I  INTRODUCTION
Yersinia Selective Medium is used in the selective isolation and differentiation of Yersinia enterocolitica from clinical and nonclinical specimens.

II  PERFORMANCE TEST PROCEDURE
1. Inoculate representative samples with the cultures listed below.
   a. Streak inoculate 1 µL (0.001 mL) from a 4 – 5 h culture of Trypticase™ Soy Broth diluted to yield 10^6 – 10^7 CFU/mL.
   b. Incubate at 25 ± 2 °C under appropriate atmospheric conditions.
   c. Include plates of a previously tested lot of TSA with 5% Sheep Blood as controls for inhibited strains.
2. Examine plates at 18 – 24 and 42 – 48 h for growth, colony color and selectivity.
3. Expected Results

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ATCC™</th>
<th>Recovery</th>
<th>Colony Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Enterococcus faecalis</td>
<td>29212</td>
<td>Inhibition (partial to complete)</td>
<td>N/A</td>
</tr>
<tr>
<td>*Escherichia coli</td>
<td>25922</td>
<td>Inhibition (partial to complete)</td>
<td>N/A</td>
</tr>
<tr>
<td>*Yersinia enterocolitica</td>
<td>9610</td>
<td>Fair to heavy growth</td>
<td>Translucent with pink to red centers (bull’s-eye appearance)</td>
</tr>
</tbody>
</table>

*Recommended organism strain for User Quality Control.

III  ADDITIONAL QUALITY CONTROL
1. Examine plates as described under “Product Deterioration.”
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.4 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 33 – 37 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV  INTENDED USE
This medium is used in the selective isolation and differentiation of Yersinia enterocolitica from clinical and nonclinical specimens.

V  SUMMARY AND EXPLANATION
Yersinia Selective Medium is based on the selective medium developed by Schiemann, called CIN Agar.1 This formulation contained a combination of inhibitory agents, including cefsulodin, Irgasan® (triclosan), novobiocin, bile salts and crystal violet, which effectively inhibited the growth of normal flora and resulted in better recovery of Y. enterocolitica compared with MacConkey Agar or other media commonly used for isolation of enteric pathogens.2 Schiemann later modified the formulation by substituting sodium desoxycholate for the bile salts and decreasing the concentration of novobiocin to improve the recovery of Y. enterocolitica.3 Yersinia Selective Medium is CIN medium without the cefsulodin and novobiocin, which makes the medium less selective and thereby increases the recovery of certain strains of Y. enterocolitica.

VI  PRINCIPLES OF THE PROCEDURE
Yersinia Selective Medium incorporates crystal violet, sodium desoxycholate and Irgasan to selectively inhibit gram-positive and gram-negative organisms. Mannitol fermentation aids in the differentiation of Yersinia species. A change in pH of the medium surrounding organisms that ferment mannitol causes the colonies to absorb the neutral red and become pigmented (pink to red). Yersinia enterocolitica produces a characteristic “bull’s-eye” colony, translucent with a deep pink to red center. Colonies of organisms that do not ferment mannitol remain colorless.

VII  REAGENTS
Yersinia Selective Medium
Approximate Formula* Per Liter Purified Water

<table>
<thead>
<tr>
<th>Peptones</th>
<th>20.0 g</th>
<th>Sodium Chloride</th>
<th>1.0 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast Extract</td>
<td>2.0 g</td>
<td>Magnesium Sulfate</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Agar</td>
<td>13.5 g</td>
<td>Sodium Pyruvate</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Mannitol</td>
<td>20.0 g</td>
<td>Neutral Red</td>
<td>0.03 g</td>
</tr>
<tr>
<td>Sodium Desoxycholate</td>
<td>0.5 g</td>
<td>Crystal Violet</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Sodium Cholate</td>
<td>0.5 g</td>
<td>Irgasan®</td>
<td>4.0 mg</td>
</tr>
</tbody>
</table>

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For in vitro Diagnostic Use.
If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.
Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens.

* “Standard Precautions”4-7 and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.
Storage Instructions: On receipt, store plates in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING
Refer to appropriate texts for details of specimen collection and handling procedures.\(^6-11\)
Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE
Material Provided: Yersinia Selective Medium
Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.
Test Procedure: Observe aseptic techniques.
The agar surface should be smooth and moist, but without excessive moisture.
Inoculate the medium as soon as the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface, and streak the remainder of the plate to obtain isolated colonies. Material not being cultured from swabs should be streaked onto the medium with a sterilized inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.
Incubate the plates in an inverted position (agar side up) at 25 °C for 24 – 48 h. This temperature is optimal for growth of *Y. enterocolitica* while inhibiting the growth of other organisms.
If a cold enrichment technique is desired to increase the recovery of *Y. enterocolitica*, inoculate the specimen into phosphate buffered saline and hold at 4 °C for up to 21 days.\(^11,12\) Periodically subculture onto plates of Yersinia Selective Medium, using the streak plate technique. Incubate plates as described above.
User Quality Control: See “Quality Control Procedures.”
Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory’s standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS
After sufficient incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.
*Yersinia enterocolitica* generally produces small to medium-sized, translucent colonies with deep pink to red centers ("bull's-eye" appearance).
Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

XI LIMITATIONS OF THE PROCEDURE
This prepared plated medium is intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and serological procedures. Consult appropriate texts for further information.\(^9,11,13\)
A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit the growth of a species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Specimens cultured on selective media should, therefore, also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY
<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>296191</td>
<td>BBL™ Yersinia Selective Medium, Pkg. of 20 plates</td>
</tr>
</tbody>
</table>
XIII REFERENCES


Technical Information: In the United States contact BD Technical Services and Support at 800-638-8663 or www.bd.com/ds.