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IDENTICULT-AE (PYR)
CAT. NO. KF4012 - 50/KIT TEST CARDS
KF4080 - 25 TEST DISCS

PURPOSE: The IDENTICULT-AE test is intended for use in the rapid identification of group A streptococci and enterococci from clinical isolates.

PRINCIPLE: IDENTICULT-AE utilizes a chromogenic substrate, L-pyrroglutamyl-peptide hydrolase, which produces a red color upon the addition of a specific reagent. PYR is a substrate which is hydrolyzed by 100% of the enterococci and group A streptococci, but not by any other streptococcal strains.[1•2•3,4.5]The PYR enzyme is detected by the hydrolysis of a color developer on a paper disk inoculated with colonies of enterococci or group A streptococci.
S. pyogenes is frequently isolated from throat cultures but may also be recovered from blood, skin, wounds, cerebrospinal fluid, vagina and rectum.[5] Typical colonies of *S. pyogenes* on blood agar are approximately 0.5 mm in diameter surrounded by a well-defined zone of beta-hemolysis. The beta-hemolytic zone is usually 2-4 times the diameter of the colony, but this may vary with the incubation temperature and the medium used. The colonies may be transparent or translucent, domed and with a smooth or semi-mat surface. Enterococci are normal inhabitants of the human gastrointestinal tract and they may spread from this site to cause bacteremia, urinary tract infections, endocarditis, and wound infection.[5] The medically important enterococci are *E. faecalis, E. faecium,* and *E. durans.* Typical enterococcal colonies are somewhat larger than *S. pyogenes* on the surface of blood agar (0.5 to 1.0 mm) and are less opaque. Although the majority of strains exhibit no hemolysis (gamma) on sheep blood agar, some strains may be alpha or beta-hemolytic.[3]

CONTENTS: **KF4012 -**Reagent #1, Buffer, 15 ml ,
Reagent #2, Color Developer, 15 ml ,
IDENTICULT-AE CARDS 50 (5 x10)
KF4080 - Reagent #2, Color Developer, 3 ml ,
IDENTICULT-AE DISCS 25

PRECAUTIONS: Precautions should be taken against the dangers of microbiological hazards. Do not touch the strips prior to or after the addition of organisms or reagent. Use of this test should be limited to individuals with appropriate bacteriological training. Sterilize prior to disposal.

STORAGE: Upon receipt store IDENTICULT-AE cards at 2-8°C. Opened bags should be resealed to reduce the possibility of moisture accumulation. Allow refrigerated product to warm to room temperature before use. Do not use this product if the paper disk is not white or if the expiration date has passed.

LIMITATIONS: It is important that testing be performed to determine that the organism is streptococcus. Only *S. pyogenes* (group A) and enterococci are positive. Other streptococci are negative; however, judgement must be made by the microbiologist to separate *S. pyogenes* from beta-hemolytic enterococci or additional testing should be performed. (It should be noted that staphylococci and most *Corynebacterium haemolyticum* strains are PYR positive as are some Enterobacteriaceae and other gram-negative bacilli.)
It should be emphasized that this product is only part of the overall schema for identification and provides a high probability for presumptive separation of S. *pyogenes* (group A) and enterococci from other streptococci. Further biochemical characterization and serological grouping may be necessary for specific identification.

PROCEDURE: Specimen Collection: Information on specimen collection and procedures for isolation and purification can be found in standard references.
1. Select colonies that morphologically resemble group A streptococci (*S. Pyogenes*) or enterococci.
2. (KF4012) Apply 3-4 drops of Reagent #1 (Buffer) (provided) to the circle on the IDENTICULT-AE test card.
(KF4080) Moisten the disc lightly with demineralized water (not provided)
3. Touch the tops of 3-5 suspect colonies with the tip of a wooden applicator, swab, loop or needle and smear the growth onto the center of the circle. Plated growth need not to be a pure culture provided morphologically similar, isolated colonies are selected. A single colony may be tested if growth is not well isolated or is sparse; however, the color reaction is stronger when a large inoculum is used.
4. Incubate the inoculated card/disc at room temp. for 2 minutes.
5. Apply 2 drops of Reagent #2 to the circle/disc.

INTERPRETATION:
Positive: Intense red color develops immediately around the colonies indicating the presence of hydrolyzed PYR.
Negative: No color or weak pink color develops slowly PYR-positive streptococci can be identified as either *S. pyogenes* or enterococci. Alpha or gamma hemolytic PYR-positive streptococci almost always are enterococci because *S. pyogenes* is almost always beta-hemolytic.[2] Beta-hemolytic enterococci and *S. pyogenes* may be differentiated by the 6.5% NaCl growth test, by the bile-esculin test or by the bacitracin test.[2] Other streptococci are PYR negative.

MATERIALS REQUIRED BUT NOT PROVIDED: Standard microbiological supplies and equipment such as loops, needles, and wooden sticks are not provided.

QUALITY CONTROL:

Microorganisms Used	(ATCC #):	Expected Results:
Streptococcus pyogenes	(19615)	+
Enterococcus faecalis	(29212)	+
Streptococcus agalactiae	(12386)	-

USER QUALITY CONTROL: Prior to using IDENTICULT-AE in test procedures, test each lot number with positive and negative controls using ATCC strains of *S. pyogenes* or enterococci and a streptococcus strain other than *S. pyogenes.*

BIBLIOGRAPHY:
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2. Ellner, P. D., D. A. Williams, M. E. Hosmer, and M. Cohenford, "Preliminary Evaluation of a Rapid Colorimetric Method forthe Presumptive Identification of a Group A Streptococci and Enterococci," J. Clin. Microbiol, 22:880-881, 1985.
3. Facklam, R. R., "Recognition of Group D Streptococcal Species of Human Origin by Biochemical and Physiological Tests," App. Microbiol, 23:1131-1139,1972.
4. Facklam, R. R., L. G. Thacker, B. Fox, and L. Eriquez, "Presumptive Identification of Streptococci with a New Test System," J. Clin. Microbiol, 15:987-990, 1982.
5. Facklam, R. R. and R. B. Carey, Streptococci and Aerococci In: Lennette, I. H., A. Balows, W. J.
Hausler, Jr., and H. J. Shadomy, Manual of Clinical Microbiology, 4th ed., Amer. Soc. for Microbiol., Washington, D. C., 1985.
*For more detailed information, consult appropriate references.