PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Urea Agar Procedure

Department of Microbiology

Effective date: 11/11/1985

Last Revision: 05/06/2014

Last reviewed: 05/2015

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1.0 Principle

Urea medium contains urea and the pH indicator phenol red. Many organisms have a urease enzyme, which is able to split urea in the presence of water to release two molecules of ammonia and carbon dioxide. The ammonia combines with the carbon dioxide and water to form ammonium carbonate, which turns the medium alkaline. The alkaline pH shift turns the indicator from its original orange-yellow color to bright pink.

2.0 Clinical Significance

The urea test can be used as part of the identification of several genera and species of *Enterobacteriaceae*, including *Proteus, Klebsiella,* and some *Yersinia* and *Citrobacter* species. It is also helpful to identify *Crytptococcus* spp., *Brucella, Helicobacter pylori, Corynebacterium urealyticum,* and many other bacteria that produce the urease enzyme.

3.0 Scope

This procedure is classified under CLIA as Highly Complex when used as part of the identification of clinical isolates. It should be carried out by technical personnel familiar with and trained to perform and interpret clinical cultures.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

To perform this procedure, you must use:

- Laboratory Coat – must be worn when handling cultures.
- Biological Safety Cabinet – must be used if *Brucella* is suspected.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

5.0 Specimen Requirements

Only well-isolated colonies should be tested.

6.0 Materials

- Aerobic incubator set at 35 ± 2°C
- Sterile swabs or inoculating loops
- Urea agar slants (Christensen)

7.0 Procedure

1. Using a sterile swab or loop, inoculate the agar slant surface from a well-isolated colony. Do not stab the butt.
2. Incubate, with the cap loosened, aerobically at 35 ± 2°C. Do not incubate in an atmosphere supplemented with carbon dioxide.
3. Observe for color change for up to 7 days.

8.0 Interpretation of Results

A positive test is indicated by the development of an intense magenta to bright pink color in 15 min to 24 h. A negative test shows no color change.

9.0 Quality Control & Quality Assurance

Inspect agar for evidence of prior freezing, contamination, cracks, and dehydration before use. Urea agar is exempt from user quality control (CLSI M22-A3).

10.0 Limitations

1. Some organisms, like *Brucella* and *H. pylori*, rapidly split urea while other react slowly.
2. Urea is light sensitive and can undergo autohydrolysis. Store at 2 to 8°C away from direct light.

11.0 References

Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

12.0 Document Control History

Reviewed by Microbiology Director, Dr. Ann Robinson: 05/17/2000, 05/06/2014

Reviewed by Medical Director, Dr. Joseph Schappert 03/10/2010

Reviewed by Microbiology Supervisor, Jerry Claridge: 05/11/1988, 10/26/1989, 04/13/1990, 06/01/1991, 06/24/1992, 02/21/1994, 11/1995, 06/1996, 05/1997, 04/1998, 09/1999, 04/2000, 07/05/2001, 04/25/2002, 03/2003, 04/2004, 11/2005, 11/2006, 10/2007, 05/2008, 05/2009, 04/01/2011, 03/2013, Jason Ammons 05/2015