use of gloves.
- For proper handling of regular and infectious waste, see LGM 8006, specifically, but not limited to proper disposal of regular and biohazardous waste.
- For proper cleaning of work area, see LGM 8007 - Cleaning Work Areas.
- For proper handling of chemicals and reagents, see the Chemical Hygiene Plan.

Clinical Decision Point

- CDP = 65,000 /μL.
- All LBC <65,000 /μL to be reflexed for confirmatory phospholipid analysis (LS & PG) by TLC (Thin Layer Chromatography).

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Specimen Requirement

The following are the specimen requirements for the procedure.

Specimen	Required Volume	Minimum Volume	
• Amniotic fluid • Vaginal pool <u>without</u> obvious mucus	10 mL	3.0 mL	

<u>Unacceptable Specimen:</u>

The following amniotic fluid samples are unacceptable for Lamellar Body testing:

- Bloody (with >5% RBC)
- Mucoid & Gelled specimen
- Contaminated with Meconium
- Vaginal Pool containing obvious mucus

Materials Needed

Equipment	Supplies
• Sysmex *pocH-100i* Analyzer • Vortex • Tube Mixer	• Thermal Paper • Transducer brush • Philips Screwdriver • 12 X 75 mm glass test tubes • Disposable transfer pipettes • Kimwipes • Beige adapter (for sample tubes) • Green adapter (for QC vials)

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Controls and Calibrator The following tables list the controls and calibrator used in this procedure, their preparation, storage, and stability.

Product	Preparation	Storage	Expiration
SYSMEX SCS-1000 Calibrator Kit	Ready to Use	Store at 2-8 °C before and after opening.	Unopened calibrators are stable until the expiration date shown on the vial. Once opened, the product is stable for 4 hours if returned to the refrigerator promptly after use.
Eightcheck 3WP X-TRA Control Set	Ready to Use	Store at 2-8 °C before and after opening.	Unopened controls are stable until the expiration date shown on the vial. Once opened, the product is stable for 14 days if returned to the refrigerator promptly after use.
Amniotic Pool Positive Control	Pool previously tested amniotic fluid (AF) samples. Run the pool on *pocH-100i*. Use negative AF samples from Genetics Lab. + previously tested LBC samples and adjust the concentration to ~ 70,000 /µL. Aliquot 2 mL each into a screw-capped plastic tube.	Store Refrigerated At 2-10 C	Stable 6 months
Amniotic Pool Negative Control	Pool negative AF samples from Genetics Lab to obtain a concentration of < ~ 3,000 /µL. Aliquot 2 mL each into a screw-capped plastic tube.	Store frozen	Stable 6 months

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

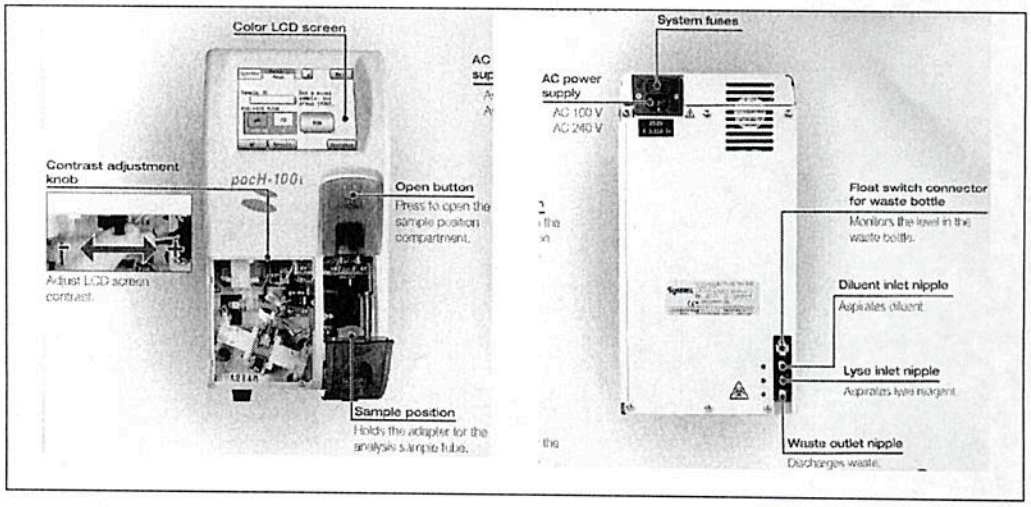
Reagents

The following tables list the reagents used in this method, their preparation, storage, and stability.

