ENDOSAFE® - PTS
RAPID MICRO METHODS
GRAM ID

DISPOSABLE CARTRIDGES FOR GRAM ID

FOR USE WITH ENDOSAFE® PORTABLE TEST SYSTEM

INTENDED USE

Disposable test cartridges are intended for use with Endosafe® Portable Test System (PTS) as an alternative to conventional Gram stain methods for preliminary identification of microorganisms.

BACKGROUND AND SUMMARY

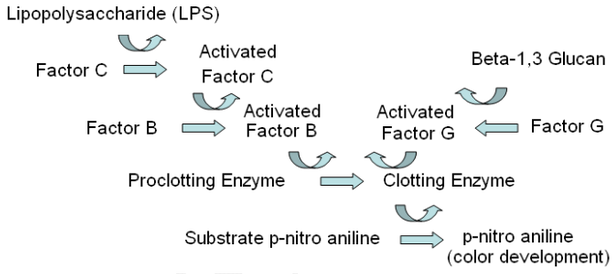
The Gram stain was devised in 1884 by Hans Christian Gram in an attempt to differentiate bacterial cells. The Gram stain is widely used today to distinguish intact, morphologically similar bacteria between Gram-negative and Gram-positive cells based on cell color after staining. Such preliminary information provides important details about the type of organism present and the further techniques required to characterize them[1].

Gram staining is based on the ability of bacterial cell walls to retain the crystal violet dye during solvent treatment. The cell walls for Gram positive microorganisms have a higher peptidoglycan and lower lipid content than Gram-negative microorganisms. Gram-negative microorganisms have lipopolysaccharide (LPS) in their cell walls. The solvent wash dissolves the LPS, affecting the integrity of the cell wall and causing the crystal violet to be washed away.

Frederick Bang observed that bacteria causes intravascular coagulation as part of the primitive immune system in the American Horseshoe Crab, *Limulus polyphemus* [2]. In collaboration, Levin and Bang[6] found that the agents responsible for the clotting phenomena are a cascading series of serine proteases residing in the crab's amebocytes, or circulating blood cells. LPS triggers the first enzyme in the cascade. The last activated enzyme in this series, the pro-clotting enzyme, cleaves a peptide from an endogenous substrate called coagulogen. Beta 1, 3 Glucan, found in the cell walls of yeast and mold, can also trigger an alternate pathway in the Limulus Enzyme Cascade and can be discriminated by time to reach color onset.[2-6]

We employ a synthetic substrate based on the coagulogen amino acid sequence that undergoes cleavage, resulting in the release of a chromophore, p-nitroaniline (pNA). PNA is a yellow color that is measured photometrically at 385 nm. The Endosafe® - PTS detects the onset of color very accurately and can precisely quantify the amount of LPS and Beta 1,3 Glucan.

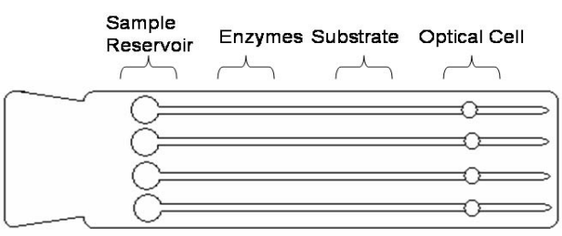
Limulus Enzyme Cascade



The Endosafe® - PTS Rapid Micro Methods Gram ID test employs the Limulus Enzyme Cascade to detect the LPS in suspensions of Gram-negative microorganisms. Since Gram-positive microorganisms lack LPS, they do not trigger the enzyme cascade and no color is produced. The Gram ID achieves comparable results of a conventional Gram stain without the need to fix, stain and view the microscope slides. In addition, the reactivity of Beta 1,3 Glucans by the Limulus Enzyme Cascade enables optional inclusion of Yeast and Mold.

CARTRIDGE REAGENTS

Each Endosafe® - PTS Rapid Micro Methods Gram ID cartridge contains four channels to which Limulus enzymes and a chromogenic substrate have been applied in the following pattern:



STORAGE CONDITIONS AND PRECAUTIONS

PTS Rapid Micro Methods Gram ID cartridges are relatively heat stable and should be stored between 2-25 ºC. If refrigerated, allow the cartridges to come to room temperature before opening the pouch and testing. Prolonged exposure to temperatures above 25 ºC should be avoided. To minimize contamination of the sample reservoirs, the cartridge should be used immediately once the foil pouch has been opened. Up to four samples may be run on each cartridge.

The cartridges are for single use only.

REAGENTS REQUIRED BUT NOT SUPPLIED

Endosafe® Limulus Amebocyte Lysate Reagent Grade Water (LRW) or LPS-free Saline (0.45% or 0.9%) must be used during the preparation of bacterial suspensions.

MATERIALS REQUIRED BUT NOT SUPPLIED

Pipettor (Endosafe® PTS 400 or equivalent) and sterile tips.

Disposable, endotoxin-free glass dilution tubes or sterile, disposable polystyrene tubes (Endosafe® T300 or equivalent).

Vortex-Type Mixer.

0.5 McFarland Equivalence Turbidity Standard or equivalent.

Sterile inoculating loops.

EQUIPMENT REQUIRED BUT NOT SUPPLIED

Endosafe® Portable Test System (PTS) Reader: The reader is a dedicated instrument that accepts the cartridge and runs the PTS Gram ID assay. The reader consists of an incubating chamber, a sample pump, four LEDs and four detectors, an alphanumeric key pad with built–in LCD, and a microprocessor. The reader operates using standard AC power or an internal rechargeable battery. Battery power also acts as automatic backup power in case of AC power failure.

