

QUALITY CONTROL PROCEDURES

I INTRODUCTION

Rapid Urea Broth is used for the presumptive identification of *Helicobacter pylori* in gastric antral biopsy specimens.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Inoculate directly from a fresh stock culture grown on **Trypticase™** Soy Agar with 5% Sheep Blood.
 - b. Incubate vials at 36 ± 1 °C for 24 h in an aerobic atmosphere with loose caps.
2. Examine vials at intervals of 1, 4 and 24 h for broth color change.
3. Expected Results

Organisms	ATCC®	Broth color
<i>Campylobacter coli</i>	834	No reaction to trace pink at 24 h
<i>Campylobacter jejuni</i>	29428	No reaction at 24 h (Pale yellow-orange)
* <i>Escherichia coli</i>	25922	No reaction at 24 h (Pale yellow-orange)
* <i>Helicobacter pylori</i>	43504	Medium red to rose red within 4 h
<i>Klebsiella pneumoniae</i>	33495	No reaction at 4 h, trace pink to medium red at 24 h
<i>Proteus vulgaris</i>	8427	Rose red at 24 h

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine vials as described under "Product Deterioration."
2. Visually examine representative vials 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 6.8 ± 0.2 .
4. Incubate uninoculated representative vials at $33 - 37$ °C and $20 - 25$ °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Rapid Urea Broth is used for the presumptive identification of *Helicobacter pylori* in gastric antral biopsy specimens.

V SUMMARY AND EXPLANATION

The presence of small, curved and S-shaped bacilli in antral biopsy specimens was first reported by Warren and Marshall in 1983.¹ Subsequently, other investigators observed an association between this organism, now known as *Helicobacter pylori*, and gastritis.²⁻⁴ However, isolating and identifying the organism on primary media may require up to 7 days, delaying treatment.⁵

McNulty developed a rapid diagnostic test for *Helicobacter*-associated gastritis. Noting that the rapid hydrolysis of urea is characteristic of *H. pylori*, he placed biopsy specimens in Christensen's 2% urea broth and observed a color change. Depending on the number of organisms present in the specimen, positive results could be observed in less than 1 h.⁶

VI PRINCIPLES OF THE PROCEDURE

The enzyme urease catalyzes the hydrolysis of urea to ammonium and bicarbonate ions. Because urease is not a human enzyme, its activity in the gastric mucosa is due primarily to the presence of *H. pylori*.⁷ The preformed urease enzyme present in biopsy specimens renders the urease broth alkaline and changes the phenol red indicator in the medium to a pink-red color.

VII REAGENTS

Rapid Urea Broth

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin.....	1.0	g
Dextrose.....	1.0	g
Sodium Chloride.....	5.0	g
Potassium Phosphate.....	2.0	g
Urea.....	20.0	g
Phenol Red.....	0.012	g

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Vials with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens.

"Standard Precautions"⁸⁻¹¹ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, specimen containers and other contaminated materials must be sterilized by autoclaving.

Storage Instructions: On receipt, store vials in the dark at $2 - 8$ °C. Avoid freezing and overheating. Do not open until ready to use.

Minimize exposure to light. Vials stored as labelled 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 vials if they show evidence of microbial contamination, discoloration, precipitation, evaporation or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens from antral biopsy may be placed directly into the Rapid Urea Broth, sterile saline, or other suitable material.^{12,13} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Rapid Urea Broth

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: After the specimen is obtained by antral biopsy, it should be placed immediately into Rapid Urea Broth. If the specimen is transported in saline to the laboratory, it should be transferred immediately to Rapid Urea Broth. Place vials in an incubator at $35 \pm 2^\circ\text{C}$ and observe for development of a red color within 1 h. If negative, continue incubation and observe periodically for up to 4 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 guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

The presence of urease is indicated by an intense pink-red color throughout the broth. A negative reaction is no color change; i.e., the broth medium remains yellowish-orange. Results should be confirmed by Gram staining and subculturing to an appropriate medium, e.g., Skirrows Medium.^{7,12,13}

XI LIMITATIONS OF THE PROCEDURE

Urea test media rely on demonstration of alkalinity; hence they are not specific for urease. The utilization of peptones by contaminating organisms or other proteins in the medium may raise the pH to alkalinity due to protein hydrolysis and release of excessive amino acid residues, resulting in false positive reactions.^{14,15}

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Rapid Urea Broth are tested to verify specific product characteristics. Samples are inoculated directly with a fresh culture of *Helicobacter pylori* ATCC 43504 and *Escherichia coli* ATCC 25922 grown on **Trypticase** Soy Agar with 5% Sheep Blood. Vials are incubated with loose caps in an aerobic atmosphere at $35 - 37^\circ\text{C}$. A positive reaction (color change from yellow-orange to rose-red) is observed with *H. pylori*. *E. coli* remains negative (no color change) after 24 h.

XIII AVAILABILITY

Cat. No.	Description
298330	BD BBL™ Rapid Urea Broth, Pkg. of 10 vials

XIV REFERENCES

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