Reagent	Preparation	Storage	Expiration
pocH-pack D (Diluent)	Ready to Use	Room Temp.	• Unopened packs are stable for 1 year. • Once opened, reagent is stable for **60 days.**
pocH-pack L (Lysing Reagent)	Ready to Use	Room Temp.	• Unopened packs are stable for 1 year. • Once opened, reagent is stable for **90 days.**
Bleach (5%)	Ready to Use	Room Temp.	• Use fresh daily.

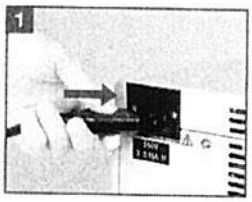
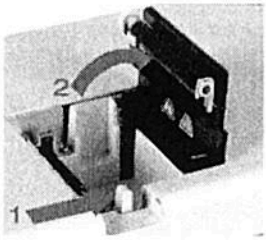
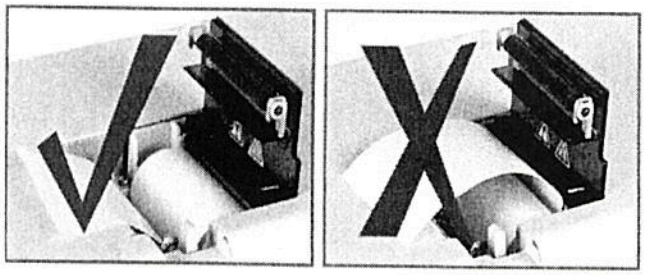
System Overview

Parts of Sysmex *pocH-100i* Analyzer



Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Startup Procedures

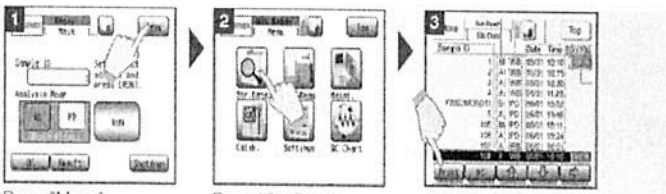
Step	Action
1	• Verify the following before powering on: • Check that the power cable is connected. 
2	• Check that there is sufficient printer paper, replace if needed.  1. Open the paper holder by pushing the knob. 2. Lift the printer cover.  **Correct** **Incorrect**
3	• Check waste fluid in waste container, discard if needed.
4	• Check and replace reagents if needed.

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

	Step	Action
Power On/ Self Check	1	• Switch power on by pressing the **power switch** located on the **right side** of the instrument. • Three automatic rinse cycles are performed and then a background check. • Should any values be out of the acceptable limit, a maximum of two extra background checks will <u>automatically</u> be performed by instrument
	2	• Review results of **background check**.

If…	Then…
PLT count is 10×10^3 /µL or less	• print & record the result on the daily PM chart. • proceed to running controls
PLT count is higher than 10×10^3 /µL	• perform another cycle of Auto Rinse. • if still outside the limits, refer to Section 6 page 71 of the Instruction Manual for more troubleshooting guidance • once background reading is within limits, proceed to running controls.

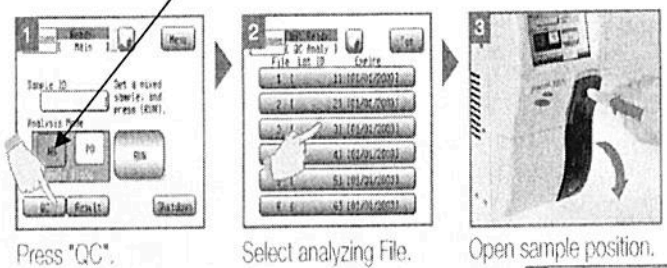
	3	• To print results of **background check** (also referred to as **Blank check**), • press "**Menu**" • press "**Str. Data**" • then select the file "**Blank check**" • results can first be viewed on screen by using the ← or → arrow. • then press "**Print**"



Press "Menu". Press "Str. Data". Choose stored data by pressing "↑" or "↓". Then press "Print".