See the *User's Guide* supplied with the Endosafe® - PTS reader for complete operations, procedures and guidelines.

SPECIMEN COLLECTION AND PREPARATION

Specimens for testing must be collected and prepared using clean, LPS-free materials. Glassware can be depyrogenated by dry heat, such as 30 minutes exposure at 250°C.[7] Plastic ware is generally clean from the manufacturer; however, care should be exercised to prevent contamination from surfaces or skin. Use aseptic technique at all times.

The use of an isolated culture less than 24 hours old is advisable for aerobic bacteria to ensure purity. Some anaerobic or other slow growing bacteria may require additional incubation up to 72 hours for sufficient colony size and growth. The colonies should be carefully removed from culture plates. Avoid contaminating the culture suspension with fragments of agar media.

Suspend the cells in approximate 2mLs of LPS-free Saline or LRW and adjust to a 0.5 McFarland Equivalence Turbidity Standard. It is recommended that the prepared cell suspension be tested within one (1) hour of preparation.

Do not use cotton tip swabs of any kind.

PERFORMANCE CHARACTERISTICS

Internally, the PTS reader measures the reaction time in each channel. An archived range specific for each lot of cartridges is constructed. See Certificate of Analysis for the lot specific archived onset times.

Example of Endosafe® PTS Rapid Micro Methods Gram ID Onset Time Parameters:

Gram Stain Result	PTS Gram ID Onset times (seconds)
Gram-negative	≤ 150 seconds
Yeast/Mold	Between 151 – 399 seconds
Gram-positive	≥ 400 seconds

LIMITATIONS OF THE PROCEDURE

The Gram ID assay provides preliminary identification information only. Intended for use with isolated pure bacterial growth and is not intended for use with mixed bacterial suspensions. Heavy inoculum of the bacterial suspension and the age of the culture may alter results. Rare Gram-negative bacterium with irregular cell wall constituents may give aberrant results.

DISPOSAL OF USED CARTRIDGES

Dispose of used cartridges following applicable procedures for the disposal of biohazard material. This involves sterilization by autoclaving before final disposal.

ROUTINE TESTS

Routine Tests: A routine PTS Rapid Micro Methods Gram ID assay is conducted by following the simple prompts on the PTS instrument.

The following represents a typical assay procedure:

Note: The PTS Rapid Micro Methods Gram ID offers two assay result formats. One assay result format will report the presence of either Gram-negative microorganisms, Yeast/Mold or Gram-positive microorganisms. The second assay result format will report the presence of Gram-negative microorganisms only. Reference the Certificate of Analysis for the calibration code to be used for each assay result format.

1. Instrument Operation
   - Press the menu key on the PTS keypad to turn instrument on (Menu 5 turns instrument off)
   - The reader then initiates a "SYSTEM SELF TEST" as it heats up to 37°C – this takes approximately 5 minutes
   - The reader displays "SELF TEST OK" and then "INSERT CARTRIDGE"

Note: If refrigerated, allow the cartridge to come to room temperature inside the pouch before opening the pouch and testing.

2. Insert the Cartridge

Remove the cartridge from the pouch and insert with the sample reservoirs facing up into the slot at the front of the PTS reader. Do not touch the sample reservoirs or optical cells.

Press cartridge gently but firmly into the slot.

3. Enter Required Information

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

* *Enter OID* (Operator ID or User Name)
* *Enter Cartridge Lot #*
* *Enter Calibration Code* (use the calibration code for the assay result format that is needed)

(See the Certificate of Analysis for the Calibration Code. If the Calibration Code for the particular lot number has already been entered, the reader does not prompt for the code again)

* Lot# #######
Enter or Cancel

(This prompt is to confirm the cartridge lot number entered. Pressing cancel will return user to cartridge lot # prompt)

Note: If alternating between calibration codes for a lot of cartridges, the user must delete all stored lot numbers in the PTS reader (see *User's Guide* for procedure)

* *Enter Sample Lot #*
* *Enter Sample ID*

(These prompts are available for entering information for 4 samples)

While the above information is being entered into the reader, the cartridge is being pre-warmed for a minimum of 30 seconds.

4. Dispense the sample

Once all test information is entered, the reader displays:

* *"ADD SAMPLE PRESS ENTER"*
* Pipette 25 µL of each sample into the sample reservoirs of the inserted cartridge and press **Enter** on the reader keypad
*The test will begin and takes about 3 to 7 minutes to produce results

Note: Use of a sterile tip for each new sample is essential to avoid cross contamination.

TEST RESULTS

When the test is complete, the PTS reader gives an audible notification that the assay is finished.

Data reporting is simple. At the conclusion of the test, the Gram identification for each sample is displayed on the screen. The reader's screen will show each sample result in an alternating pattern. It will display one of the following results for each sample:

Gram Negative
Gram Positive
Yeast/Mold

The reader continues to display the assay results until the cartridge is removed. Once the assay is complete and the results are noted, remove the cartridge promptly from the reader.

Retrieving Results Options:

- Download results directly to your PC and retrieve from the designated location file.
- Use the Seiko® DPU-414 Printer (available from Charles River Endosafe) to print the last test result, all results from a particular date, or up to a maximum of one hundred stored test results.

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PATENT INFORMATION

U.S. Patent No: US D472,324 S
Other Patents Pending

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