National Cancer Institute-Frederick, Frederick, MD Biopharmaceutical Development Program	STANDARD OPERATING PROCEDURE	Effective Date Nov 5, 2008	Procedure Number 23125
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Title: Rapid Gram Stain ID using the Charles River Portable Test System (PTS)

Author/Date: [redacted]

Approvals/Date: [redacted]

SOP References: 21531	Supersedes: Revision 00

Purpose: The PTS Gram ID measures the presence of the cell wall in a microbial isolate. The measurement is interpreted by the software to indicate whether a sample contains Gram negative or positive bacteria or yeasts/molds.

Scope: Process Analytics (PA) qualified personnel will perform this procedure.

Contents:

1.0 Authority and Responsibility

1.1 The Director, Process Analytics (PA) has the authority to define this procedure.

1.2 PA is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

1.3 PA personnel are responsible for the performance of this procedure.

1.4 BQA is responsible for quality oversight of this procedure

2.0 Materials/Equipment

2.1 Endosafe PTS Cartridge, BDP Part # 30857

2.2 Eppendorf pipette tips, BDP Part # 20469, or BDP approved equivalent

2.3 LAL Reagent Water, BDP Part # 30328

2.4 0.5 McFarland Equivalence Turbidity Standard

2.5 Sterile Loops, BDP Part # 20675 or BDP approved equivalent

2.6 Pyrogen-Free Test Tubes, 10 x 75 mm, BDP Part # 20277 or BDP approved equivalent

2.7 Isolated culture

2.8 PTS, BDP Part # 81770 or 81780

2.9 Endosafe- PTS mini pipettor, BDP Part # 21860 or BDP approved equivalent

2.10 Epson TM-U22OD Printer

2.11 Mini Vortexer

3.0 Sample Preparation

NOTE: Do not use cotton tip swabs of any kind during sample preparation.

3.1 Dispense approximately 2 mLs of LAL Reagent Water in test tube.

3.2 From an isolated pure culture, remove colonies from agar surface. Avoid contaminating the culture with fragments of agar media. Suspend cells in LAL Reagent Water and adjust to a 0.5 McFarland Equivalence Turbidity Standard.

NOTE: Organisms up to 72 hours can be used but using a culture less than 24 hours old is advisable for best results.

4.0 PTS Operation

4.1 Press the menu key on the PTS keypad to turn the instrument on. The reader initiates a system self test as it heats up to 37°C. This will take approximately 5 minutes.

4.2 The reader will display “SELF TEST OK” and then “INSERT CARTRIDGE.” If the reader fails the self test, do not use it, and notify the Supervisor.

NOTE: Each PTS cartridge lot will be tested using ATCC organisms prior to the use of the lot.

4.3 Remove the cartridge from the pouch and insert with sample reservoirs facing up into slot at front of the PTS reader. Press cartridge firmly into slot.

4.4 Once the cartridge has been firmly inserted into the reader, the reader will prompt the user to enter the following information:

4.4.1 Enter OID (Operator ID).

4.4.2 Enter Lot number of cartridge.

4.4.3 Enter calibration code. The calibration code can be found on the COA. See Attachment 2. If the calibration code for the particular lot number has already been entered, the reader does not prompt for the code again. To erase all stored lot number’s and corresponding calibration codes, select menu, 2, and then 4 from the initial menu.

4.4.4 Lot number. (Confirms cartridge lot number entered)

4.4.5 Enter Sample #1 ID (This prompt is available for all four samples). Selecting and scrolling with the menu key under the sample ID header allows for fifty samples to be entered and stored.

4.4.6 Once all the test information is entered, the reader displays: “ADD SAMPLE; PRESS ENTER.”

NOTE: Each channel in the gram ID cartridge can be used to identify 4 different isolates.

4.5 Pipette 25 µL of first sample into one sample reservoir of the cartridge. Change pipette tip to avoid cross contamination between each sample. After all four (4) sample reservoirs are filled; press enter on the reader keypad. Sample must be delivered to each sample port without contamination or cross contamination between samples.

4.6 Pumps draw samples aliquots into the test channels, thereby initiating the test. Results will be obtained in approximately 3 minutes for Gram-negative and Gram-positive identification and approximately 7 minutes for Yeast/Mold identification.

5.0 Test Results

5.1 When the test is complete, the PTS reader gives an audible notification that the assay is finished and the gram identification is displayed on the screen for each sample as Gram Negative, Gram Positive, Yeast/Mold.

5.2 The reader display alternates between the following results:

- Sample #1
- Sample #2
- Sample #3
- Sample #4
- Remove Cartridge

NOTE: The reader continues to display the assay results until the cartridge is removed.

