STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: Coagulase – Tube	**Revision Date:** 20-April-2018	**Issue Date:** 20-April-2016
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Distribution: Microbiology Test Manual	**Page:** 1 of 5	
Approved by: S. Asmussen, Manager of Diagnostic Services	**Signed by:** 	

INTRODUCTION:

Identification of staphylococci is based on microscopic examination, colonial morphology and cultural and biochemical characteristics. Staphylococci associated with acute infection (*Staphylococcus aureus* in humans) can clot plasma. The most widely used and generally accepted criterion for identification of these pathogenic organisms is based on the presence of the enzyme coagulase.

PURPOSE:

This test is used to differentiate *Staphylococcus aureus* from other *Staphylococcus spp.* by determining the ability of an isolate to clot plasma by producing the enzyme coagulase.

PRINCIPLE:

Coagulase is a thermostable thrombin-like substance that activates plasma fibrinogen to form fibrin, resulting in a fibrin clot. *Staphylococcus aureus* produces two types of coagulase, free and bound. Free coagulase is an extracellular enzyme produced when the organism is cultured in broth. Bound coagulase, also known as the clumping factor, remains attached to the cell wall of the organism. In the direct tube test, both free and bound coagulase are detected.

SAMPLE INFORMATION:

Type	Well isolated colonies that are: • Gram-positive cocci in clusters • catalase positive • from a 18-36 hour culture

	• grown on non-selective media

REAGENTS and/or MEDIA:

Type	BBL Coagulase Rabbit Plasma with EDTA (lyophilized rabbit plasma with 0.15% EDTA and 0.85% sodium chloride)
Storage Requirements	• Store unopened lyophilized at 2-8°C. • Store reconstituted coagulase plasma at 2-8°C or aliquot and freeze at -20°C.
Stability	• Unopened lyophilized coagulase plasma is stable until the date of expiration on the label • Reconstituted plasma is stable at 2-8°C for 14 days or 30 days at -20°C. • Frozen aliquots should not be thawed and refrozen.
Reagent Preparation	• Rehydrate coagulase plasma by adding 15 mL of sterile purified water to the vial. • Mix by gentle end-over-end rotation of the vial.

SUPPLIES:

- BBL Coagulase Rabbit Plasma
- Sterile water
- Inoculating loop or wire
- Wooden sticks
- Pipettes
- Small glass test tubes (10X75 mm)
- 35-37°C 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.

QUALITY CONTROL:

Quality control is set up per run/per bottle:

Positive:	*Staphylococcus aureus*	ATCC 25923
Negative:	*Staphylococcus epidermidis*	ATCC 12228

- A TQC order is automatically generated in the TQC system to record results

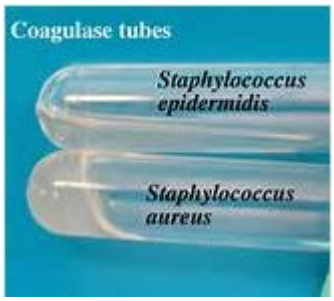
PROCEDURE INSTRUCTIONS:

Step	Action
Performing a Tube Coagulase Test	
1	In the plate log – Order **^TC**
2	Using a sterile 1 mL pipette, add 0.5 mL of rehydrated coagulase plasma to a 10 X 75 mm glass test tube supported in a rack.
3	Using a sterile loop/wire or wooden stick, thoroughly emulsify 2-4 colonies from a non-selective agar plate in the tube of plasma.
4	Mix gently.
5	Incubate in an ambient air incubator at 35-37°C for up to 4 hours.
6	Examine the tubes after 4 hours, observing for clot formation.
7	If there is no visible clot formation, leave at room temperature overnight (for a total of 24 hours) and observe for clot formation.

INTERPRETATION OF RESULTS:

Positive test: Clot formation

Negative test: No clot formation



NOTES AND PRECAUTIONS:

1. Do not use if the product is caked, discolored or shows other signs of deterioration.
2. Examine reconstituted reagents for evidence of contamination, evaporation or other signs of deterioration, such as cloudiness or partial clotting.
3. When observing the tube, do not shake or agitate the tube as it may lead to breakdown of clot formation.
4. The coagulase clot can be destroyed by *S.aureus* fibrinolysin or staphylokinase, a plasmid-carried enzyme which is more active at 35°C than at 25°C. This is why it is important to incubate at 35°C for a maximum of 4 hours, after which any negative tests should be left at room temperature overnight. If a 4 hour incubation period is not feasible, incubate for the time available and leave at room temperature overnight.
5. Some species of organisms utilize citrate in their metabolism and will yield false positive reactions for coagulase activity. Be sure the isolate is Gram positive and catalase positive before interpreting the test.
6. *S. intermedius* and *S.hyicus* may be positive in the tube test; these species are generally found only in dogs and pigs, respectively, but are as infectious as *S.aureus* when they infect humans. Both form nonhemolytic colonies on fresh plates and are Voges-Proskaeur negative, which separates them from *S.aureus*. *S.intermedius* is also PYR positive.

REFERENCES:

- BD BBL Coagulase Plasmas package insert, 2003
- Clinical Microbiology Procedures Handbook, 2nd Edition, Henry D. Isenberg, 2004, p.3.17.14

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	14Nov12	Initial Release	A. Darrach
1.1	31Jul13	Added Illustration	A. Darrach
1.2	10Mar14	Changed from Document control number MTE10515 to MIC50515	C. Russell
2.0	31Mar16	Update of “Special Safety Precautions” to reflect risk assessment recommendations.	C. Russell