



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Endo Agar is a slightly selective and differential medium for the isolation, cultivation and differentiation of gram-negative microorganisms from both clinical and nonclinical specimens.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with dilutions of the cultures listed below.
 - Streak the plates for isolation using 5-h broth cultures diluted to yield 10^3 – 10^4 CFU/plate.
 - Incubate plates at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere.
 - Include Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
- Examine plates after 18–24 and 48–72 h for amount of growth, colony size, pigmentation and selectivity.
- Expected Results

Organisms	ATCC™	Recovery	Colony Color
* <i>Escherichia coli</i>	25922	Growth	Colonies pink to rose-red with green metallic sheen. Marked reddening of the medium may occur.
* <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	Growth	Colonies colorless to faint pink
<i>Shigella flexneri</i>	12022	Growth	Colonies colorless to faint pink and slightly more pink than <i>Salmonella</i> colonies.
* <i>Enterococcus faecalis</i>	29212	Inhibited. Moderate growth acceptable.	Colonies small, pink to rose-red. Trace sheen may be evident.

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine plates as described under "Product Deterioration."
- Visually examine representative plates to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of 7.5 ± 0.2 .
- Note the firmness of plates during the inoculation procedure.
- Incubate uninoculated representative plates aerobically at $35 \pm 2^\circ\text{C}$ for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Endo Agar is a differential and slightly selective culture medium for the detection of coliform and other enteric microorganisms.

V SUMMARY AND EXPLANATION

The majority of the enteric plating media developed in the early years of the 20th century utilized either mixtures of bile salts or individual salts as selective agents to achieve inhibition of gram-positive species. In 1904, Endo reported the development of a culture medium for the differentiation of lactose fermenters from the nonfermenters in which no bile salts were used.¹ Inhibition of gram-positive microorganisms was achieved by the sodium sulfite and basic fuchsin contained in the formulation. Endo's Fuchsin Sulphite Infusion Agar was the original name for this medium² which is known today as Endo Agar. It was initially developed in order to facilitate the isolation and identification of the typhoid bacillus.

The original formula has been modified extensively since its introduction. The meat infusions have been replaced by a peptic digest of animal tissue. The dye composition and concentration also have been adjusted.

Over the years, Endo Agar has been an important medium in the microbiological examination of potable water and wastewater, dairy products and foods; however, the current compendia of standard methods for the examination of these materials recommend alternative media formulations.³⁻⁵

VI PRINCIPLES OF THE PROCEDURE

The selectivity of Endo Agar is due to the sodium sulfite/basic fuchsin combination which results in the suppression of gram-positive microorganisms. It is classified as only slightly selective as other media contain more potent inhibitors of the gram-positive microorganisms. Coliforms ferment the lactose producing pink to rose-red colonies and similar coloration of the medium. The colonies of organisms which do not ferment lactose are colorless to faint pink against the pink background of the medium.

VII REAGENTS

Endo Agar

Approximate Formula* Per Liter Purified Water

Dipotassium Phosphate.....	3.5 g
Peptic Digest of Animal Tissue	10.0 g
Agar	15.0 g
Lactose	10.0 g
Sodium Sulfite	2.5 g
Basic Fuchsin	0.5 g

*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"⁶⁻⁹ 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

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{10,11} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Endo 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. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

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, protected from light, at 35 ± 2°C in an aerobic atmosphere for 18–24 h.

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 (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After incubation most plates will show an area of confluent growth. 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 these 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 colonial morphology on Endo Agar is as follows:

<i>E. coli</i>	Pink to rose-red, green metallic sheen
<i>Enterobacter/Klebsiella</i>	Large, mucoid, pink
<i>Proteus</i>	Colorless to pale pink
<i>Salmonella</i>	Colorless to pale pink
<i>Shigella</i>	Colorless to pale pink
<i>Pseudomonas</i>	Irregular, colorless
Gram-positive bacteria	No growth to slight growth

XI LIMITATIONS OF THE PROCEDURE

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.¹⁰⁻¹⁵

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
221167	BBL™ Endo Agar, Pkg. of 20 plates
221265	BBL™ Endo Agar, Ctn. of 100 plates

XIII REFERENCES

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