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

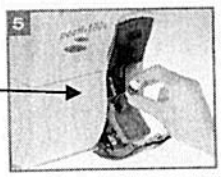
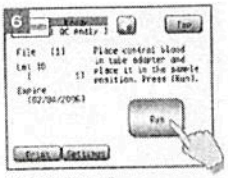
	Step	Action
Performing Quality Control	1	• Run 1 set of EIGHTCHECK Control (Low, Normal, and High) on each day of instrument use. • Remove control vials from the refrigerator at least 15 minutes before use. • **Roll** 10 times, **Turn upside down** 10 times, then **Roll again** for another 10 times. • Examine vial bottom. If there is red cell pellet still formed on the bottom, **then repeat** the whole mixing procedure!
		• Include Amniotic Pool Positive & Pool Negative controls with every run of patient sample. • Negative Pool control is stored frozen. **Thaw** at <u>least 30 minutes</u> before use to bring to room temperature. • Positive pool control is stored in the refrigerator, bring to room temperature before use. • **Vortex** well before running.
	4	• To run **QC** →from the main screen • Verify that **"WB"** mode is selected, if not, press **"WB"**



- Press "**QC**"
- Select the **QC file** (filename is the **lot #** for Low, Normal, or High control).
- Open the sample door (also known as sample position) and insert the **green** adapter

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Performing Quality Control

Step	Action
5	• **Mix** the control **10 more times** then **REMOVE THE CAP!**  Insert control blood and close door.  Press "Run". • Check the vial for bubbles. • Press "**Run**". • At the end of the run, the "**Quit**" button will appear on screen. • Press "**Quit**" to print results and to go back to main screen.

If…	Then…
PLT count is within acceptable performance for Low, Normal, and High controls	• Proceed to running patient samples.
Control results <u>are not acceptable</u>	• Verify that the right QC file was selected. • Check that there is no cell pellet on the bottom, mix the vial, then re-run control. • If still outside the limits, refer to Section 6 page 71 of the Instruction Manual for more troubleshooting guidance. • Document all problems and corrective action(s) taken on the QC/QI book. • **STOP!** Do not proceed to patient testing until QC is acceptable.

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Maintaining Patient and Sample Identity

Step	Action
1	• Check that all patient's information on the worksheet or requisition match those on sample tube(s) (Name, Accession #, MR #, gender, etc).
2	• Always use barcode labels when labeling secondary tubes.
3	• Use barcode scanner to scan the Accession # for sample ID when running pocH 100i.

Performing Sample Analysis

Step	Action
1	• To run pool or patient sample →from the **main screen** • Verify that **"WB"** mode is selected. • Press "**Sample ID**" • the alphanumeric keypad will appear on screen.

If…	Then…
running Pool controls	• manually type in the ID (Pool). • press enter.
running patient sample	• scan the barcode ID. • press enter.

NOTE: Always verify that button **(WB)** is selected.

2	• **Vortex** the pool controls and patient samples for 5-10 sec for a well mixed sample.
3	• Transfer at least 2 mL to a 12 X 75 mm test tube.
4	• If processing more than one patient sample, a **Pool Negative** must be analyzed after each patient to verify no carryover exhibited

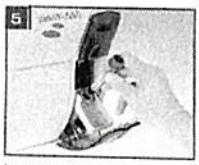
Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Performing Sample Analysis, *continued*

Step	Action
5	The following sampling sequence **must be followed exactly** 1. **Pool Positive** Control (PPC). 2. **Pool Negative** Control (PNC). 3. Sample **1** (patient) 4. **Pool Negative** Control (PNC) 5. Sample **2** (patient) 6. **Pool Negative** Control (PNC) 7. Sample **3**, (patient), etc. ***Note: The pool negative sampling sequence is to ensure no carryover exhibited from previous patient or control samples***
6	**If…** / **Then…** PNC result is **"0"** → • Proceed to running sample. PNC is giving any value → • **STOP!** • Do not run sample, perform a **"Clean Transducer"** cycle. • See page 12 for stepwise procedure on **How to clean the transducer.**
7	• **Open** the sample door (also known as sample position) • Insert the **beige sample adapter**, **load sample** and **close door.**
8	• Press "**Run**" The results will automatically print at the end of the run.

Push the top part to open the door

Insert the correct adapter.



