UREA MEDIA, RAPID METHODS

PRODUCT:

Tube Media:

Rapid Urea Medium, item no. F4000
Stat Urease, item no. F5820

PURPOSE:

Rapid Urea Medium is used to determine the ability of *Helicobacter (Campylobacter) pylori* to split urea rapidly by the action of the enzyme urease.

Stat Urease is designed for the rapid detection of urea hydrolysis by microbial enzymes, including these produced by *H. pylori*. Stat Urease can be used for detecting the presence of urease in biopsy specimens as well as microbial cultures.

PRINCIPLE:

Some organisms possess the enzyme urease which is capable of hydrolyzing urea to yield ammonia and ammonium carbonate as end products. Urea is a diamide, and the enzyme urease, an amidase, is capable of breaking the bond of nitrogen and carbon by hydrolysis to form alkaline end products.

In 1989 Goldie et al.[5] developed a medium which is nontoxic and produces rapid and specific results. The detection of urease produced by the organism provides the basis for this test. The urea substrate is suspended in a monosodium phosphate buffer, and phenol red serves to indicate the pH change that results from urease activity.

Helicobacter pylori, formerly *Campylobacter pylori*, has recently been established as the etiological agent of chronic active antral gastritis (type B) and may play a role in gastric and duodenal ulcers. The diagnosis of an *H. pylori* infection has been based on either the isolation of the organism from biopsy specimens obtained by endoscopy or by the identification of the organism in stained sections. Both of these methods are time consuming. Thus, a rapid test that could specifically identify the presence of *H. pylori* would expedite therapeutic decision making. *H. pylori* has a very high endogenous urease activity.[1,8] This property has been used to simplify the process of identification and allows the differentiation of *H. pylori* from other organisms, such as *Proteus* species, which are capable of splitting urea, but at a much slower rate.

FORMULAS:

Approximate, per liter deionized filtered water.

(1) Rapid Urea Medium

Urea	20.0 g
Monosodium Phosphate	0.7
Agar	4.0
Phenol Red, Aqueous 0.4%	25.0 ml

Final pH 6.4 ± 0.2 at 25°C

(2) Stat Urease

Same as (1) above, except no agar is added, approximate formula in kit format.

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking or discoloration), or if the expiration date has passed.

Limitations: Inoculating loops made of nichrome generate an immediate, spuriously positive test result; a platinum loop or wooden stick should be used.

The substrate medium is very slightly buffered at the pH of gastric tissue; the yellow color of the medium should not change upon addition of biopsy tissue. Any color change to pink upon addition of biopsy tissue which persists over 5 minutes or which develops at a later time may be attributed to urease activity. However, an inoculum of culture colonies may result in an immediate increase in pH and development of a red color due to carryover of extraneous alkaline materials. This red color will fade to yellow within minutes and will remain yellow unless urease activity is present.

Biopsy specimens that are negative after 30 minutes should be reincubated and re-examined at convenient intervals for up to 24 hours. If desired, the cup may be sent to the laboratory for monitoring and/or subculturing. The bag included in the kit provides a convenient transport method.

When Stat Urease is used for detection of urease activity in microbial cultures, it should be noted that some microorganisms are more prolific producers of urease than others. Hence, substrate cups, which are negative at 30 minutes, should be reincubated and re-examined at 24 hours.

With biopsy specimens, incubation of the urease medium at 35EC rather than at room temperature may yield positive results faster than at room temperature. Also, incubation times longer than 24 hours may allow contaminating organisms to produce a positive reaction that would yield confusing results. A negative urease reaction does not completely rule out the possibility of *Helicobacter pylori* colonization.

The use of saline has been shown to be the preferred transport and holding medium (at 2-8°C) for biopsy specimens.[10]

PROCEDURE:*

Specimen Collection: Biopsy specimens are placed into 0.5 ml of lactated Ringer's solution (pH 6.5) or saline and transported to the laboratory on wet ice. Biopsies are ground in a sterile tissue grinder. A portion of the ground tissue is then transferred to the medium. Alternatively, the biopsy can be placed directly into the medium at the time of endoscopy.

Method of Use, Rapid Urea Medium: Submerge the biopsy specimen in the medium. Incubate the inoculated tube aerobically at 35EC or room temperature for up to 24 hours. A strong positive reaction may be noted within 30 minutes of inoculation.

Method of Use, Stat Urease:

1. Remove cap from cup and add 10 drops of reagent. Dried substrate may be dissolved by replacing cap and shaking or by mixing with the inoculating loop or stick in step #2.

2. Inoculate test medium in cup with specimen. If shaking did not previously dissolve dried substrate, be sure to mix liquid well at this point with inoculating loop or device to achieve dissolution of substrate.

Biopsy Specimen: *Helicobacterpylori* is often embedded in the mucosal tissue of gastric biopsy specimens. Steps must be taken to expose the microorganisms to the urea substrate for the test to react efficiently. These steps may be performed as follows:

A. Place biopsy specimen into cup and replace cap tightly. Vortex vigorously to mix tissue and substrate.

OR

B. Macerate biopsy specimen in tissue grinder or other similar process to expose tissue surfaces. Place tissue fragments into test cup.

Microbiology Culture: Select 2-3 isolated colonies and mix with loop to assure uniform suspension of cells.

3. Replace cap and place cup into reclosable plastic bag or into a rack.

4. Record patient information on label area of bag or on cup if a rack is used.

5. Hold at room temperature and read for color changes within 30 minutes. Incubation times may decrease when placed in 35°C incubator.

Interpretation: Positive: Intense pink-red (red-violet) color.
Negative: No color change, Rapid Urea Medium; Yellow, Stat Urease.

Materials Required but Not Provided: Standard microbiological supplies and equipment such as loops, needles, incubator, incinerator, and inoculation system are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC#):	**Expected Results:**
Helicobacter pylori (43504)	(+)
Proteus mirabilis (12453)	(+); slower reaction time than *H. pylori* (18-24 hours)
Escherichia coli (25922)	(-)
	Key: See "Interpretation"

User Quality Control: Prior to using the Rapid Urea Medium or Stat Urease kit, test each lot in accordance with standard laboratory practice using known positive and negative control organisms.

BIBLIOGRAPHY:

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10. Coudron, P. E. and D. F. Kirby, 1988, "Abstracts of the Annual Meeting," Am. Soc. Microbiol., C-104.

* For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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