

INTENDED USE

ID- AE™ kit is intended for use as a rapid colorimetric test for the presumptive identification of group A streptococcal and enterococcal bacteria from clinical isolates.

SUMMARY AND EXPLANATION

PYR is a substrate which is hydrolyzed by 100% of the enterococci and group A streptococci, but not by any other streptococcal strains.¹⁻⁵ *S. pyogenes* are isolated from throat cultures, blood, skin, wounds, vagina, and rectum. Typical colonies of *S. pyogenes* on blood agar are approximately 0.5 mm in diameter surrounded by a zone of beta-hemolysis. Colonies appear translucent, domed, and smooth or semi-mat surface. Enterococci are normal inhabitants of the human gastrointestinal tract and may spread from this site to cause urinary tract infections, and wound infections. Enterococci are typically larger in size than *S. pyogenes* on blood agar medium.

PRINCIPLES

The ID- AE™ test utilizes filter paper strips impregnated with the substrate specific for PYRase. The usefulness of pyroglutamyl aminopeptidase (PYRase) activity as an aid in the detection of group A streptococci and enterococci is well documented.¹⁻⁵ This test is based on the ability of group A streptococci and enterococci to hydrolyze the chromogenic substance PYR (L-pyrrolidonyl-beta-naphthylamide). The hydrolysis of PYR is detectable by the presence of the color red when a Color Developer reagent is added.

REAGENTS

Each ID- AE™ kit contains the following reagents sufficient for 50 tests:

1-15 ml bottle Buffer Reagent

1-15 ml bottle Color Developer A

50 Test Cards

PRECAUTIONS: This test is intended for use by those trained in appropriate laboratory and bacteriological procedures. This test is for IN VITRO DIAGNOSTIC USE only. Precautions should be taken against the dangers of microbiological hazards. Specimens, containers, media, and test cards should be sterilized after use. Reagents should not come into contact with skin, eyes, or clothing. Do not inhale or ingest reagents. In case of an accident, seek medical attention immediately. The sodium azide buffer reagent may react with copper and lead in plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent buildup of azides.

STORAGE INSTRUCTIONS: This kit must be stored at 2-8C to prevent breakdown of the substrates and reagents. The kit must be at room temperature before use, and may be left at room temperature for up to one hour.

EVIDENCE OF DETERIORATION: Substrates and Reagents provided with this kit should not be used if the expiration date has passed. Discard product if the white filter paper within the test circle shows signs of discoloration. If any deficiencies are noted with this product notify the manufacturer.

SPECIMEN COLLECTION

Clinical specimens should be protected from excessive heat and cold and should be delivered to the laboratory without delay. The specimen should be collected prior to the initiation of therapy. If the specimen is collected after the initiation of therapy, the microbiologist should be notified on the data form. Additional information on collection of clinical specimens may be found in standard reference texts. Fresh cultures grown overnight on nonselective media give the best results. Use Gram positive and catalase negative colonies which morphologically resemble group A streptococci and/or enterococci. Inoculate the test circle with approximately eight, 0.5 mm or larger colonies.

PROCEDURES

OTHER MATERIAL REQUIRED BUT NOT SUPPLIED: The standard clinical microbiological equipment such as loop, burner, and incubator are needed for procedures involving the use of this product. Other materials required include the following: Gram Stain Reagents, Catalase Reagent, Microscope Slides, and Culture media.

Use one card for each specimen tested. Before performing the test make sure the colonies are gram positive and catalase negative.

1. Apply 3-4 drops of the buffer reagent to the circle.
2. Using a swab, wooden applicator or an inoculating loop, apply approximately four to five suspect colonies 0.5 mm or larger to the center of the filter paper within the circle. The smear should be visible to the naked eye.
3. Incubate the inoculated card at room temperature for 2 minutes.
4. Apply 2 drops of Color Developer to the filter paper with the test circle. The appearance of a red color

indicates positive PYRase activity.

INTERPRETATION OF RESULTS

The presence of PYRase activity in Gram positive and catalase negative colonies presumptively identifies an organism as either group A streptococci or enterococci. The confirmation of group A streptococci and enterococci must be performed using additional biochemical and/or serological procedures.

Interpretation Chart:

Group A Strep	Red (positive)
Enterococcus	Red (positive)
Non-Enterococcus	No Color (negative)

USER QUALITY CONTROL

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

	Red Circle
<i>Streptococcus pyogenes</i> (Group A) ATCC 19615	Red (PYRase +)
<i>Enterococcus faecalis</i> (Group D) ATCC 29212	Red (PYRase +)
<i>Streptococcus agalactiae</i> (Group B) ATCC 13813	No Color/Yellow (PYRase -)

LIMITATIONS OF PROCEDURE

The ID- AE™ test is intended only for the presumptive identification of Gram positive, catalase negative cocci which are morphologically similar to streptococci isolated from primary and secondary plated media. Some species of staphylococci may produce a positive PYR test. Streptococci may be differentiated from staphylococci by the catalase test and the benzidine test.¹ Streptococci are catalase-negative and, lacking cytochromes, are benzidine negative. Staphylococci are catalase-positive and yield a positive benzidine test.

It should be noted that recently described gram-positive cocci with negative or weak catalase reactions (*Lactococcus*, *Gemella*, *Helcococcus*, *Globicatella*, and *Stomatococcus*) may produce results similar to group A streptococci.⁶

Klebsiella (gram negative rod) may also produce a positive PYRase reaction, a gram-stained smear should be examined to differentiate this organism. Further biochemical and/or serological procedures are required to identify the colonies.

PERFORMANCE CHARACTERISTICS

The ID- AE™ test was tested on 180 clinical isolates at two different laboratories. The isolates consisted of 50 group A streptococci, 50 Enterococci, and 80 non group A streptococci. The ID A.E. tested correctly 178 out of the 180 isolates to show a 98.8% sensitivity and 100% specificity.

REFERENCES

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3. Facklam, R.R. 1972. Recognition of Group D Streptococcal Species of human origin by biochemical and physiological tests. *Appl. Microbiol.* 23:11311-1139.
4. Facklam, R.R., L.G. Thacker, B. Fox, and L. Eriquez. 1982. Presumptive identification of Streptococci with a new test system. *J. Clin. Microbiol.* 15:987-990.
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6. Murray, B.E. 1990. The life and times of the Enterococcus. *Clin. Microbiol. Rev.* 3:46-65.