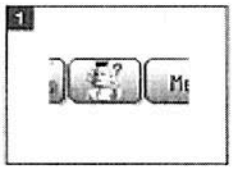
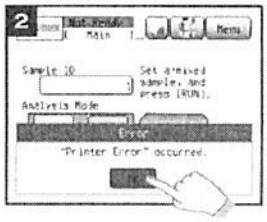
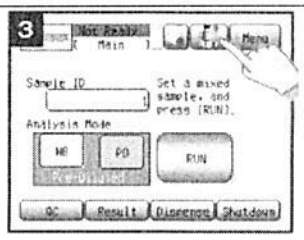
Insert sample tube and close the door.

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

How to Perform Clean Transducer

Step	Action
1	• From Main screen at the "**Ready**" status, 1. press "**Menu**". 2. press "**Maint.**" 3. select **"Clean Transducer"**
2	• Fill 12 x 75 mm test tube with **concentrated bleach**. • Push the sample door to open the sample position. • Place the tube with **bleach** on the beige adapter. • Press **"Execute"**

How to fix Error Messages

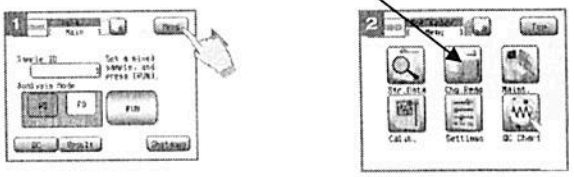
Step	Action
1	• When an error occurs, • Alarm will sound • Alarm button will flash. 
2	• Error message(s) will appear on screen. • In case of multiple errors, they will be displayed on screen ranked by importance. • Press the **"OK"** button on error message screen to stop the alarm and to close error dialog. 
3	• Press the **HELP** button (also called **alarm** button). • This will show on screen the kind of error that had occurred. • Press **"Detail"** to see instructions on how to fix the error. 

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

How to fix Error Messages, *continued*

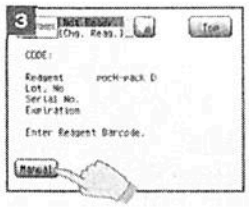
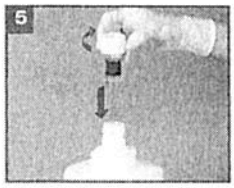
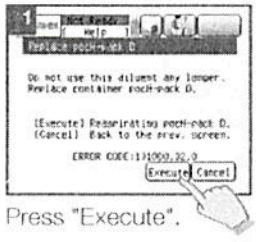
Step	Action
4	• Refer to Page 67 (Chapter 6) of the Instructions Manual for more troubleshooting guidance.
5	• If unable to solve the problem, call 1(866)-8SYSMEX for technical assistance.
6	• *NOTE:* In case of power failure during operation, turn the main power switch OFF.

How to Replace Reagents

Step	Action
1	• Changing reagent from Main screen at the "**Ready**" status, • press "**Menu**". • press "**Chg. Reag**" 
2	• The **reagent screen** will appear, (for example): **Lot. No.** **Expiration** pocH-pack D A8073 07/14/2010 pocH-pack L A8014 12/18/2009 • **Choose** the reagent to replace pocH-pack D | pocH-pack L | Reag. Log
3	• **Press** the appropriate button for the **reagent pack to replace**.

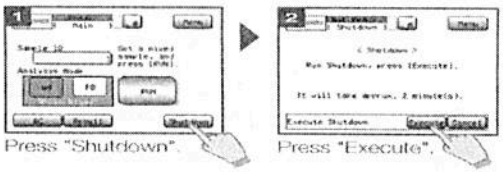
Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

How to Replace Reagents, *continued*

Step	Action
4	• The screen below opens up.  • Reagent lot & exp. date can be entered manually or by scanning the reagent barcode. • **Scan the reagent barcode**, the lot # & exp. date will automatically register on screen.
5	• Press "**OK**" to confirm the Lot # & exp. date.  Press "OK".
6	• Insert the container spout kit into the new bottle. • Tighten the cap then press "**Execute**".   Press "Execute".
7	• If the reagent runs out in the **middle of run**, an error message will appear on screen prompting the operator to replace the depleted reagent. • Press "**Execute**" • Follow steps 4-6 above.  Press "Execute".

