COLUMBIA HORSE BLOOD BILAYER

INTENDED USE
Remel Columbia Horse Blood Bilayer is a solid medium recommended for use in qualitative procedures for the isolation and identification of *Listeria monocytogenes.*

SUMMARY AND EXPLANATION
Columbia Agar Base was first described by Ellner, Stoessel, Drakeford, and Vasi in 1966.[1] It was found to be capable of growing fastidious and nonfastidious organisms. Lee developed Horse Blood Overlay Agar for increased visualization of hemolytic *Listeria* colonies.[2,3] *L. monocytogenes* is hemolytic on Columbia Horse Blood Bilayer, forming translucent colonies with a narrow zone of hemolysis. This medium is recommended by the United States Department of Agriculture (USDA) and the American Public Health Association (APHA) for the isolation of *Listeria monocytogenes* from foods.[4,5]

PRINCIPLE
Peptones and beef extract supply nitrogen, amino acids, and peptides necessary for the growth of *Listeria*. Yeast extract and corn starch serve as energy sources. Sodium chloride is a source of essential electrolytes and maintains osmotic equilibrium. Horse blood is added to demonstrate hemolytic activity in certain microorganisms and provides the X factor (heme).

REAGENTS (CLASSICAL FORMULA)*
Bottom Layer:

Casein Peptone	12.0 g	Yeast Extract	3.0 g
Meat Peptone	5.0 g	Corn Starch	1.0 g
Sodium Chloride	5.0 g	Agar	13.5 g
Beef Extract	3.0 g	Demineralized Water	1000.0 ml

Top Layer:

Casein Peptone	12.0 g	Yeast Extract	3.0 g
Meat Peptone	5.0 g	Corn Starch	1.0 g
Sodium Chloride	5.0 g	Horse Blood	5 %
Beef Extract	3.0 g	Agar	13.5 g
		Demineralized Water	1000.0 ml

pH 7.3 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS
1. This product is For Laboratory Use only. It is not intended for use in the diagnosis of disease or other conditions.

PROCEDURE
Methods for detection of *L. monocytogenes* vary with the material under examination. Choose an appropriate method based on the type of sample to be tested. Follow established laboratory procedures for sample preparation, media inoculation, and incubation. Consult appropriate references for further instructions.[3-5]

QUALITY CONTROL
Each lot number of Columbia Horse Blood Bilayer has been manufactured, packaged, and processed in accordance with current Good Manufacturing Practice regulations. All lot numbers 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.

CONTROL	INCUBATION	RESULTS
Listeria monocytogenes ATCC® 7646	Aerobic, up to 48 h @ 33-37°C	Growth w/ beta hemolysis

LIMITATIONS
1. *Listeria* spp. other than *L. monocytogenes* may grow on Columbia Horse Blood Bilayer agar.[5]
2. This test is only part of the overall scheme for identification of *L. monocytogenes.* Additional testing may be required for definitive identification of *L. monocytogenes*. Consult appropriate references for further instructions.[3-5]

BIBLIOGRAPHY

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4. U.S. Department of Agriculture Food Safety and Inspection Service. 1998. Microbiology Laboratory Guidebook. 3rd ed. Appendix: Media and Reagents. (updated 01/28/08). Retrieved October 21, 2009 from http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/.
5. Downes, F.P. and K. Ito. 2001. Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA, Washington, D.C.

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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