5.3 Printing test results using the Epson Printer

5.3.1 Connect serial port cable to the printer and the RJ45 plug in back of the PTS reader.

5.3.2 Press the MENU key on the PTS reader keypad.

5.3.3 Select 4 for the print menu. Selecting one of the following choices on the reader keypad will send results to the printer. See attachment III for sample printout.

- 1- Print Last Test
- 2- Print by Date
- 3- Print all Tests

Attach results to form 23125-1

NOTE: Printing data will automatically download results to the PTS Logger software.

6.0 Documentation

6.1 Attach appropriate results to the QC test request form and forward to PA Supervisor for PA review.

6.2 Each report is signed and dated by the analysts and the reviewer.

6.3 All usage, maintenance, calibration, cleaning, etc. should be documented in the instrument logbook as per ***SOP 21531, Equipment/Facility Logs***.

ATTACHMENT I

NCI-Frederick
Form No.: 23125-01
SOP No.: 23125
Revision 01:

PTS Gram ID Analysis

Requestor:	QC Number:

Reagent	BDP Lot #/ Expiration
PTS Cartridge	
LAL Reagent Water	
Pipette ID Number/BDP Number	
PTS BDP Number/Calibration Due	
Other	

Sample:	Gram ID:

Attach PTS printout

Analyst/Date: _____

Process Analytics Review By/Date: _____

ATTACHMENT II

ENDOSAFE® - PTS

RAPID MICRO METHODS GRAM ID CARTRIDGES

CERTIFICATE OF ANALYSIS

Cartridge Lot #: 7311935 Expiration Date: 5-2009

Calibration Code for Determination of Gram Negative and Gram Positive Isolates: 515015143077

Calibration Code for Determination of Gram Negative, Yeast/Mold and Gram Positive Isolates: 515040043099

Archived Onset Times For Isolates:

Gram Negative	≤150 seconds
Yeast/Mold	151-399 seconds
Gram Positive	≥400 seconds

This lot of Gram ID Cartridges has been tested with the following microorganisms and met quality requirements:

Escherichia coli ATCC 8739 Gram-negative
Candida albicans ATCC 10231 Yeast
Bacillus cereus ATCC 14579 Gram-positive

Store Gram ID Cartridges between 2 – 25 °C (DO NOT FREEZE). Gram ID Cartridges should be used immediately once the foil pouch seal has been opened. Gram ID Cartridges are for single use only.

Qualified Analyst _____ Date 12 NOV 2007

Reviewed By _____ Date 12 NOV 2007

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RMM-COA-RMMGI100-01

ATTACHMENT III

Example of Gram-negative, Yeast/Mold or Gram-positive assay result format report:

```
********* ENDOSAFE Test Record *********
          V7.09A 02/14/05
DateTime: ......... 02/16/05 @ 10:44:09
Device: ............................ 0012
OperatorID: ........................ DMN
Cartridge: ....................... Gram ID
Temperature: .. Start: 37.0C  End: 37.0C
Method: ......................... GSX-401
Cartridge Lot#: ................. 5047910
Onset Time (sec): ...  292 <150 >400 238
Sample #1 Lot: ................... 10231
Sample #1 ID: ............. CALBICANS
Sample #2 Lot: ..................... 8739
Sample #2 ID: ............. ECOLI
Sample #3 Lot: ..................... 6538
Sample #3 ID: ............. SAUREUS
Sample #4 Lot: ................... 10231
Sample #4 ID: ............. CALBICANS
Sample #1: ................ Yeast / Mold
Sample #2: ............... Gram Negative
Sample #3: ............... Gram Positive
Sample #4: ................ Yeast / Mold
:
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Example of Gram-negative or Gram-positive assay result format report:

```
********* ENDOSAFE Test Record *********
          V7.09A 02/14/05
DateTime: ......... 02/15/05 @ 16:27:36
Device: ............................ 0012
OperatorID: ........................ DMN
Cartridge: ....................... Gram ID
Temperature: .. Start: 37.0C  End: 37.0C
Method: ......................... GSX-401
Cartridge Lot#: ................. 5044922
Onset Time (sec): .  <150 >151 <150 >151
Sample #1 Lot: ..................... 8739
Sample #1 ID: ............. ECOLI
Sample #2 Lot: ..................... 6538
Sample #2 ID: ............. SAUREUS
Sample #3 Lot: ..................... 8739
Sample #3 ID: ............. ECOLI
Sample #4 Lot: ..................... 6538
Sample #4 ID: ............. SAUREUS
Sample #1: ............... Gram Negative
Sample #2: ............... Gram Positive
Sample #3: ............... Gram Negative
Sample #4: ............... Gram Positive
:
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ATTACHMENT IV

Image of the Endosafe PTS System