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Performing Daily Shutdown Procedure

Step	Action
1	• It is very important to **perform shutdown** procedure at the end of the day to remove deposits in the tubing. • Deposits in the instrument tubing can cause incorrect results or it may damage the instrument.
2	• Run **concentrated bleach** as sample to clean the transducer of residual protein.
3	• Perform shutdown cycle, from Main screen at the "**Ready**" status, • press "**Shutdown**". • press "**Execute**" 
4	• After the shutdown cycle (2 minutes), **turn OFF** the instrument.

Reporting/ Results Interpretation

Step	Action
1	• Lamellar Body Counts are read as **PLT** on *pocH-100i* . **If…** / **Then…** PLT count is ≥ **65** x $10^3/\mu L$ → • Release in LMS, it will be reported as **MATURE.** PLT count is < **65** x $10^3/\mu L$ → • Release in LMS, it will reflex to **L/S & PG** confirmation by TLC.

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

How to Calibrate

Step	Action
1	• Calibration interval on *pocH-100i* is every 6 months.
2	• The calibration is always performed in the **"WB"** mode. • The type of calibration performed is **"Calibrator calibration for whole blood (WB) mode"** *page 65, instructions manual.*
3	• A precision check is performed prior to calibration. • When performing this calibration, the first analysis data is not included in the calculation. • It is overwritten by the second analysis data.
4	• If an error occurs during precision check analysis or calibrator analysis, an alarm sounds and an error dialog appears • The results of the analysis where the error occurred are displayed in reverse video, as "---.-". • Press "**OK**" to close the error dialog, then press "**Run**" again.
5	• Take out the calibrator from refrigerator at least 30 minutes before use to warm the vial to room temperature. • Mix each vial by gentle end-to-end inversion until the cell button in the bottom of the vial is completely suspended. • Allow the vial to rest on flat surface 15 seconds prior to analysis to allow for the dispersion of micro-bubbles.

6	• From Main screen,
	  • press "**Menu**"
	  • press "**Calib**"
	  • select "**Calibrator (WB)**"

7	• Insert the **green** adapter into the sample position then load the calibrator vial.
8	• Press "**Run**", let the analysis finish; then repeat this step **10 more times.** • This step should be performed a total of 11 times for precision check.

9	• After the completion of precision check,
	  • press "**Next**"
	  • press "**Quit**"

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

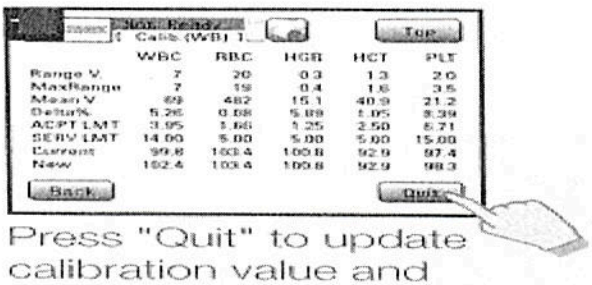
How to Calibrate, *continued*

Step	Action

10	• The calibration screen opens up.
	• Manually enter the target values listed on calibrator insert and verify that they are correct
	  • press "**Enter**"
	  • press "**Next**"
	  • press "**OK**"

11	• Press "**Run**", let the analysis finish; then repeat this step **5 more times.** • This step should be performed a total of **6 times** to obtain required calibration data.
12	• After analysis, press **"Next".** • The calibration results show on screen.
13	• Press **"Quit"** to update calibration value and calibration history.
14	• Document calibration on calibration log, CLS initials & date.

Reviewing Calibration Results

Step	Action
1	• Image below shows an example result of calibration run. 

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Reviewing Calibration Results, *continued*

2	• Explanation of displayed items on calibration result screen.

Range V.	• The highest value minus the lowest value within five consecutive analysis. •
MaxRange	• The maximum Range Value allowed.
Mean V.	• Mean value of five consecutive analysis results.
Delta %	• $\frac{\text{Assay Target} - \text{Mean Value}}{\text{Mean Value}} \times 100$ (this is automatically calculated and displayed).
ACPT LMT	• Maximum Upper Limit of Delta Percent (%).
SERV LMT	• If the Delta Percent exceeds this limit, technical assistance may be necessary and instrument system needs service.
Current	• Current calibration value which was obtained in the previous calibration procedure.
New	• New calibration value calculated from the calibrator analysis.

