

ENDOSAFE® - PTS RAPID MICRO METHODS GLUCAN

SINGLE-TEST, DISPOSABLE CARTRIDGES FOR
GLUCAN DETECTION

FOR USE WITH ENDOSAFE® PORTABLE TEST SYSTEM

INTENDED USE

Disposable test cartridges are intended for use with Endosafe® Portable Test System (PTS) to perform quantitative detection of (1→3)-β-D-Glucan by kinetic chromogenic methods for the Bio/pharmaceutical Industry.

The Endosafe® - PTS Glucan is intended for research purposes only and is not to be used for diagnostic testing purposes and is not to be used for end product release of Pharmaceutical Drugs, Devices, or Biologics that are regulated by FDA.

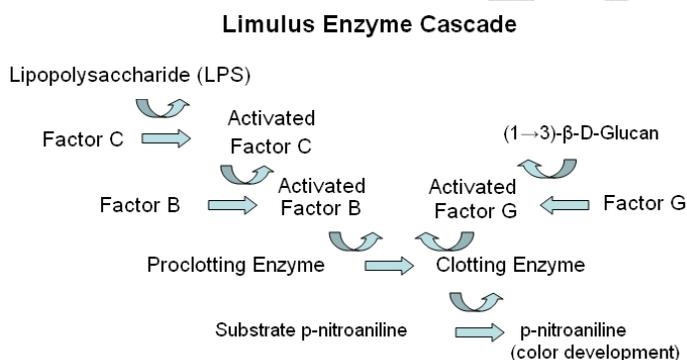
BACKGROUND AND SUMMARY

Frederick Bang observed that bacteria causes intravascular coagulation as part of the primitive immune system in the American Horseshoe Crab, *Limulus polyphemus*.¹ In collaboration, Levin and Bang^{2,7} found that the agents responsible for the clotting phenomena are a cascading series of serine proteases residing in the crab's amoebocytes, or circulating blood cells, and that pyrogens (bacterial endotoxin) triggered the enzymes involved in the clotting cascade. The coagulation of Limulus Amebocyte Lysate (LAL) use to be considered specific to endotoxins, but an alternative pathway triggered by (1→3)-β-D-Glucans was discovered in LAL.³⁻⁵

The presence of (1→3)-β-D-Glucan in samples can contribute to false positive signals in Bacterial Endotoxin Tests (BET), and this is why it is necessary to have an easy method to verify the source of the LAL activation to determine if it is (1→3)-β-D-Glucan-derived. (1→3)-β-D-Glucans with β-1,6-glucosidic branches are commonly found in fungi, yeast, algae and plants.

BIOLOGICAL PRINCIPLES

In this assay, (1→3)-β-D-Glucans initiate the activation of a cascading series of serine proteases in LAL. The last activated enzyme in this series, the pro-clotting enzyme, cleaves a peptide from an endogenous substrate called coagulogen. 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 -410 nm. The Endosafe® - PTS detects the onset of color very accurately, and can precisely quantify the amount of (1→3)-β-D-Glucan.



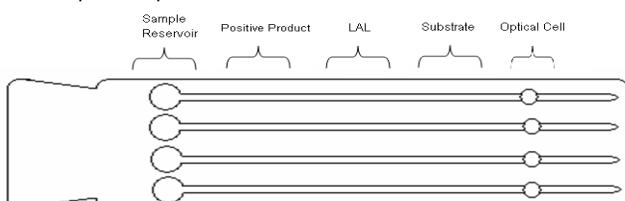
The Endosafe® - PTS Glucan test employs the Limulus Enzyme Cascade to detect (1→3)-β-D-Glucan in a sample. The Endosafe® - PTS cartridge and its interface with the reader have been designed to mimic Kinetic Chromogenic Methods by measuring color intensity directly related to the (1→3)-β-D-Glucan concentration in a sample. Each cartridge contains precise amounts of glucan specific LAL formulation, chromogenic substrate, and glucan standard.

APPLICATIONS

Assay applications include, but are not limited to the following: LAL Endotoxin Out of Specification Results (OOS), Cell Culture Fluid, and rDNA Yeast Protein Production.

CARTRIDGE REAGENTS

Each Endosafe® - PTS Glucan cartridge contains four channels to which Glucan specific LAL reagent and a chromogenic substrate have been applied. Two of the four channels also contain a glucan spike and serves as the positive product controls.



STORAGE CONDITIONS AND PRECAUTIONS

PTS Glucan cartridges are relatively heat stable and should be stored between 2-25 °C. If refrigerated, allow the cartridges to come to room temperature before opening the pouch and testing. Prolonged exposure to temperatures above 25 °C should be avoided. To minimize contamination of the sample reservoirs, the cartridge should be used immediately once the foil pouch has been opened. Cartridges are for single-test use only.

