Ministry of Health

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Document Type	Procedure
Directorate/Institution	The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH)
Targeted Group	Medical laboratories
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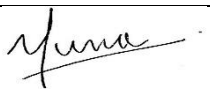
Signature		Signature	
Date	May 2023	Date	June 2023

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Acknowledgment

The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH) would like to thank and appreciate the great effort of the Microbiology documents development team. Participated and contributed personnel are:

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Acronyms:

ATCC	American Type Culture Collection
SOP	Standard operating procedure
WHO	World Health Organization

1. **Purpose:**

   The purpose of document is to provide instructions on how to carry catalase test.

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 Catalase is an enzyme that breaks down hydrogen peroxide (3%) into water and oxygen

4. **Procedure:**

   4.1. Clinical background:

   Catalase test is used to determine gram positive cocci whether it is catalase enzyme producer (e.g. *Staphylococcus sp*.) or non-catalase enzyme producer (e.g. *Streptococcus sp*.).

   4.2. Principle:

   Some Organisms to survive rely on defence mechanisms to repair or escape oxidative damage caused by hydrogen peroxide (H_2O_2). Few organisms produce the enzyme catalase, which facilitates cellular detoxification. Catalase is an enzyme that breaks down hydrogen peroxide (3%) into water and oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$). It should be tested on organisms grown on media without blood e.g. MacConkey and C.L.E.D. The blood agar can give a false positive reaction.

   4.3. Pre – analytical stage:

   4.3.1. Sample:

   4.3.1.1 This test is performed using 18-24 hours pure growth isolated colonies on solid medium.

   4.3.1.2 Colonies on medium containing whole red cells are not acceptable because they contain catalase.

   4.3.1.3 Colonies taken from chocolate agar plate may be tested as the blood cells have been destroyed.

4.3.2. Materials:

Reagents	**Consumables/Supplies**	**Equipment**
Hydrogen peroxide reagent concentration**:** • 30%H_2O_2 for *Neisseria*. • 15% H_2O_2 for anaerobes. • 3% H_2O_2 for other bacteria (purchase or dilute 30% 1:10 in deionized water prior to use). • Stored in a dark bottle under refrigeration.	• Transfer pipette. • Glass slides / covers slip. • Test tubes glass/plastic. • Wooden applicator. • Bacteriological Disposable plastic /loop. • Solid medium agar/slant without red blood cells.	Fridge 2-8°C

4.3.3. Reagent stability:

4.3.3.1 Hydrogen peroxide is unstable and decomposes quickly. If sealed and protected from light it is stable for 3 years from manufacture.

4.3.3.2 The rate of decomposition of Hydrogen peroxide increases with rise in temperature, concentration, and pH.

4.3.3.3 If opened or diluted it is stable at 2-8 °C in caped dark bottle for 6 months.

4.3.4. Safety Precautions:

4.3.4.1 Performing catalase assays on bacteria can be hazardous due to the release of bacteria-laden aerosols that result from liberation of oxygen. All work likely to produce aerosols needs to be performed in microbiological safety cabinets.

4.3.4.2 Hydrogen peroxide is a highly corrosive chemical and therefore appropriate personal protective equipment shall be worn.

4.3.4.3 Refer to risk assessment, appropriate COSHH and MSDS documents.

4.3.5. Quality Control:

4.3.5.1 Reagent shall be labeled properly with open / expiry date once in use.

4.3.5.2 Because hydrogen peroxide is unstable, it should undergo a quality control check every day or immediately before use.

4.3.5.3 Both positive and negative controls should be run simultaneously before testing patient's isolates and result recorded on quality control sheet (see appendix #1 Daily microbiology identification test quality control sheet).

4.3.5.3.1 Bacteria control:

- Positive control:
  - *Staphylococcus aureus* ATCC @ 29213.
  - *Staphylococcus epidermidis* ATCC @ 12228.
- Negative control:
  - *Enterococcus faecalis* ATCC @ 29212.
  - *Streptococcus pyogenes* ATCC @ 19615

4.4. Analytical stage:

4.4.1 Tube Method:

4.4.1.1 Add 1 ml of hydrogen peroxide into test tube.

4.4.1.2 Remove colonies from the agar medium using plastic /disposable loop/wooden stick and insert it in the test tube.

4.4.1.3 Immediately observe for the release of Oxygen bubbles.

4.4.2 Slide Method:

4.4.2.1 Place a loopful of hydrogen peroxide on a glass slide.

4.4.2.2 Pick up a colony of the test organism and smear it on the center of a cover slip.

4.4.2.3 Place the coverslip onto the slide, and observe for bubbles.

4.4.3 H_2O_2 Dilution method:

Calculation formula to make dilution:

IC × IV = FC × FV

- IC = Initial concentration.
- IV = Initial Volume.
- FC = Final Concentration.
- FV = Final Volume.

Example: to prepare 3% Hydrogen peroxide from 30% Manufacture concentration.

IC = 30%, FC=3%, FV= 10 ml.

$$IV = \frac{(FC \times FV)}{IC} = \frac{3 \times 10}{30} = \textbf{1 ml.}$$

- Therefore, pipette 1 ml of 30% H_2O_2 and top up with 9 ml deionized water to get 10 ml of 3% H_2O_2.

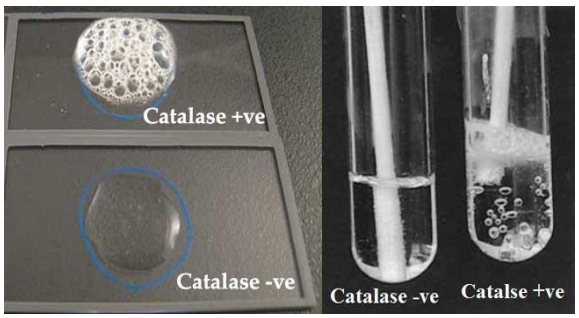
4.5.Post – analytical stage:

4.5.1 Interpretation of expected quality control results:

4.5.1.1 Positive reaction: any bubbling indicates the presence of catalase.

4.5.1.2 Negative reaction: lack of bubbling.

4.5.1.3 When expected results were met QC is passed and the test can performed on clinical isolate.



4.5.2 Limitation:

4.5.2.1 There is a possibility that older cultures will give false negative results. Therefore, do not process the test on culture older than 24 hours.

4.5.2.2 Be careful not to transfer blood containing catalase as it is found in RBCs.

4.5.2.3 Some *lactobacilli* grown in low concentrations (0.05%) of glucose may produce a false catalase reaction.

4.5.2.4 A few species e.g. *Aerococcus viridans* produce a weakly positive reaction, which may be easily missed.

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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Catalase test. UK Standards for Microbiology Investigations. TP 8 Issue 4. https://www.gov.uk/uk-standards-for-microbiologyinvestigations-smi-quality-and-consistency-in-clinical-laboratories	**Public Health England**	2019	
A Study Monitoring the Hydrogen Peroxide Stability for Shelf-Life Determination, vol. 9 Visited web site on 01-06-2021 @ (https://www.texwipe.com/Content/Images/uploaded/documents/Technical-Data/TechNote_Hydrogen_Peroxide_Shelf_Life.pdf).	**Postlewaite J. and Taraban L.**	2015	

8. Annexes # 1 Daily microbiology identification test quality control sheet:

Test Name: Catalase 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																														