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)
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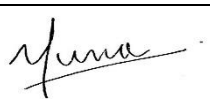
Signature		Signature	
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Acknowledgment

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Acronyms:

BA	Blood agar
CA	Chocolate agar
MAC	MaConkey
ATCC	American Type Culture Collection
ID	Identification
IQC	Internal Quality Control
SOP	Standard operating procedure
GC	Gonococcus
STIs	Sexually Transmitted Diseases
SAB	Sabouraud Agar

1. Purpose

This document describes the procedure for culture and isolation of organisms known to cause urethritis in male patients.

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 Urethritis: is the inflammation of the urethra (lower part of the urinary tract). The most common symptoms include dysuria and urethral discharge.

4. Procedure

4.1. Clinical background:

Urethritis in men is clinically characterized by a urethral discharge and/or dysuria. Asymptomatic infection with Neisseria gonorrhoeae (NG) or Chlamydia trachomatis (CT) occurs frequently. Rectal and oropharyngeal infection with N. gonorrhoeae and C. trachomatis may occur in homosexual men. Urethritis caused by organisms other than N. gonorrhoeae is called nongonococcal urethritis and caused by C. trachomatis, Ureaplasma urealyticum or Trichomonas vaginalis and human herpesvirus. Bacterial agents such as staphylococci, various Enterobacteriaceae, Acinetobacter spp. and Pseudomonas spp. can be isolated from the urethra of healthy men, but have not been shown to cause urethritis. The detection of C. trachomatis requires non-culture methods such as enzyme immunoassays, immunofluorescence assays and nucleic acid amplification tests have recently become available and will not be discussed in this document.

4.2. Pre – analytical stage:

4.2.1. **Sample:**

- Use aseptic technique.
- Collect swabs into Amies transport medium with charcoal and transport in sealed plastic bags at room temperature to the laboratory.
- Specimens should be transported and processed as soon as possible preferably within 4 hrs. Samples collected more than 48hrs before reaching lab, should be rejected.

- If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48hr are undesirable.
- Collect specimens before antimicrobial therapy where possible.
- Contamination with micro-organisms from the foreskin should be avoided.
- The patient should not have passed urine for at least 1 hour. If a discharge is not apparent, attempts should be made to "milk" exudate from the penis. The swab is gently passed through the urethral meatus and rotated.

4.2.2. **Material:**

Reagents	**Consumables/Supplies**	**Equipment**
Blood Agar plate Chocolate plate GC Sabouraud Agar Gram stain reagents Oxidase API NH Rapid Neisseria identification test	Microscopic slides Sterile loops	Microscope Slide dryer Safety cabinet class II Incubators

4.2.3. **Safety precaution:**

- All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times. Universal Precautions must be practiced at all stages of these procedures.

4.2.4. **Quality control:**

- Check the expiry dates of all media, reagents and stains before use.
- All media, reagents, kits, and stains **MUST** be quality controlled before use.
- Identification tests should be run with appropriate controls.
- Record the quality control results in the appropriate QC sheet.

4.3. Analytical stage:

4.3.1. **Sample reception:** after checking the Urethral swab acceptability criteria, add urethral microscopy as a reflex test.

4.3.2. **Microscopy (Gram stain):**

4.3.2.1. Gram stain has excellent specificity for diagnosis of Neisseria gonorrhea.

4.3.2.2. Prepare a thin smear on a clean microscope slide for Gram stainingafter inoculating the culture media.

4.3.3. Perform Gram Stain.

4.3.4. Examine the slide for bacteria, pus cells, epithelial cells and yeast.

4.3.4.1. Examine the specimen for polymorphs and Intracellular Gram Negative Diplococci under high power field (HPF) with the 100x objective and proceed as follows in table (1):

Table (1): Quantification of WBC/ Bacterial count under HPF microscopy

Gram stain reporting (HPF)		
WBC, or organisms count	1- 5, some fields without WBC/ organisms	Scanty
	5- 10/ HPF	+
	10- 25/HPF	++
	>25 /HPF	+++

4.3.5. Culture of Urethral Swab:

4.3.5.1. Inoculate the swab on the surface of BA, CA and GC media and streak using sterile loop. Add Sabouraud Agar (SAB) if yeast seen in the Grams stain.

4.3.5.2. Incubate the BA in CO_2 at $35^o – 37\ ^oC$ for 24 hours,

4.3.5.3. Incubate CA and GC in CO_2, and SAB in O_2 at $35^o – 37\ ^oC$, x 48 hrs.

4.3.5.4. Examine BA and CA at 24 hours.

4.3.5.5. Examine GC, CA & SAB plates at 48 hrs.

4.3.6. Identification and Isolation:

4.3.6.1. Gram stain: Quantitate the presence or absence of pus cells and organisms.

4.3.6.1.1. Report any intracellular gram negative diplococci.

4.3.6.2. Culture:

4.3.6.2.1. Any suspected growth of *Neisseria gonorrhoea* needs further investigation with Oxidase and Grams stain.

4.3.6.2.2. Confirm the identification of the organism by different methods.

4.3.6.2.3. Refer any suspected *Neisseria gonorrhoea* to reference laboratory for conformation.

4.3.6.2.4. Discuss any other pure growth with microbiologist to decide about the need for further processing.

4.3.7. Susceptibility Testing:

As per the national antibiotic susceptibility testing guidelines.

4.4. Post – analytical stage:

4.4.1. Reporting:

4.4.1.1 Gram stain: Report the presence or absence of pus cellsand organisms such as gram negative diplococci with quantification as per table (1).

4.4.1.2 Culture:

- Negative report: "No significant growth"
- Positive Report: report significant isolate
- For *Neisseria gonorrhoea* refer the isolate, release provisionally with the comment "presumptive *Neisseria gonorrhoea*, confirmation and susceptibilities from Central Public Health Laboratory to follow"

4.4.1.3 A presumptive identification of N. gonorrhoeae is based on a positive oxidase reaction and a Gram-stained smear showing Gram-negative diplococci.

4.4.1.4 Telephone all presumptive Neisseria gonorrhoea isolate to physician or clinical location and recommend the E-Notification of Communicable Diseases.

4.4.1.5 Recommend screening the patient and his/ her partner for other STIs.

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
Clinical microbiology procedures Handbook, 4th edition.	Amy L. Leber.	2016	
Genital Tract Culture Manual, MOUNT SINAI HOSPITAL, DEPARTMENT OF MICROBIOLOGY	QA Committee	2022	
UK Standards for Microbiology Investigations	Standards Unit, Public Health England	2017	