

VISI-SPOT

A RAPID COLORMETRIC TEST TO AID IN THE PRESUMPTIVE IDENTIFICATION OF ENTEROCOCCI AND GROUP "A" STREPTOCOCCUS FROM CULTURE MEDIA

BACKGROUND & PRINCIPLE

Pyroglutamyl aminopeptidase (PYRase) activity aids in the detection of enterococci (1-5). Hydrolysis of L-pyrrolidonyl-beta-naphthylamide by PYRase is as specific as the 6.5% NaCl tolerance test when identifying enterococci (5). Enterococci can be distinguished enzymatically from other streptococci species by esculin hydrolysis.

The esculin relies on the ability of beta-D-glucosidase (GLCase) to hydrolyze esculin into esculetin and free glucose (9). The fact that GLCase activity occurs in the enterococci is the basis of identification.

The Visi-Spot test utilizes filter paper strips impregnated with substrates specific for PYRase and GLCase. The hydrolysis of these substrates by bacterial enzymes produces visible end products which presumptively identifies enterococci colonies from culture media.

MATERIALS SUPPLIED

Each Visi-Spot kit contains the following reagents for 50 tests:

- Test Cards: Fifty test cards each containing filter paper impregnated with substrates specific for PYRase and GLCase localized within the red and blue circles respectively.
- Buffer Reagent: One dropper bottle containing 28 ml of saline solution with sodium azide.
- Color Reagent: One dropper bottle containing 14 ml of color developer.
- Wooden applicators.

MATERIALS REQUIRED BUT NOT PROVIDED

Culture media

PRECAUTIONS

Visi-Spot is for in vitro diagnostic use only. Test materials and test specimens should be handled by trained individuals only. Avoid skin contact with COLOR REAGENT. Do not use the product past expiration date and discard product when the white filter paper within the red and blue circles on the strips show signs of discoloration. Sterilize all used strips prior to disposal.

Buffer Reagent contains 0.1% sodium azide as a preservative. It may react with copper or lead plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide buildup.

STABILITY & STORAGE

Store the Visi-Spot kits at 2-8°C. The kit must be brought to room temperature before use, and may be left at room temperature (15-30°C) for up to one (1) hour.

SPECIMENS

Fresh primary or secondary cultures grown overnight on non-selective media such as blood agar gives best results. Use Visi-Spot only for the presumptive identification of enterococcal-like colonies. Colonies tested must be gram positive, catalase negative, and morphologically resemble enterococci. Apply several colonies to each test circle from primary or secondary culture plate. In case of insufficient growth, a subculture should be made.

TEST PROCEDURE

Use one card for each specimen to be tested.

1. Pick several suspect colonies 0.5mm or larger in size using wooden applicator supplied in kit. Place tip of wooden applicator on test area at a 45 degree angle and rotate the wooden applicator in place within the red and blue circle. Do not smear the colonies across test area. These colonies should morphologically resemble enterococci, be gram positive and catalase negative.
2. Moisten each test area with 3-4 drops of Buffer Reagent.
3. Incubate the inoculated test card at room temperature (15-30°C) for 5 minutes. Dispense 3-4 drops of Color Reagent onto reaction area within the red circle only. The reaction in the red circle forms a red color immediately around the colonies confirming PYRase activity. If no color develops, the PYR test is negative.
4. Wait an additional 15 minutes before reading the color in the blue circle. Development of blue color on or around the colonies within the blue circle confirms GLCase activity.
5. To presumptively identify test organisms, color reactions in both sites (test areas) must be read.

INTERPRETATION OF TEST RESULTS

The presence of GLCase activity along with PYRase classifies organisms as Enterococci (10).

Interpretation Chart:

	RED CIRCLE	BLUE CIRCLE
Enterococci	Red	Blue
Group A Strep	Red	No Color
Non-Enterococci	No Color	Blue/No Color

QUALITY CONTROL PROCEDURE

Daily quality control should be performed in accordance with good laboratory procedures using organisms that will produce known positive and negative reactions. The following American Type Culture Collection strains are recommended:

E. faecalis (Group D)

ATCC # 29212 (PYRase + (Red) & GLCase + (Blue))

S. agalactiae (Group B)

ATCC # 13813 (PYRase – GLCase -)

LIMITATIONS

Visi-Spot is intended only for the presumptive identification of gram positive, catalase negative cocci which morphologically resemble enterococci when isolated from a primary and/or secondary culture. Both reactions (PYRase and GLCase) must be positive.

The occurrence of GLCase in other gram positive, catalase negative organisms such as *Aerococcus*, *Lactococcus*, *Pediococcus*, *Leuconostocs*, *Lactobacillus* (11) and *Listeria* may cause the hydrolysis of the substrate within the blue circle to yield a positive reaction (blue color).

Some less commonly encountered isolates of lactococci and aerococci may be positive in both PYRase (red) and GLCase (blue) activities, members of lactococci and aerococci should not react with Group D antisera. The confirmation of enterococci is best achieved by biochemical and serological procedures.

SPECIFIC PERFORMANCE CHARACTERISTICS

Over 96 isolates of enterococci and 89 isolates of non-enterococci were tested with Visi-Spot. Reactions were confirmed with commercially available biochemical and serological assays. Visi-Spot results demonstrated 100% agreement with other commercial tests.

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