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Reviewing Calibration Results, *continued*

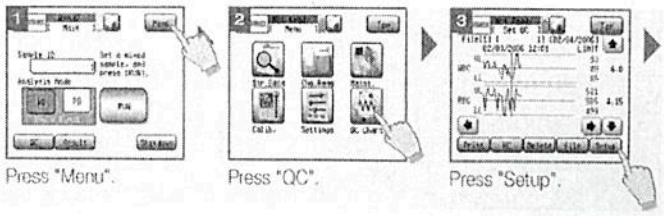
Step	Action
3	• Table below shows how the calibration data is used by the system.

If…	Then…
Delta % is within ACPT LMT	• Calibration factor will not be changed, i.e. instrument will continue using the "**Current**" calibration value.
Delta % <= ACPT LMT	• The "**New**" calibration value will become the "**Current**" calibration value.
Range V. > MaxRange	• The "**New**" calibration value will become the "**Current**" calibration value.
Delta % > SERV LMT	• Calibration will not be allowed. • Instrument needs service. • Call 1(866)-8SYSMEX for technical assistance.

Step	Action
4	• "NEW" calibration value = (Target/Mean V.) x Current (automatically calculated & stored by instrument)

How to Setup QC File

Step	Action
1	• To setup QC file, from Main screen, • press "**Menu**". • press "**Chg. Reag**" • press **"Setup"**

