



Enterococcus/Group A Screen

INTENDED USE

Remel Enterococcus/Group A Screen is recommended for use in qualitative procedures for presumptive identification of Group A streptococci and enterococci.

SUMMARY AND EXPLANATION

Rochaix first demonstrated the value of esculin hydrolysis in the identification of enterococci.¹ In 1970, the L-pyroglyutamic acid β -naphthylamide (PYR) hydrolysis test was described by Mulczyk and Szweczek.² This combination of tests has proven to be useful in the identification of *Streptococcus pyogenes*, *Enterococcus* species, and other gram-positive cocci.³

PRINCIPLE

L-pyroglyutamic acid β -naphthylamide (PYR) serves as the substrate for the detection of pyrrolidonyl peptidase. Following substrate hydrolysis by the peptidase enzyme, the resulting β -naphthylamine produces a red color upon the addition of p-dimethyl-aminocinnamaldehyde (Cinnamaldehyde Reagent). The esculin compound is hydrolyzed enzymatically at the β -glucose linkage to yield two products, esculetin and glucose. Esculetin reacts with the ferric ammonium citrate in the medium to form a phenolic iron complex which is brown-black in color.

REAGENTS (CLASSICAL FORMULA)*

Enterococcus/Group A Screen Tubes:

Heart Infusion Agar	30.0 g
Esculin	0.5 g
Ferric Ammonium Citrate	0.25 g
Pyroglyutamyl- β -naphthylamide.....	0.15 g

Cinnamaldehyde Reagent:

Reactive ingredient: p-dimethylaminocinnamaldehyde

*Adjusted as required to meet performance standards.

PRECAUTIONS

CAUTION! May cause irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact. Refer to Material Safety Data Sheet for additional information.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C in the dark until used. A precipitate formed at storage temperatures or color variation from yellow-orange to red-orange in the Cinnamaldehyde Reagent is common and does not affect performance. Allow product to equilibrate to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of contamination or dehydration, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, TRANSPORT

Specimens should be collected and handled following recommended guidelines.⁴

MATERIALS SUPPLIED

(1) Enterococcus/Group A Screen tubes and (2) Cinnamaldehyde Reagent.

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Wooden applicator sticks.

PROCEDURE

Enterococcus/Group A Screen is designed for testing catalase-negative, gram-positive cocci, morphologically typical of enterococci and streptococci. Test isolates should be 18-24 hours old and in pure culture or have well isolated colonies.

1. Obtain a visible, heavy inoculum of the test isolate using a wooden applicator stick or sterile loop.
2. Stab the Enterococcus/Group A Screen tube near one side next to the glass.
3. Incubate the tube aerobically at 35-37°C for 30 minutes to 4 hours.
4. Observe for a color change in the medium to dark brown indicating a positive esculin reaction.
5. Add two (2) drops of Cinnamaldehyde Reagent to tube after reading the esculin hydrolysis reaction.
6. Observe for up to two (2) minutes for a color change to red indicating a positive PYR test.

INTERPRETATION

Esculin Test:

Positive Test - Dark brown color formation
Negative Test - No color change

PYR Test:

Positive Test - Red color development
Negative Test - No color development, light orange, or light pink orange

EXPECTED VALUES

ORGANISM	ESC	PYR
<i>Enterococcus</i>	+	+
<i>Streptococcus</i> (Group A)	-	+
<i>Streptococcus</i> (Group B,C,F,G)	-	-
<i>Streptococcus</i> (Group D)	+	-

QUALITY CONTROL

All lot numbers of Enterococcus/Group A Screen have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS	
		ESC	PYR
<i>Enterococcus faecalis</i> ATCC® 29212	Ambient, up to 4 h @ 33-37°C	+	+
<i>Streptococcus agalactiae</i> ATCC® 12386	Ambient, up to 4 h @ 33-37°C	-	-
<i>Streptococcus gallolyticus</i> ATCC® 9809	Ambient, up to 4 h @ 33-37°C	+	-
<i>Streptococcus pyogenes</i> ATCC® 19615	Ambient, up to 4 h @ 33-37°C	-	+

LIMITATIONS

1. The performance of this test is dependent on a proper inoculum.
2. The test for esculin hydrolysis does not incorporate bile tolerance of the organism and thus does not directly correlate to results obtained with bile esculin agar or broths.
3. This test is part of the overall scheme for identification of *Enterococcus* and group A *Streptococcus*. Definitive results should be obtained by serological methods.⁵




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PACKAGING

Enterococcus/Group A Screen:
REF R21134850 Tests/Kit

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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