REAGENTS REQUIRED BUT NOT SUPPLIED

LAL Reagent Grade Water (LRW).

MATERIALS REQUIRED BUT NOT SUPPLIED

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

All materials and glassware must be free of interfering glucans.

Vortex-Type Mixer (if necessary).

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 Glucan assay and other designated assays. 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 depyrogenated materials. Glassware must be depyrogenated by validated conditions, such as a minimum of 30 minutes at 250°C.⁸

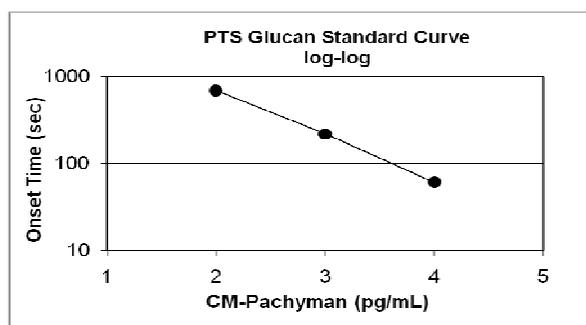
Use aseptic technique at all times.

PERFORMANCE CHARACTERISTICS

Acceptance criteria for a valid assay is an archived curve correlation coefficient of ≥ 0.980 and a positive product control (spike) recovery of 50-200%. A coefficient of variation (CV) of less than 25% is expected.

Internally, the PTS reader measures the reaction time in each channel. An archived standard curve specific for each lot of cartridges is constructed using the log of the reaction time vs. the log of the concentration. The sample and spike values are calculated by interpolation of the standard curve using the reaction times. See Certificate of Analysis for the lot specific archived curve range.

Example of the archived PTS Glucan standard curve.



See the below table for specific activity of the Endosafe®-PTS Glucan.

Sample	Solvent	Relative Activity	Remarks
CM-Pachyman	NaOH	100.0%	β-1,3-;β-1,6-glucan, DS: 0.2
CM-Pachyman	Water	31.0%	β-1,3-;β-1,6-glucan, DS: 0.2
CM-Pachyman	Hot Water	58.0%	β-1,3-;β-1,6-glucan, DS: 0.2
Pachyman	NaOH	11.0%	β-1,3-;β-1,6-glucan, DP: 255
Curdlan	NaOH	14.0%	β-1,3-glucan, DP: 455
CM-Curdlan	NaOH	13.0%	β-1,3-glucan, DS: 0.4-0.8
Lentinan	NaOH	16.0%	β-1,3-;β-1,6-glucan, DP: 5800-6500
Lentinan	Water	0.3%	β-1,3-;β-1,6-glucan, DP: 5800-6500
Laminarin	NaOH	0.7%	β-1,3-;β-1,6-glucan, DP: 20-30
Zyosan A	NaOH	0.8%	β-1,3-;β-1,6-glucan, Particle
Lichenan	NaOH	0.6%	β-1,3-;β-1,4-glucan, DP: 100-200
Glucan from Baker's yeast	NaOH	24.0%	β-1,3-;β-1,6-glucan
Lipopolysaccharide	Water	<0.005%	derived from <i>E. coli</i> O55:B5
Lipoteichoic Acid	Water	<0.0001%	derived from <i>Bacillus sp</i>
Peptidoglycan	Water	<0.0001%	derived from <i>Micrococcus luteus</i>

The Endosafe® - PTS Glucan test is intended for research purposes only. Not for *in-vitro* diagnosis of humans or animals.

TYPES OF ASSAYS

Initial Qualification: Each new lot of cartridges must be qualified upon receipt. The initial qualification testing requires one cartridge with LAL Reagent Water as a sample. The evaluation must demonstrate no detectable beta glucan and acceptable spike recovery (50-200%).

Inhibition / Enhancement: Absence of interference is demonstrated by achieving acceptable spike recovery (50-200%) on a given sample preparation.

Inhibition is usually concentration dependent and can be overcome by dilution with LAL reagent water. The most common sources of inhibition are 1) conditions that interfere with the enzyme activity due to ionic strength and / or pH, 2) those that alter the dispersion of the positive control⁶, and 3) when high quantities of beta glucans are present. If the positive product control fails and the pH related problem is suspected, the pH of the test specimen should be measured. Do not arbitrarily adjust the pH of unbuffered solutions.

ROUTINE TESTS

Routine Tests: A routine PTS Glucan assay is conducted by following the simple prompts on the PTS instrument. See the User's Guide supplied with the Endosafe®- PTS reader for complete operations, procedures, and guidelines. The following represents a typical assay procedure:

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*

(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)

* Enter *Sample Lot #*

* Enter *Sample ID*

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 15 minutes to produce results

TEST RESULTS

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

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 or the Epson TM-U220D Printer (available from Charles River Laboratories) 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

U.S. Patent No: US 7,329,538 B2

Other Patents Pending

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