STREP ID QUAD

INTENDED USE
Remel Strep ID Quad is comprised of solid media recommended for use in qualitative procedures for the differentiation and presumptive identification of streptococci and enterococci.

SUMMARY AND EXPLANATION
Quadrant I contains Tryptic Soy Agar supplemented with the antibiotic bacitracin. In 1953, Maxted demonstrated the use of bacitracin to differentiate group A β-hemolytic streptococci (sensitive) from non-group A streptococci (resistant).[1] Quadrant II contains Tryptic Soy Agar supplemented with 5% sheep blood. Streptococcal isolates are grouped according to their hemolytic pattern on Sheep Blood Agar.[2] Sheep blood also provides the erythrocytes needed for performing the CAMP test which presumptively identifies group B streptococci.[3] Quadrant III contains Bile Esculin Agar which provides a reliable means of identifying group D streptococci and enterococci. Rochaix first demonstrated the value of esculin hydrolysis in the identification of enterococci.[4] Meyer and Schonfeld found that 61 of 62 strains of enterococci hydrolyzed esculin in a bile-containing medium.[5] Facklam and Moody tested 700 strains of streptococci representing all known serological groups on a bile esculin medium developed by Swan and determined that 100% of the group D streptococci were bile esculin positive.[6,7] Quadrant IV contains Brain Heart Infusion Base supplemented with 6.5% sodium chloride to differentiate enterococci which grows in the presence of salt, from group D streptococci which does not.

PRINCIPLE
The base medium in Quadrants I and II contains casein and soy peptones which supply organic nitrogen, amino acids, and peptides necessary for bacterial growth. Sodium chloride supplies essential electrolytes and maintains osmotic equilibrium. Quadrant I contains bacitracin which serves to presumptively identify group A streptococci (sensitive, no growth). Quadrant II with 5% sheep blood is used to detect the CAMP factor which differentiates group B streptococci (positive) from other β-hemolytic streptococci. Group B streptococci produce a protein-like compound called CAMP factor that acts synergistically with the beta lysin produced by some strains of *Staphylococcus aureus* to produce an area of complete lysis that is arrowhead- or crescent-shaped. Quadrant III contains Bile Esculin Agar which aids in the identification of group D streptococci and enterococci. Bile is incorporated in the medium as a selective agent. Group D streptococci and enterococci hydrolyze esculin, resulting in the production of esculetin and dextrose. Esculetin reacts with the ferric ions in the medium to form a brown-black complex which surrounds the colonies. Quadrant IV contains Brain Heart Infusion Base with 6.5% sodium chloride. Enterococci are salt-tolerant and will usually grow heavily on this medium while non-enterococcal species will not. About 80% of group B streptococci are salt-tolerant and will also grow in Quadrant IV but are differentiated based on the production of CAMP factor in Quadrant II. A bile-esculin positive species which is also salt-tolerant may be presumptively identified as enterococci.

REAGENTS (CLASSICAL FORMULAE)*
Base Medium (Quadrant I and II):

Casein Peptone	15.0 g
Sodium Chloride	5.0 g
Soy Peptone	5.0 g
Agar	15.0 g
Demineralized Water	1000.0 ml

pH 7.3 ± 0.2 @ 25°C

The following ingredients are added per liter of medium:

Quadrant I:

Bacitracin	50 I.U.

Quadrant II:

Sheep Blood	5 %

Bile Esculin Agar (Quadrant III):

Oxgall (Bile)	40.0 g
Gelatin Peptone	5.0 g
Beef Extract	3.0 g
Esculin	1.0 g
Ferric Citrate	0.5 g
Agar	15.0 g
Demineralized Water	1000.0 ml

pH 6.6 ± 0.2 @ 25°C

BHI with 6.5% NaCl (Quadrant IV):

Sodium Chloride	65.0 g
Casein Peptone	14.5 g
Brain Heart Infusion Solids	6.0 g
Meat Peptone	6.0 g
Dextrose	3.0 g
Disodium Phosphate	2.5 g
Agar	15.0 g
Demineralized Water	1000.0 ml

pH 7.4 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Implement appropriate procedures to verify the test isolate is catalase-negative gram-positive cocci in chains, morphologically characteristic of streptococci.
2. Select 3-4 well-isolated colonies from a pure, 18-24 hour culture. Streak the colonies across the agar surface of Quadrants I, III, and IV.
3. In Quadrant II perform the test for CAMP factor according to one of the following methods:
   a. Inoculate the agar with a streak of β-lysin-producing *S. aureus* (e.g., *S. aureus* ATCC® 33862). Follow with a streak of the test isolate perpendicular to the *S. aureus* leaving a 1-2 mm space in between.
   b. Place a Beta Lysin Disk (REF R21120) on the uninoculated medium. Follow with a streak of the test isolate perpendicular to the disk leaving a 1-2 mm space in between.
4. Incubate the plate aerobically at 33-37°C for 18-24 hours.
5. Observe Quadrant II for the formation of an arrowhead or crescent-shaped area of complete lysis at the juncture of the test isolate and the *S. aureus* or the Beta Lysin Disk.
6. Observe Quadrant III for a brown to black color development in the area surrounding growth.
7. Observe Quadrant IV for growth.

INTERPRETATION OF THE TEST

Quadrant I (Bacitracin Test):
Positive Test - No growth (sensitive)
Negative Test - Growth (resistant)

Quadrant II (CAMP Test):
Positive Test - An arrowhead or crescent-shaped zone of hemolysis at the juncture of the test isolate and the *S. aureus* streak or the Beta Lysin Disk
Negative Test - No enhanced hemolysis

Quadrant III (Esculin Hydrolysis):
Positive Test - Brown-black pigmentation of the medium
Negative Test - No blackening of the medium

Quadrant IV (Salt Tolerance):
Positive Test - Growth (salt-tolerant)
Negative Test - No growth (salt-intolerant)

QUALITY CONTROL

All lot numbers of Strep ID Quad 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.

		RESULTS			
CONTROL	**INCUBATION**	**I (BAC)**	**II (CAMP)**	**III (ESC)**	**IV (NACL)**
Enterococcus faecalis ATCC® 29212	Aerobic, 24 h @ 33-37°C	R	-	+	+
Streptococcus agalactiae ATCC® 12386	Aerobic, 24 h @ 33-37°C	R	+	-	V
Streptococcus bovis ATCC® 9809	Aerobic, 24 h @ 33-37°C	R	-	+	-
Streptococcus pyogenes ATCC® 19615	Aerobic, 24 h @ 33-37°C	S	-	-	-

R = resistant (growth); S = sensitive (no growth); V = variable

LIMITATIONS

1. Do not incubate Strep ID Quad in an anaerobic atmosphere or in CO_2 because some group A streptococci may produce a false-positive CAMP test.
2. This test is only part of the overall scheme for identification of *Streptococcus* spp. Further biochemical and/or serological testing may be required for definitive identification. Consult appropriate references for further instructions.[2,8]
3. Test only catalase-negative gram-positive cocci which are morphologically characteristic of streptococci on Gram stain. The hemolytic pattern on sheep blood agar must be considered in the identification of streptococci.[2,8]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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