





Ministry of Health

Document Title	API 20E Test SOP
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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Release Date	May 2023
Review Frequency	Three Year

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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:

API	Analytical Profile Index
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization Time-of-Flight mass spectrometry
CPHL	Central Public Health Laboratories
ONPG	O-Nitrophenyl-b-D-galactopyranoside
TDA	Tryptophan Desaminase
VP	Voges-Proskauer test
ADH	Arginine Dihydrolase
LDC	Lysine Decarboxylase
ODC	Ornithine Decarboxylase
URE	Urea
H ₂ S	Hydrogen Sulfide
IND	Indole
GEL	Gelatin
GLU	Glucose (hexose sugar)
MAN	Mannose (hexose sugar)
INO	Inositol (cyclic polyalcohol)
SOR	Sorbitol (alcohol sugar)
RHA	Rhamnose (methyl pentose sugar)
SAC	Sucrose (disaccharide)
MEL	Melibiose (disaccharide)
AMY	Amygdalin (glycoside)
ARA	Arabinose (pentose sugar)

1. Purpose:

The purpose of this document is to identify the bacteria of Oxidase negative gram negative *Enterobacteriaceae*.

2. Scope:

This document is applicable to all laboratory technologist staff in Oman.

3. Definition:

3.1 Incubation: the act of keeping an organism in favorable conditions for the growth and development.

4. Details: procedure:

4.1 Pre- analytical stage:

4.1.1 Background:

4.1.1.1 Use of API 20E:

API 20E is a standardized identification system for *Enterobacteriaceae* and other non-fastidious Gram-negative rods, which incorporates 20 miniaturized biochemical identification tests and a database.

4.1.1.2 Principle:

The API 20E strip comprises 20 micro tubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension, which reconstitutes the media. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the interpretations table and the identification is obtained by referring to the Identification table or the Analytical Profile Index or online API-web (<https://apiweb.biomerieux.com/login>).

4.1.2 Sample:

4.1.2.1 Sample type: well young growth of isolated colonies.

4.1.2.2 Sample source: solid culture medium.

4.1.2.3 Amount of sample required, including minimum requirements: 0.5 McFarland.

4.1.2.4 Sample stability and storage requirements: 18-24 hours.

4.1.2.5 Criteria for unacceptable samples and follow-up action: Culture older than 24 hours are not acceptable.

4.1.3 Materials:

Reagents	Consumables/Supplies	Equipment
Reagents : <ul style="list-style-type: none">○ MacConkey agar○ API 20E strips.○ James reagent.○ TDA reagent.○ VP 1 and VP2 reagents.○ Saline readymade (not supplied with kit).	5 ml sterile plastic container 10 µl sterile plastic loop Microscopic Oil immersion.	Incubator 37°C Fridge (Storage: 2-8°C).

Note:-

- The package of strips, once opened, must be sealed with the supplied clip seal.
- All reagents and strips must be stored at 2-8°C.

4.1.4 Quality control:

- API boxes, and reagents shall be labelled properly with opening date once in use.

The Strips and reagents should be used before its expiry date, and saved appropriately as manufacturer instructions.

Quality control shall be used once for every Lot number / Batch number.
- Use minimum of two types of Enterobacteriaceae ATCC strains as controls as follows:
 - 1st control: *E. coli*.
 - 2nd control: *Proteus mirabilis*.
 - Check the purity plate of the controls before the interpretation of the strip.
 - Record quality control result on the quality control sheet (see Annex #1, API 20E Quality control record sheet).

In case of QC failure of API strip, repeat and initiate investigation with correction.

4.2 Analytical stage:

4.2.1 Prepare the strip:

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

4.2.1.2 Place the strip in the tray and label it with the sample ID, Isolate number, the type of fermentation date.

4.2.2 Prepare the inoculum:

4.2.2.1 Use a 5ml bijou of sterile water or Saline to prepare the inoculum and label it.

4.2.2.2 Take pure colonies which are well isolated to the saline and emulsify to create a homologous suspension.

4.2.2.3 Subculture the suspension on labelled purity plate (MacConkey agar) and incubate at 37C for 18-24 hrs.

4.2.3 Inoculate the strip:

4.2.3.1 With a sterile pipette, fill both the tube and cupule of tests **CIT**, **VP**, **GEL** with the bacterial suspension.

4.2.3.2 Fill only the tubes of the other tests.

4.2.3.3 Create anaerobiasis in the tests ADH, LDC, ODC, URE, and, H2S by overlaying with mineral oil.

4.2.3.4 Close the incubation tray and incubate in air at 35-37C for 18-24 hours.

4.2.4 Results:

4.2.4.1 Check purity plate for purity. If not pure, repeat the test.

4.2.4.2 If the plate is pure, proceed by reading the strip through the

Interpretation Table of API 20E.

4.2.4.3 If less than 3 tests are negative on the API strip, Re-incubate the strip for 24 hours without adding any reagent.

4.2.4.4 If 3 tests or more are positive on the API strip add the reagents for VP, TDA, IND to reveal the reaction.

4.2.4.5 If necessary for identification, NO₂ reagents can be added to the glucose tubule; motility, Mac growth, and fermentation of glucose, can be checked.