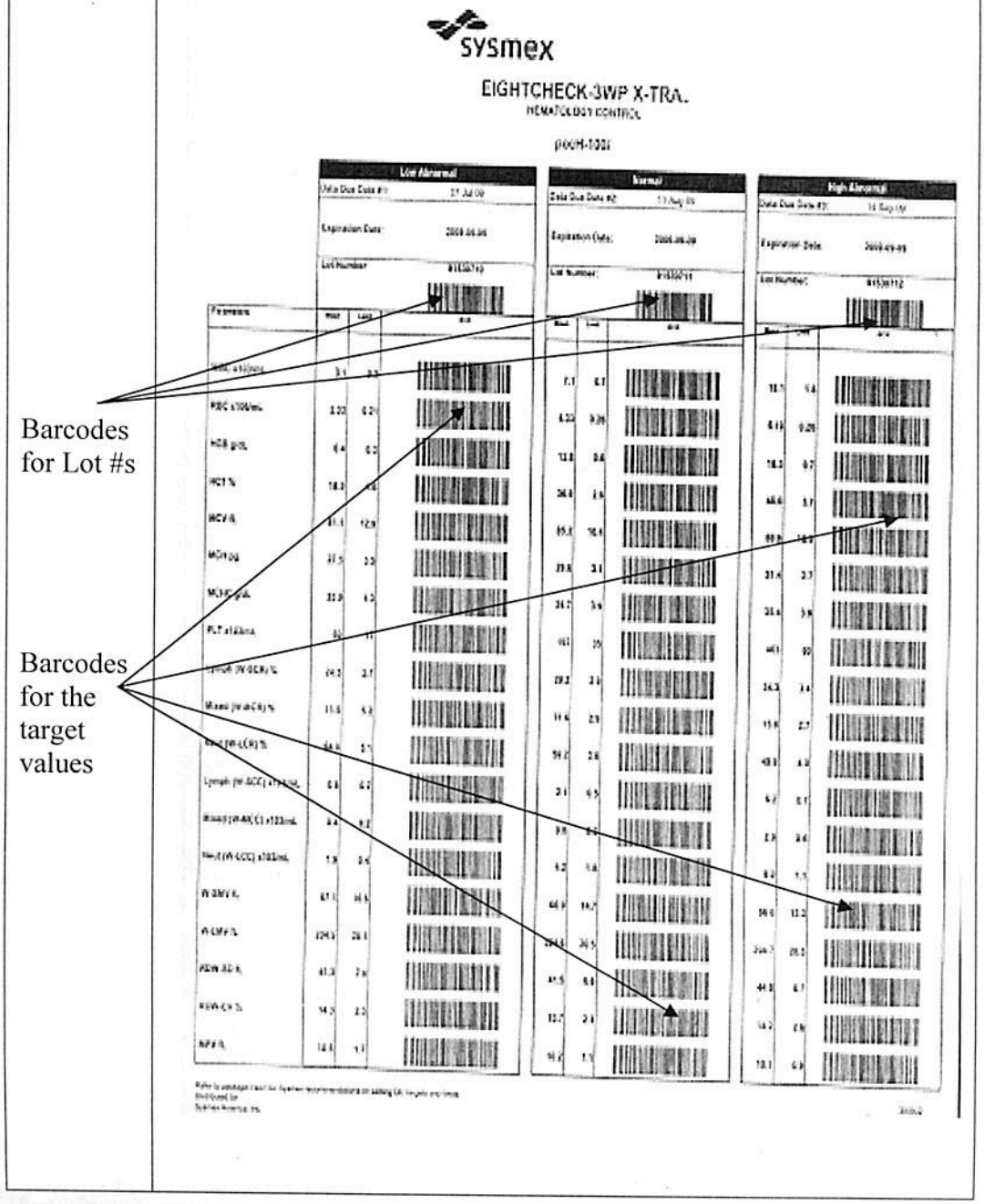


Step	Action
2	• Scan the barcode label for the Lot #. (the first set of barcodes on top of the QC package insert-refer to step 3). • **Expiration date has to be manually entered.**

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

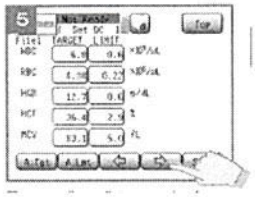
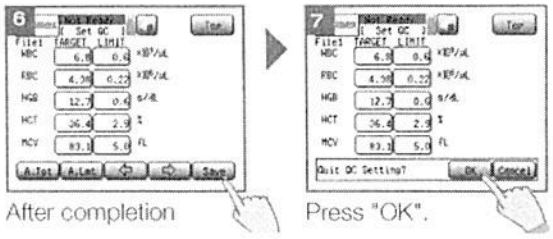
How to Setup QC File, *continued*

3	• QC package insert for use in defining QC File.



Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

How to Setup QC File, *continued*

Step	Action
4	• Press → to go to the next 5 QC file setting screens. • Scan **all 19 barcode labels** for each level (Low, Normal, and High). 
5	• Once all the parameter have been entered/ scanned, • press "**Save** • press "**Ok**  After completion Press "OK".

Quality Control Evaluation

Step	Action
1	• When calculating ranges for new lot of whole blood controls or new batch of amniotic pool positive control, use the same historical SD & CV as the Regional Reference Laboratory.

Notes and Limitations

Step	Action
1	• For sub-optimal samples that are unacceptable for Lamellar Body Count obtain L/S& PG order and send specimen to the Regional reference laboratory for L/S & PG detection by TLC.
2	• If the AF hematocrit (HCT) exceeds 1%, reflex the specimen for L/S & PG detection by TLC.

Procedure for Lamellar Body Counts in Amniotic Fluid On SYSMEX *pocH-100i* Analyzer, *continued*

Alternate Method

Step	Action
1	• In the event of instrument malfunction and the problem could not be resolved, *samples should be sent straight for L/S & PG Detection by TLC.*

Non-Controlled Documents The following non-controlled documents support this procedure.

Reference	
SYSMEX *pocH-100i* Analyzer, Instructions 2005-2006	Non-controlled
SYSMEX package inserts (reagents, calibrators, controls)	Non-controlled
Preventive Maintenance for *pocH-100i* Analyzer	Non-controlled
Lamellar Body Count Inventory	Non-controlled
Calibration Log	Non-controlled
Operator Training Checklist	Non-controlled

Controlled documents

	Title
LGM8000	Standard precautions and safety practices in the laboratory
LGM8005	Universal Body Substance precautions
LGM8010	Proper hand-washing
LGM8006	Infection Control
LGM8007	Cleaning Work Areas
LGM8012	Proper storage and disposal of chemical hazardous waste

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Procedure for Lamellar cody Counts in Amniotic Fluid on SYSMEX pocH-100i Analyzer

Reviewed and approved by:

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Procedure for Lamellar cody Counts in Amniotic Fluid on SYSMEX pocH-100i Analyzer, Continued

Document History Page

Effective Date:

Change type: New, Major, Minor etc.	Changes Made to SOP – describe	Signature responsible person/date	Med. Dir. Reviewed/ Date	Lab Manager reviewed/ date	Date change implemented
New		10/29/12	12/11/12	12/10/2012	