

# COLUMBIA CNA AGAR w/ 5% SHEEP BLOOD w/ VANCOMYCIN (6 µg/ml)

## INTENDED USE

Remel Columbia CNA Agar w/ 5% Sheep Blood and 6 µg/ml Vancomycin is a solid medium recommended for use in qualitative procedures as a primary, selective isolation medium for gram-positive cocci with reduced susceptibility to vancomycin.

## SUMMARY AND EXPLANATION

Vancomycin-resistant enterococci, first detected in 1986, are being recovered from clinical specimens with increasing frequency.<sup>1</sup> Various phenotypes responsible for acquired resistance in enterococci have been described with high- to low-level vancomycin resistance and cross-resistance to teicoplanin.<sup>2</sup> The *vanA* gene, in particular, is frequently plasmid-borne and has been transferred *in vitro* from enterococci to a variety of gram-positive microorganisms, including *Staphylococcus aureus*.<sup>3</sup> Prevention and control of the spread of vancomycin-resistant enterococci by surveillance screening may facilitate earlier identification of colonized patients leading to more efficient containment. The frequency and intensity of surveillance should be based on the size of the population at risk and the specific hospital unit(s) involved. The cost of screening can be reduced by inoculating specimens onto selective media containing vancomycin and restricting screening to those patients who have been in the hospital long enough to be at risk for colonization or admitted from a facility where vancomycin-resistant enterococci have been identified.<sup>4,6</sup>

Ellner introduced Columbia CNA Agar with 5% sheep blood for use as a selective medium for isolating streptococci and staphylococci from specimens containing mixed flora.<sup>7</sup> In 1995, Auld evaluated Columbia CNA with 5% sheep blood and 6 µg/ml Vancomycin as a method to screen stool cultures for the presence of vancomycin-resistant enterococci and found it to be a sensitive screening method.<sup>8</sup>

## PRINCIPLE

Peptones in this medium provide essential growth factors, such as nitrogen, carbon, vitamins, and trace elements necessary for bacterial growth. Beef extract and cornstarch serve as energy sources and yeast extract supplies B-complex vitamins. Sheep blood provides the X factor (hemin) necessary for the growth of many bacteria and enables the demonstration of hemolytic activity. Colistin and nalidixic acid inhibit most gram-negative bacilli. Vancomycin (6 µg/ml) is included to select for gram-positive organisms with reduced susceptibility to vancomycin.

## REAGENTS (CLASSICAL FORMULA)\*

Casein Peptone.....	12.0 g	Colistin.....	10.0 mg
Meat Peptone.....	5.0 g	Nalidixic Acid.....	10.0 mg
Sodium Chloride.....	5.0 g	Vancomycin.....	6.0 mg
Beef Extract.....	3.0 g	Sheep Blood.....	5 %
Yeast Extract.....	3.0 g	Agar.....	13.5 g
Cornstarch.....	1.0 g	Demineralized Water.....	1000.0 ml

pH 7.3 ± 0.2 @ 25°C

\*Adjusted as required to meet performance standards.

## PROCEDURE

1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. If material is being cultured directly from a swab, roll the swab over a small area of the agar and streak for isolation.
3. Incubate plates aerobically or in 3-10% CO<sub>2</sub> at 33-37°C for 24-48 hours.
4. Examine plate for typical colony morphology and hemolytic reaction.

## QUALITY CONTROL

All lot numbers of Columbia CNA Agar w/ 5% Sheep Blood and 6 µg/ml Vancomycin 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

*Enterococcus faecalis* ATCC® 51299  
*Enterococcus faecalis* ATCC® 29212

## INCUBATION

Aerobic, 24-48 h @ 33-37°C  
Aerobic, 24-48 h @ 33-37°C

## RESULTS

Growth  
No growth

## LIMITATIONS

1. Columbia CNA Agar w/ 5% Sheep Blood and 6 µg/ml Vancomycin is recommended for selective isolation of gram-positive cocci with reduced susceptibility to vancomycin and is not intended for use as a method of antimicrobial susceptibility testing.
2. Gram-positive organisms other than enterococci with reduced susceptibility to vancomycin (e.g., *Pediococcus*, *Leuconostoc*, and *Lactobacillus*) may grow on this media. Further biochemical and serological testing is required for definitive identification.<sup>3</sup>
3. Confirmation of vancomycin-resistance by an approved method is recommended, as some organisms on initial isolation may overcome the inhibitory effects of this medium.<sup>9</sup>
4. The absence of suspect colonies does not rule out the presence of gram-positive organisms with reduced susceptibility to vancomycin.

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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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