4.2.4.6 Record all spontaneous reactions on the sheet.

4.2.5 Interpretation Table API 20E:

<u>TESTS</u>	<u>REACTIONS</u>	<u>RESULTS</u>	
		<u>NEGATIVE</u>	<u>POSITIVE</u>
ONPG	BETA-GALACTOSE	COLOURLESS	YELLOW (1)
<u>ADH</u>	ARGININE DIHYDROLASE	YELLOW	RED/ORANGE (2)
<u>LDC</u>	LYSINE DECARBOXYLASE	YELLOW	ORANGE
<u>ODC</u>	ORNITHINE DECARBOXYLASE	YELLOW	RED/ORANGE (2)
<u>CIT</u>	CITRATE UTILIZATION	PALE GREEN/YELLOW	BLUE- GREEN/BLUE (3)
<u>H₂S</u>	H ₂ S PRODUCTION	COLOURLESS/GR EYISH	BLACK
<u>URE</u>	UREASE	YELLOW	RED/ORANGE
TDA	TRYPTOPHANE DESAMINASE	1 DROP TDA REAGENT/IMMEDIATE	
		YELLOW	DARK BROWN
IND	INDOLE PRODUCTION	INDOLE REAGENT—WAIT TWO MIN.	
		YELLOW	RED
<u>VP</u>	ACETOIN PRODUCTION	1 DROP VP1+VP2—WAIT 10 MIN.	
		COLOURLESS	PINK/RED
<u>GEL</u>	GELITINASE	NO DIFFUSION OF BLACK	DIFUSION OF BLACK
GLU MAN INO SOR RHA SAC MEL AMY ARA	FERMENTATION/OXIDATION(4)	BLUE/BLUE- GREEN	YELLOW
OX	ON FILTER PAPER CYTOCHROME OXIDASE	OXIDASE REAGENT -30 SEC.	
		COLOURLESS	PURPLE
NO₃-NO₂	NO ₂ PRODUCTION	1 DROP NIT1+NIT2/WAIT 2-3 MIN.	
		COLOURLESS	RED
	REDUCTION TO N ₂ GAS	IF NO ₂ NEGATIVE ADD ZINC	
		RED	YELLOW
MOB	MOTILITY	NON-MOTILE	MOTILE
MAC	GROWTH	NO GROWTH	GROWTH
OF	(API OF) FERMENTATION –CLOSED OXIDATION - OPEN	GREEN GREEN	YELLOW YELLOW

- 1) A very pale yellow should be considered positive
- 2) An orange colour after 24 hours is negative
- 3) Reading made in the cupule
- 4) Fermentation begins in the lower portion, oxidation begins in the cupule

4.3 Post-analytical stage:

- 4.3.1 On the API 20E result sheet, the tests are separated into groups of 3 and a value 1, 2 or 4 is indicated for each. By adding together the values corresponding to positive reactions within each group, a 7-digit profile number is obtained for the 20 tests of the API 20 E strip.
- 4.3.2 Use the online API-web (<https://apiweb.biomerieux.com/login>) to enter the API profile index number to get identification of the bacteria.
- 4.3.3 If API failed to identify the bacteria, the isolate (Only from sterile sites) should be referred out to CPHL-Darsit for MALDI-TOF.
- 4.3.4 Subtype *Salmonella* sp. and *Shigella* sp. isolates with anti-sera.

5. Responsibility:

- 5.1. Responsible staff shall:
 - 5.1.1 To ensure the adherence to this procedure.
 - 5.1.2 To perform and record the QC.
- 5.2. Quality manager /officer:
 - 5.2.1 To follow up the implementation of the procedure
 - 5.2.2 To monitor regularly QC performance of the test and raise non-conformance with corrective action in case of any QC failure.

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
Package insert “ <u>API 20E</u> ”, http://www.biomerieux.com	bioMérieux	2002	

8. Annexes #1 API 20E Quality control record sheet:

Manufacture Name: bioMérieux	Date:	Done By:	Signature:	Supervisor sig.
Name of reagent	Lot Number	Expiry Date	Date opened	QC Status
API 20E Kit strip				
TDA				
IND				
VP				

AT CC	ON PG	AD H	LD C	OD C	CI T	H ₂ S	UR E	TD A	IN D	VP	GE L	GL U	MA N	IN O	SO R	RH A	SA C	ME L	A M Y	AR A	N O ₂	N 2*
1	+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	+	+	-
Test																						
3	+	+	-	+	+	-	-	-	-	+	-	+	+	-	+	+	+	+	+	+	+	-
Test																						
4	-	-	-	+	V	+	+	+	-	-	V	+	-	-	-	-	V	-	-	-	+	-
Test																						
5	+	-	+	-	+	-	V	-	-	V	-	+	+	+	+	+	+	+	+	+	+	-
Test																						

1. *Escherichia coli* ATCC 25922.
1. *Stenotrophomonas maltophilia* ATCC 51331.
2. *Enterobacter cloacae* ATCC 13047.
3. *Proteus mirabilis* ATCC 35659.
4. *Klebsiella pneumoniae* ssp *pneumoniae* ATCC 35657.