



STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: DNase	Revision Date: 10-March-2016	Issue Date: 10-March-2014
Document Number: MIC50700	Status: Approved	
Distribution: Microbiology	Page: 1 of 4	
Approved by: Cheryl Case, Manager of Diagnostic Services	Signed by: <i>Cheryl Case</i>	

PURPOSE:

This test determines the ability of an organism to produce deoxyribonuclease (DNase). This test is used, in conjunction with others, for the identification of *S. aureus*, *M. catarrhalis* and *Serratia* species.

PRINCIPLE:

DNases are enzymes that hydrolyze DNA and release free nucleotides and phosphate. Methyl green combines with highly polymerized DNA at pH 7.5 to produce a green colour complex in the agar. When the organism produces DNase the DNA is hydrolyzed, and the methyl green fades resulting in a clearing of the agar around the colony.

SAMPLE INFORMATION:

Type	Well isolated colonies
Source	18-24 culture

REAGENTS and/or MEDIA:

Type	DNase Agar with methyl green
Source	Oxoid Cat#MP0420
Stability	Stable until expiration date indicated on the package
Storage Requirements	Store at 2-8°C, away from direct light.

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FILENAME: MIC50700DNASEPRO.doc	PRINT DATE: 10 March 2014

TITLE: DNase	Revision Date: 10-March-2016	Issue Date: 10-March-2014
Document Number: MIC50700	Status: Approved	
Distribution: Microbiology	Page: 2 of 4	

Criteria for rejection and follow up action	Media should not be used if there are signs of contamination or deterioration (shrinking, cracking or discolouration).
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SUPPLIES:

- Sterile sticks, needles or inoculating loops
- DNase agar plate

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 with the following control organisms:

Positive: *Staphylococcus aureus* ATCC # 29213
 Negative: *Escherichia coli* ATCC # 25922

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

PROCEDURE INSTRUCTIONS:

Step	Action
Performing a DNase Test	
1	After touching several colonies, inoculate a segment of the agar surface with a very visible, heaping amount of organism equivalent to an entire colony.

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FILENAME: MIC50700DNASEPRO.doc	PRINT DATE: 10 March 2014

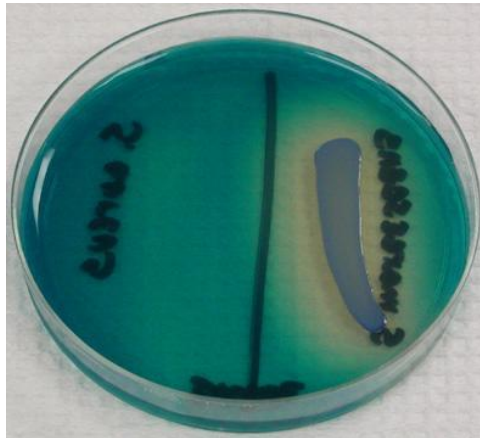
TITLE: DNase	Revision Date: 10-March-2016	Issue Date: 10-March-2014
Document Number: MIC50700	Status: Approved	
Distribution: Microbiology	Page: 3 of 4	

2	Use either circular or line method for inoculation. (Do not streak the entire plate, as it will be difficult to see the reaction.)
3	Incubate aerobically (without CO ₂) at 35°C for 18 to 24 hours.
4	Examine the plate against a white background for colour change.

INTERPRETATION OF RESULTS:

Positive: A colourless zone around the inoculum

Negative: No colour change around the inoculum



NOTES AND PRECAUTIONS:

1. An inoculum that is too broad may result in complete decolourization of the media, due to the reduction of the dye. If this occurs, the test must be repeated.

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TITLE: DNase	Revision Date: 10-March-2016	Issue Date: 10-March-2014
Document Number: MIC50700	Status: Approved	
Distribution: Microbiology	Page: 4 of 4	

REFERENCES:

- DNase Media Technical Data Sheet, Dalynn Biologicals, Inc., 2003, Revised Feb 2006
- Clinical Microbiology Procedures Handbook, Henry D. Isenberg-Editor in Chief, 2004

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31-Dec-2013	Initial Release	A.Darrach