PURPOSE: The urea test is used to determine the ability of an organism to split urea by the action of the enzyme urease forming two molecules of ammonia with resulting alkalinity.

SAMPLE INFORMATION:

Type	One, well isolated colony

REAGENTS and/or MEDIA:

- Urea tube

SUPPLIES:

- Disposable inoculation needles
- 35° ambient air incubator

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

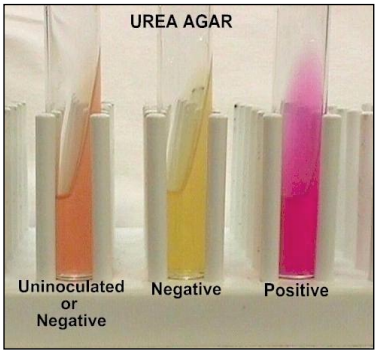
- Inspect the media for cracks, freezing, contamination and bubbles when received into the laboratory. Refer to MIC60090 – Entering New Media and Reagents into TQC for recording media acceptance.
- This medium is considered exempt as per CLSI document M22-A3.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the urea test	
1	Remove the urea tube from the refrigerator and bring to room temperature.
2	Using a disposable inoculating needle, touch the center of a well-isolated colony.
3	Inoculate the agar slant surface. Do not stab the butt.
4	Loosely replace the cap on the tube. DO NOT tighten the cap.
5	Incubate in the O_2 incubator.
6	Check for color change to bright pink after 3 hours and again at 18 to 24 hours. For slower growing organisms such as *Nocardia*, the color change can take up to 7 days.

INTERPRETATION OF RESULTS:

IF	THEN
Intense pink or red colour	**Urea = Positive**
No colour change or pale yellow colour	**Urea = Negative**



PROCEDURE NOTES:

- The urea test is used as part of the identification of several genera and species of Enterobacteriaceae, including *Proteus, Klebsiella* and some *Yersinia* and *Citrobacter* species, as well as some *Corynebacterium* species, *Brucella, Helicobacter pylori* and many other bacteria that produce the urease enzyme.
- The test can also be used as part of the identification of *Cryptococcus* species.

LIMITATIONS/PRECAUTIONS:

1. *Proteus* spp. will show positive reactions within 1 to 6 hours.
2. The cap of the urea tube must not be tightened.
3. Urea-positive, oxidase-positive, Gram-negative coccobacilli that are isolated from the urinary tract may be *Oligella ureolytica*.
4. Some organisms rapidly split urea (*Brucella* and *H.pylori*) while others react slowly.
5. When performing overnight tests from medium that contains peptone, the alkaline reaction may be due not to urease but to hydrolysis of peptone.
6. Urea is light sensitive and can undergo auto hydrolysis. Store at 2°C to 8°C in the dark.
7. Urea-positive, oxidase-positive, Gram-negative coccobacilli that do not grow on MacConkey agar in 24 hours are presumptively identified as *Brucella*, unless they are isolated from urine. *Immediately transfer cultures to a biosafety cabinet.*

REFERENCES:

- Oxoid Urea Agar product details, 12/30/2018
- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- CLSI. *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard—Third Edition.* CLSI document M22-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2004

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	8 APR 19	Initial Release	L. Steven