TITLE: Disc Diffusion-Novobiocin	**Revision Date:** 20-April-2018	**Issue Date:** 20-April-2016
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INTRODUCTION:

Staphylococcus saprophyticus is recognized as a significant cause of urinary tract infections, especially in young women. Males may also develop nonspecific urethritis. Resistance to novobiocin can be used as presumptive identification for this organism.

PURPOSE:

The novobiocin disc diffusion method is used to differentiate *Staphylococcus saprophyticus* from other coagulase negative *Staphylococcus species*.

SAMPLE INFORMATION:

Type	Well isolated coagulase negative S*taphylococcus* colonies

REAGENTS and/or MEDIA:

Type	Oxoid Novobiocin 5 µg disk
Storage Requirements	• Discs must be stored at -20°C if kept for long periods. • Storage at 2-8°C is suitable for discs currently being used or to be used very soon. • Discs should be returned to the refrigerator as quickly as possible after use. The most common cause of moisture reaching the discs and causing destruction of labile antimicrobials is condensation of warm laboratory air on cold discs removed from the refrigerator. • It is important to allow the cartridge blister pack to reach room

	temperature before exposing the discs, a period of one hour is generally sufficient. • Once a cartridge has been opened it needs to be stored in a desiccated environment at 2-8°C.

SUPPLIES:

- 5 ug Novobiocin disc
- Tweezers
- Blood agar plate
- Wire or loop

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 each time the test is performed using the following control organisms:

Positive:	*Staphylococcus saprophyticus*	ATCC 15305
Negative:	*Staphylococcus epidermidis*	ATCC 12228

- A TQC order is automatically generated to record the QC results

PROCEDURE INSTRUCTIONS:

Step	Action
Performing a Novobiocin Disc Diffusion	
1	In the plate log – Order **^NV**
2	Select one suspect coagulase negative *staphylococcus* colony with a flamed wire or loop and quadrant streak on a blood agar plate (with a thick 1st and 2nd quadrant).
3	Place a 5 ug disc of novobiocin on the surface in between the 1st and 2nd quadrant and tap down lightly.
4	Incubate for 16 to 18 hours at 35°C aerobically.
Alternate Method	
1	In the plate log – Order **^NV**
2	Prepare a suspension in sterile saline equivalent to a 0.5 McFarland standard
3	Moisten a sterile swab with the suspension and spread evenly over a Mueller Hinton Agar plate, as for Kirby Bauer testing
4	Place a 5 ug disc of novobiocin on the plate and incubate at 35C for 16-18 hours
5	Examine and measure the zone of inhibition of growth around the disc.

INTERPRETATION OF RESULTS:

IF	THEN
Less than or equal to 12mm	**Resistant:** *Staphylococcus saprophyticus* See Procedure for reporting requirements
13-16mm	**Intermediate:** Repeat testing using the Alternate Method. If repeat is the same, perform a VITEK GPI
Greater than 16mm	**Susceptible:** NOT *Staphylococcus saprophyticus.* Report as *"Coagulase Negative Staphylococcus NOT saprophyticus"*

NOTES AND PRECAUTIONS:

1. Strains of staphylococci, other than *S. saprophyticus*, that are resistant to novobiocin include *S. xyloses*, *S. kloosi*, and *S. cohnii*. Any strain may become resistant to novobiocin, including *S. aureus*; reporting *S. saprophyticus* should be limited to urinary isolates unless further tests for identification to the species level are performed.

REFERENCES:

1. Clinical Microbiology Procedures Handbook, 2nd edition, Henry D. Isenberg – Editor in Chief 2004, p. 3.17.4.1 – 3.17.4.5
2. Oxoid Antimicrobial Susceptibility Test Disc package insert, 2004
3. Color Atlas and Textbook of Diagnostic Microbiology, 5th edition, Elmer W. Koneman, Stephen D. Allen, William M. Janda, Paul C. Schreckenberger, Washington C. Winn, Jr., 1997, Chart 55.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	25/01/11	Initial Release	J Whitson
2.0	31Dec2013	LIS updates	A.Darrach
3.0	31Mar16	Update of “Special Safety Precautions” to reflect risk assessment recommendations.	C. Russell