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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Acknowledgment

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Acronyms:

API 20 NE	Analytical Profile Index twenty non-*Enterobacteriaceae*
MAC	MacConkey Agar
ATCC	American Type Culture Collection
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
SOP	Standard operating procedure

1. **Purpose:**

   This document describes the procedure for API 20 NE 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 Non-fastidious: bacteria that does not require any special requirements substances or conditions essential to grow on the culture medium.

4. **Procedure:**

   4.1. Clinical background:

   API 20 NE test which is a standardized identification system for non-fastidious Gram-negative rods (bacilli) not belonging to the *Enterobacteriacae* family and used generally for the oxidase positive, non-fermentative groups of organisms. This test combines 8 conventional tests and 12 assimilation tests.

   4.2. Principle:

   The API 20 NE strip comprises 20 micro tubes containing dehydrated substrates. These conventional tests are inoculated with a saline bacterial suspension that reconstitutes the media. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. The assimilation tests are inoculated with a minimal medium (20AUX) and the bacteria grow if they are capable of utilizing the substrates. The reactions are read according to the Reading Table and the identification is obtained by referring to the Analytical Profile Index.

   4.3.Pre – Analytical stage:

   4.3.1. Sample:

   4.3.1.1 Sample type: API 20 NE is not for use directly with clinical or other specimens. The microorganisms to be identified must first be isolated on a suitable culture medium according to standard microbiological techniques. Pure identical single young growth colonies can be used.

   4.3.1.2 Amount of sample required, including minimum requirements: 1-4 colonies.

   4.3.1.3 Sample stability and storage requirements: to be processed immediately.

   4.3.1.4 In case of delay, store the sample in 2-8°C.

4.3.2. Material:

Reagents	Consumables/Supplies	Equipment
• MacConkey plate. • Api 20NE kit (strips, incubation boxes and inoculation medium). • Sterile Saline. • James supplied separately stored in the dark at 2-8°C. • NIT 1& 2 supplied separately is stored at 2-30°C. • Zinc powder supplied separately. Stored at 8-30°C • mineral oil	• Sterile loops. • Sterile 5 ml container. • Sterile disposable pasture pipette 1–3 ml.	• Safety cabinet class II. • Incubators at 30 °C.

4.3.3. 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.

4.3.4. Quality control:

- Check the expiry dates of API, media, reagents and stains before use.
- All API, media, reagents, kits, and stains **MUST** be quality controlled before use.
- Each LOT of API must be QC before usage.
- Identification tests should be run with appropriate ATCC controls (*Pseudomonas aeroginosa* ATCC 27853 and / or *Aeromonas hydrophilia* ATCC 35654).
- Record the quality control results in the appropriate QC sheet.

- In case of any QC failure, stop using the API, investigate, fill the (laboratory reagent / chemicals/ disposables/quality reporting) form after informing the supervisor.

4.4. Analytical stage:

4.4.1 Prepare the strip:

4.4.1.1 Prepare a tray and lid and distribute 5-ml.of water into the tray wells to create a humid atmosphere.

4.4.1.2 Place the strip in the tray.

4.4.2 Prepare the inoculum:

4.4.2.1 Use an ampoule of NaCl 0.85% medium to prepare the inoculum.

4.4.2.2 Add 1-4 well isolated colonies and emulsify to create a homologous suspension (turbidity must be 0.5 McFarland standard).

4.4.3 Inoculate the strip:

4.4.3.1 With a sterile pipette, fill tubes of tests NO3 to PNPG with the bacterial suspension.

4.4.3.2 Open an ampoule of AUX , add 200 µl (6-8 drops) of the saline suspension, homogenize.

4.4.3.3 Fill the tubes and cupules of tests **GLU** to **PAC** with the suspension. Leave cupule meniscus flat or slightly convex.

4.4.3.4 Create anaerobiasis in the tests **ADH**, , **URE**, and, **GLU** by overlaying with mineral oil.

4.4.3.5 Close the incubation tray and incubate in air at 29±2°C for 24 hours.

4.4.3.6 Prepare Mac as purity plates for the bacterial suspension.

4.5.Post – analytical stage:

4.5.1. Interpretation table of API 20NE:

TESTS	REACTIONS	RESULTS	
		NEGATIVE	POSITIVE
NO_3-NO_2	NO_2 PRODUCTION	1 DROP NIT1+NIT2/WAIT 2-5 MIN.	
		COLOURLESS	PINK/RED
	REDUCTION TO N_2 GAS	IF NO_2 NEGATIVE ADD ZINC-WAIT 5 MIN.	
		PINK	COLOURLESS
TRP	INDOLE PRODUCTION	INDOL/WAIT 2 MINUTES	
		COLOURLESS, PALE GREEN/YELLOW	PINK
GLU	ACIDIFICATION	BLUE TO GREEN	YELLOW
ADH	ARGININE DIHYDROLASE	YELLOW	ORANGE/PINK/RED
URE	UREASE	YELLOW	ORANGE/PINK/RED
ESC	HYDROLYSIS (β-GLUCOSIDASE)	YELLOW	GREY/BRN/BLACK
GEL	HYDROLYSIS (PROTEASE)	NO PIGMENT DIFFUSION	PIGMENT DIFFUSION
PNPG	β-GALACTOSIDASE	COLOURLESS	YELLOW
GLU	ASSIMILATION	TRANSPARENT	OPAQUE
ARA		TRANSPAREN	OPAQUE
MNE		TRANSPAREN	OPAQUE
MAN		TRANSPAREN	OPAQUE
NAG		TRANSPAREN	OPAQUE
MAL		TRANSPAREN	OPAQUE
GNT		TRANSPAREN	OPAQUE
CAP		TRANSPAREN	OPAQUE
ADI		TRANSPAREN	OPAQUE
MLT		TRANSPAREN	OPAQUE
CIT		TRANSPAREN	OPAQUE
PAC		TRANSPAREN	OPAQUE
OX	ON FILTER PAPER CYTOCHROME OXIDASE	OXIDASE REAGENT -30 SEC.	
		COLOURLESS	PURPLE

4.5.2. Reading of the Strip

- After the incubation period, read the strip by referring to the Reading Table from software ATB NEW or APIWEB website.
- Record all spontaneous reactions (GLU, ADH, URE, ESC, GEL, and PNPG) on the result sheet.

- The reading of the two tests NO3 and TRP should be performed while protecting the assimilation tests from airborne contamination (to do this, cover the assimilation tests with the incubation box lid during the reading of the NO3 and TRP tests).

5. Responsibilities

5.1.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.

5.2.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
API 20 NE kits insert	BIOMERIEUX	2019	

8.1.Annexes: API 20NE Procedure bench guide: