QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION
CIN (cefsulodin-Irgasan™-novobiocin) Agar is used for the selective isolation of *Yersinia enterocolitica*.

II PERFORMANCE TEST PROCEDURE
1. Inoculate representative samples with the cultures listed below.
   a. Streak the plates for isolation using 5-h Trypticase™ Soy Broth cultures diluted to yield 10³–10⁴ CFU/plate.
   b. Incubate plates at 25 ± 2 °C in an aerobic atmosphere.
   c. Include Trypticase Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 and 48 h for amount of growth, pigmentation, colony size, and selectivity.
3. Expected Results

<table>
<thead>
<tr>
<th>CLSI Organisms</th>
<th>ATCC®</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>9610</td>
<td>Growth; deep red center, transparent border (bull’s-eye)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>Inhibition (partial to complete)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>27853</td>
<td>Inhibition (partial to complete)</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>29212</td>
<td>Inhibition (partial to complete)</td>
</tr>
</tbody>
</table>

Additional Organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC®</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>49397</td>
<td>Fair to heavy growth. Colonies flat with confined deep-red center (bull’s-eye) surrounded by a transparent border.</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>12453</td>
<td>Inhibition (partial to complete)</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 aerobically at 35 ± 2 °C for 72 h and examine for microbial contamination.

IV INTENDED USE
CIN (cefsulodin-Irgasan-novobiocin) Agar is a differential and selective medium used in qualitative procedures for the isolation of *Yersinia enterocolitica* from a variety of clinical and nonclinical specimens.

V SUMMARY AND EXPLANATION
CIN Agar was first described by Schiemann as an alternative to MacConkey Agar and other commonly used media for isolation of *Yersinia enterocolitica*, a causative agent of gastroenteritis.¹ CIN Agar has been found to be far superior to MacConkey, SS, CAL or Y agars.²

VI PRINCIPLES OF THE PROCEDURE
Fermentation of mannitol in the presence of neutral red results in a characteristic “bull’s-eye” colony, colorless with red centers. Selective inhibition of gram-negative and gram-positive organisms is obtained by means of crystal violet, sodium desoxycholate and the antimicrobial agents, cefsulodin, Irgasan (triclosan) and novobiocin.

VII REAGENTS

**CIN Agar**
Approximate Formula* Per Liter Purified Water

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Gelatin</td>
<td>10.0 g</td>
<td>Sodium Desoxycholate</td>
</tr>
<tr>
<td>Peptic Digest of Animal Tissue</td>
<td>5.0 g</td>
<td>Agar</td>
</tr>
<tr>
<td>Beef Extract</td>
<td>5.0 g</td>
<td>Crystal Violet</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>2.0 g</td>
<td>Neutral Red</td>
</tr>
<tr>
<td>Mannitol</td>
<td>20.0 g</td>
<td>Cefsulodin</td>
</tr>
<tr>
<td>Sodium Pyruvate</td>
<td>2.0 g</td>
<td>Irgasan</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>1.0 g</td>
<td>Novobiocin</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>0.001 g</td>
<td></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”³-⁶ 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 incubation.

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
Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts. Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE
Material Provided: CIN Agar
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.
Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.
Incubate plates at 25 °C for 24–48 h.
If a cold enrichment procedure is desired, inoculate specimen into phosphate-buffered saline and hold at 4 °C for up to 21 days. Periodically subculture onto plates of CIN Agar, streaking for isolation. Incubate plates as stated above.

User Quality Control: See “Quality Control Procedures.” Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory’s standard quality control procedures.

X RESULTS
After incubation most plates will show an area of confluent growth in the first quadrant inoculated. Because the streaking procedure is in effect, a “dilution” technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of the subsequent areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the medium.

Typical Yersinia enterocolitica colonies will have deep-red centers surrounded by a transparent border giving the appearance of a “bull’s-eye.”

XI LIMITATIONS OF THE PROCEDURE
Although certain strains of Yersinia can be recovered by direct plating, others may require cold enrichment (4 °C) in phosphate-buffered saline. However, cold enrichment may not be practical because of the long incubation time and because it selects for nonpathogenic strains of Y. enterocolitica and other Yersinia species.

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY
Cat. No. Description
221848 BD BBL™ CIN Agar, Pkg. of 10 plates
299579 BD BBL™ CIN Agar, Ctn. of 100 plates
XIII REFERENCES


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