Ministry of Health

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Directorate/Institution	Diagnostic Laboratories Services at Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH)
Targeted Group	Medical laboratories
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Date	May 2023	Date	June 2023

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Acknowledgment

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Acronyms:

DNase	Deoxyribonuclease
ATCC	American Type Culture Collection
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
SOP	Standard operating procedure
HCL	Hydrochloric acid

1. **Purpose:**

   This document describes the DNase test SOP for the identification of *Staphylococcus aureus*.

2. **Scope:**

   This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. **Definitions:**

   3.1 DNase: an enzyme which is capable of degrading deoxyribonucleic acid (DNA).

4. **Procedure:**

   4.1.Clinical background:

   The DNAse test on an agar plate is used to identify the bacteria that produce DNAse enzymes as part of their normal metabolism. This test is used to differentiate between different bacterial species, as different bacteria can produce different types and levels of DNAse activity.

   DNase test positive bacteria are:

   - *Serratia marcescens (Serratia fonticola is the only Serratia sp. that is DNase negative.).*
   - *Staphylococcus aureus.*
   - *Campylobacter jejuni.*
   - *Moraxella catarrhalis.*
   - *Corynebacterium diphtheria.*

   In clinical microbiology, this medium is not used as an isolation medium on which specimens are streaked directly but requires the use of pure cultures such as those previously isolated from clinical specimens.

   4.2.Principle:

   DNase agar is a type of differential medium that is used to test the ability of an organism to produce an enzyme called deoxyribonuclease (DNase). This enzyme has the ability to hydrolyze DNA. To determine if an organism can produce this enzyme, the DNase agar is inoculated with the organism and then incubated. If the organism is able to produce DNase, it will hydrolyze the DNA in the agar, resulting in a clear zone around the growth on the agar. The presence of the enzyme is confirmed by adding hydrochloric acid (HCL) to the agar, which causes any unhydrolyzed DNA to precipitate and make the medium

opaque. If the organism is able to produce DNase and hydrolyze the DNA, the medium will remain clear due to the absence of unhydrolyzed DNA.

4.3. Pre – analytical stage:

4.3.1. Sample:

4.3.1.1 Sample type: Pure and young growth bacterial colonies that are growing separately on a solid medium.

4.3.1.2 Amount of sample required, including minimum requirements: 2-3 colonies.

4.3.1.3 Sample stability and storage requirements: to be processed immediately within 18-24 hours.

4.3.2. Material:

Reagents	Consumables/Supplies	Equipment
Dnase Agar plate. 1M HCL.	Bacteriological straight wire/loop. Disposable Pasteur pipette.	Incubator at 35°C ±1. Fridge 2-8°C.

4.3.3. Safety precaution:

4.3.3.1 All specimens need to be treated as potentially infectious.

4.3.3.2 Standard procedures for handling of biohazard material must be followed at all times.

4.3.3.3 Universal Precautions must be practiced at all stages of these procedures.

4.3.3.4 During HCL preparation add Acid to water and never do the opposite.

4.3.4. Quality control:

4.3.4.1 Check the expiry dates of all media, reagents and stains before use.

4.3.4.2 All media, reagents, kits, and stains MUST be quality controlled before use.

4.3.4.3 Identification tests should be run with appropriate controls.

- Positive Control: ATCC *Staphylococcus aureus* ATCC @ 29213.
- Negative Control: *Staphylococcus epidermidis* ATCC @ 12228.

4.3.4.4 Record the quality control results in the appropriate QC sheet. Refer to Daily microbiology identification test quality control sheet in appendix 1.

4.4. Analytical stage:

- There are two techniques to do the test (spot inoculation & Band or line streak inoculation).

4.4.1 Spot inoculation:

4.4.1.1 Obtain a loop and use it to gently touch a single colony of the organism under examination.

4.4.1.2 Inoculate the loop onto a small area in the middle of one of the marked sections of the DNase test agar plate. This will create a thick plaque of growth, approximately 5-10 mm in diameter, after incubation.

4.4.1.3 It can also be helpful to gently stab the agar with the loop to ensure that the inoculum is well-distributed.

4.4.1.4 Place the agar plate on the surface for incubation at 35°C ±1.

4.4.2 Line streak inoculation:

4.4.2.1 Use a large amount of inoculum and draw a line that is 3-4 cm long from the edge of the plate to the center.

4.4.2.2 Incubate the plate at 35°C ±1 for 18-24 hours.

4.4.3 Detection of DNase activity by flooding with HCL:

4.4.3.1 Flood the plate with 1M hydrochloric acid to precipitate any unhydrolyzed DNA.

4.4.3.2 Allow the plate to sit for a few minutes to allow the reagent to be absorbed into the agar.

4.4.3.3 Remove any excess hydrochloric acid and examine the plate against a dark background.

4.4.3.4 Compare the zone around the test strain with the control zones.

4.4.3.5 Unhydrolyzed DNA will be precipitated and will appear as a white, cloudy area in the agar due to the reaction between the HCl and DNA salts in the agar.

4.5. Post – analytical stage:

4.5.1. Interpretation / Results / Alerts:

- Positive result: Colonies surrounded by clear zones comparable in width to that around the DNase positive control.

- Negative result: No zone of clearing or a zone narrower than the DNase positive control.

4.5.2. Reporting:

- Dnase Positive: The colonies on the plate are surrounded by clear zones
- Dnase Negative: There is no clear zone on the plate

4.5.3. Limitation:

- It can only detect the presence of DNAse, not the amount or activity level of the enzyme. This can make it difficult to accurately determine the extent of DNA degradation or the effectiveness of DNAse inhibitors.
- The test relies on the presence of intact DNA, which can be damaged or degraded in certain samples, such as those that have been exposed to extreme temperatures or chemicals. This can lead to false negative results.
- The Dnase test requires a specific set of conditions and protocols to be followed, such as specific temperatures and incubation times, which can vary depending on the specific enzyme being used. Any deviations from these protocols can affect the accuracy of the test.
- The Dnase test can be affected by the presence of other enzymes or substances that may interfere with the reaction, such as proteases or other nucleases. This can lead to false positive or false negative results.

5. Responsibilities:

5.1.Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

5.2.Quality manager /officer

- To follow up the implementation of the procedure
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3.All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended
- To report test failures or incident

6. Document History and Version Control:

Version	Description	Review Date
1	Initial Release	May 2026

7. References:

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
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The Deoxyribonuclease Test. Merck Manuals. Retrieved from https://www.merckmanuals.com/professional/clinical-laboratory/general-laboratory-tests/deoxyribonuclease-test	Gebert, C., et al.	2011	
Deoxyribonucleases: general properties and some practical considerations. Microbiology and Molecular Biology Review, Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC99048/	Storz, G.	2000	64(1), 192-215
Detection, Analysis, and Applications. In Nucleases. Springer, Berlin, Heidelberg. Retrieved from https://link.springer.com/chapter/10.1007/978-3-642-21541-5_12	Walsh, C. J., et al.	2011	329-364

8. Annexes: Daily microbiology identification test quality control sheet:

Test Name: Dnase Test																															
Kit Manufacture Name:																															
Reagent Lot No:																															
Reagent Exp. Date:																															
Reagent Open date:																															
Month: ……																															
Positive Control result (P)																															
Negative Control result (N)																															
Quality Control (Pass/Failed)																															
Initials																															