

STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: Disc Diffusion-Novobiocin	Revision Date:	Issue Date:
	10-March-2016	10-March-2014
Document Number: MIC50615	Status: Approved	k
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Approved by:	Signed by:	11. 1 Carol
Cheryl Case, Manager of Diagnostic Services		Theyl Case

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 *Staphyloccus* species.

SAMPLE INFORMATION:

Туре	Well isolated coagulase negative Staphylococcus colonies

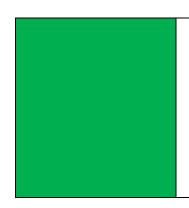
REAGENTS and/or MEDIA:

Туре	Oxoid Novobiocin 5 µg disk	
	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 	
Storage	to be used very soon.	
Requirements	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	

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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 dessicated environment at 2-8°C.

SUPPLIES:

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

SPECIAL SAFETY PRECAUTIONS:

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:

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:

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Step	Action		
Perfo	Performing a Novobiocin Disc Diffusion		
1	In the plate log – Order ^NV		
2	Select one suspect coagulase negative <i>staphyloccus</i> colony with a flamed wire or loop and quadrant streak on a blood agar plate (with a thick 1 st and 2 nd quadrant).		
3	Place a 5 ug disc of novobiocin on the surface in between the 1 st and 2 nd 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:

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1. Strains of staphylococci, other than *S. saprophyticus*, that are resistant to novobiocin include *S. xylosus*, *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:

- 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
- 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
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