

HiCrome™ *Listeria* Agar Base / Modified

Recommended as a selective and differential agar medium recommended for rapid and direct identification of *Listeria* species.

M1417F/
M1417

| Composition ** | M1417 | M1417F |
|---------------------|-------------|-------------|
| Ingredients | Grams/Litre | Grams/Litre |
| Peptone special | 23.00 | - |
| Peptone | - | 30.00 |
| Sodium chloride | 5.00 | - |
| Yeast extract | 1.00 | 1.00 |
| HM extract# | 5.00 | 5.00 |
| Lithium chloride | 5.00 | 9.00 |
| Rhamnose | 10.00 | - |
| D-xylose | - | 10.00 |
| Phenol red | 0.12 | 0.12 |
| Chromogenic mixture | 5.13 | 5.13 |
| Agar | 13.00 | 13.00 |

Final pH (at 25°C) 7.3 ± 0.2 7.3 ± 0.1

** Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract

Directions

Suspend 33.62 grams of M1417 or 36.63 grams of M1417F in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Add rehydrated contents of 1 vial of HiCrome™ *Listeria* Selective Supplement (FD181) aseptically. Mix well and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ *Listeria* Agar Base Modified medium is a modification of a medium first developed by Notermans et al. (3) and Mengaud et al. (2) for the detection of *Listeria* species from food stuffs. HiCrome™ *Listeria* Agar Base Modified allows growth of *Listeria* species and gives a presumptive identification of *L. monocytogene* within 24-48 hours after pre-enrichment. HiCrome™ *Listeria* Agar Base Modified (M1417) is based on rhamnose fermentation while HiCrome™ *Listeria* Agar Base (M1417F) is based on xylose fermentation. HiCrome™ *Listeria* Agar Base (M1417F) is in accordance with FDA BAM (1) where D-Xylose is the fermentable carbohydrate. This medium is based on the specific chromogenic detection of β -glucosidase activity and also sugar fermentation. *Listeria* species hydrolyse the purified chromogenic substrate in the medium forming bluish green coloured colonies. Since β -glucosidase activity is specific for *Listeria* species, other organisms cannot utilize the chromogenic substrate and therefore form colourless colonies. Differentiation between *Listeria* species is based on the property of rhamnose or xylose fermentation. The colonies of *L. monocytogenes* and *L. innocua* appear bluish green with a yellow halo (rhamnose

positive) while the colonies of *L. ivanovii* appear blue without a yellow halo (rhamnose negative) in M1417. In case of M1417F, the colonies of *L. ivanovii* appear bluish green with yellow halo (xylose positive) while *L. monocytogenes* and *L. innocua* appear bluish green without a yellow halo (Xylose negative).

Peptone special, peptone, yeast extract and HM extract provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients. Rhamnose or xylose are the fermentable carbohydrates with phenol red as an indicator. Sodium chloride maintains the osmotic equilibrium. Lithium chloride and the added HiCrome™ *Listeria* Selective Supplement (FD181) inhibit growth of most gram positive bacteria, gram negative bacteria, yeasts and moulds.

Type of specimen

Food samples

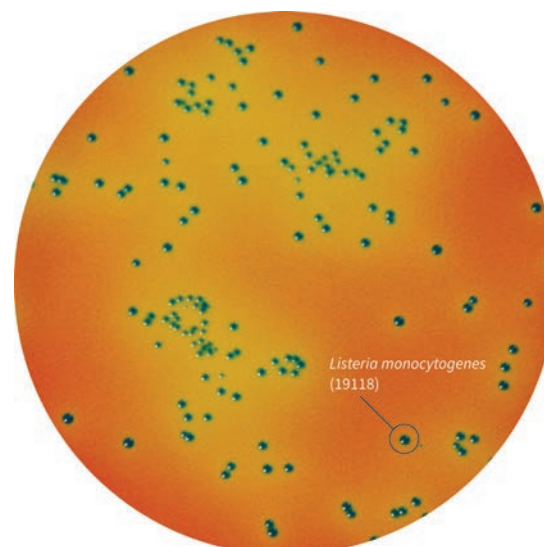
Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets



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Limitations

1. Some species may show poor growth due to nutritional variations.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the recommended temperature.

Quality Control

- Appearance of powder** : Light yellow to pink coloured, homogeneous, free flowing powder.
- Gelling** : Firm, comparable with 1.3% Agar gel.
- Colour and Clarity of prepared medium** : Red coloured, clear to slightly opalescent gel forms in Petri plates.
- Reaction** : Reaction of 6.72% w/v aqueous solution of M1417 at 25°C. pH : 7.3 ± 0.2.
 Reaction of 7.32% w/v aqueous solution of M1417F at 25°C. pH : 7.3 ± 0.1.
- Cultural Response** : Cultural characteristics observed with added HiCrome™ *Listeria* Selective Supplement (FD181) after incubation at 35-37°C for 24-48 hours.

| Organisms (ATCC) | Inoculum (CFU) | Growth | Recovery | Colour of colony | (M1417) Rhamnose fermentation | (M1417F) Xylose fermentation |
|--|------------------|-----------|----------|------------------|-------------------------------|------------------------------|
| <i>Listeria monocytogenes</i> (19118) | 50-100 | luxuriant | ≥50% | bluish-green | + | - |
| <i>Listeria ivanovii</i> (19119) (00018*) | 50-100 | luxuriant | ≥50% | bluish-green | - | + |
| <i>Listeria innocua</i> (33090) (00017*) | 50-100 | luxuriant | ≥50% | bluish-green | + | - |
| <i>Escherichia coli</i> (25922) (00013*) | ≥10 ³ | inhibited | 0% | | | - |
| <i>Bacillus spizizenii</i> sub <i>spizizenii</i> (6633) (00003*) | ≥10 ³ | inhibited | 0% | | | - |
| <i>Pseudomonas aeruginosa</i> (27853) (00025*) | ≥10 ³ | inhibited | 0% | | | - |
| <i>Candida albicans</i> (10231) (00054*) | ≥10 ³ | inhibited | 0% | | | - |

Key : + = positive reaction, - = negative reaction.

* = corresponding WDCM Numbers

Storage and Shelf-life

Store between 2-8°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label.

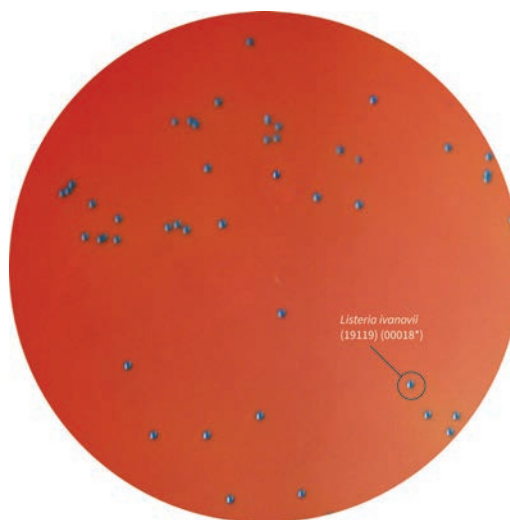
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5, 6).

References

1. FDA U.S., Bacteriological Analytical Manual 8 ed. Gaithersburg, MD, AOAC international, 1998.
2. Mengaud J., Braun-Breton C. and Cossart P., (1991), Molecular Microbiology, (2): 367-372.
3. Notermans S.H. and Dufrenne J., (1991), Applied and Environmental Microbiology, 57(09):2666-70.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S. and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.



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