

SOP: API 20E Microbial Identification

Approvals:

Preparer: Dr. Maggie Bryans
Reviewer: Jason McMillan
Reviewer: Winsome Grenyion

Date: 17MAR14
Date: 18MAR14
Date: 05OCT18

1. Purpose:

1.1. To perform a microbial identification assay.

2. Scope:

2.1. This procedure is intended as a standardized identification system for *Enterobacteriaceae* and non-fastidious Gram-negative rods included in the database.

3. Responsibilities:

- 3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
- 3.2. It is the responsibility of the students to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

4. References:

- 4.1. API 20E System Brochure
- 4.2. Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – cGMP (FDA publication, September 2004)
- 4.3. United States Pharmacopeia 25
- 4.4. Gram Stain SOP
- 4.5. Bergey's Manual of Systematic Bacteriology
- 4.6. Oxidase Test SOP

5. Definitions:

5.1. *Enterobacteriaceae*: Family of Gram-negative, rod bacteria that inhabit soil, water and are commonly found in the large bowel of humans. Most common organisms isolated from clinical specimens.

6. **Precautions:** Aseptic technique and standard precautions for handling microbial cultures.

7. Materials:

Test Setup

- 7.1. API 20E strip, API Suspension Medium, incubator tray and cover
- 7.2. Bacterial cultures including *E. coli* control (ATCC #25922)
- 7.3. Disposable gloves
- 7.4. Test tube rack and sterile disposable pipettes
- 7.5. McFarland Standard # 0.5
- 7.6. Sterile wooden sticks
- 7.7. Sterile mineral oil
- 7.8. 37°C Incubator
- 7.9. Squirt bottle - approx. 10 ml of tap water

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Development

- 7.10. Oxidase Dropper, filter paper/cotton swab
- 7.11. Kovac's (James) reagent
- 7.12. VPI and VP2 reagents
- 7.13. 10% ferric chloride
- 7.14. Nitrate reagents (I&II)
- 7.15. Zn dust
- 7.16. API 20E Result sheets
- 7.17. API 20E Quick Index Booklet or the API 20E Profile Recognition System

8. Procedure:

- 8.1. Using the Gram-stain technique (Gram-stain SOP) determine that the bacterial culture is a Gram-negative rod.
- 8.2. **Oxidase test:**
 - 8.2.1. Perform the Oxidase test according to the Oxidase Test SOP.
 - 8.2.2. Record the result on the API 20E Results sheet (21st identification test).
- 8.3. **Preparation of the inoculum:**
 - 8.3.1. Open an ampule of API Suspension Medium (5 ml).
 - 8.3.2. Using a sterile wooden stick, remove a well-isolated colony from the streak plate and transfer to the API Suspension Medium. Mix to emulsify and obtain a homogenous suspension.
 - 8.3.3. Check the turbidity of the suspension to that of the McFarland Standard # 0.5. If necessary, add more bacteria.
 - 8.3.4. The suspension must be used promptly after preparation.
- 8.4. **Preparation of the API strip:**
 - 8.4.1. Obtain an incubation box (tray and lid).
 - 8.4.2. Using a squirt bottle, add 5 ml of water to the bottom of the incubation tray.
 - 8.4.3. Record your name, date and strain reference on the elongated flap of the incubation tray.
 - 8.4.4. Remove the strip from the sealed pouch and place it in the incubation tray.
- 8.5. **Inoculation of the API strip:**
 - 8.5.1. Gently shake the 5 ml of bacterial suspension.
 - 8.5.2. Remove the cap and fill a sterile disposable pipette with the bacterial suspension.
 - 8.5.3. Tilt the incubation strip to avoid forming air bubbles.
 - 8.5.4. Using the filled disposable pipette, fill both the tube and the cupule of the tests CIT, VP and GEL.
 - 8.5.5. Fill only the tube (NOT the cupule) of the other tests.
 - 8.5.6. Use the sterile mineral oil to completely overlay and fill the cupule section of the tests ADH, LDC, ODC, H₂S and URE.
 - 8.5.7. Place the lid on the incubation tray and incubate at 36°C ± 2°C for 18-24 hours.
 - 8.5.8. Make an isolation streak on a TSA plate with a portion of the bacterial suspension to ascertain the purity of the sample.

8.6. Reading the API strip:

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- 8.6.1. After the incubation period, read the strip by referring to the Reading Table.
- 8.6.2. If **3 or more tests** are positive, record all reactions not requiring the addition of reagents (do NOT read TDA, VP, IND). Record the results by placing a (+) for a positive reaction and a (-) for a negative reaction and continue with step 8.6.3. If the number of positive tests is **less than 3**, reincubate the strip for a further 24 hours (\pm 2 hours) before continuing to step 8.6.3.
- 8.6.3. Reveal the tests which require the addition of reagents and add the following test reagents in the order listed. In all cases, read the results immediately after adding the reagents and waiting the proper length of time. Do not replace the lid on the tray until all results have been collected. Record results.
 - 8.6.3.1. **TDA test:** Add 1 drop of 10% ferric chloride to the TDA tube. A reddish brown color indicates a positive reaction. A negative reaction is yellow.
 - 8.6.3.2. **VP test:** Add 1 drop each of VP1 (40% KOH) and VP2 (α -naphthol) solutions to the VP microtube. The KOH solution should be added first. Wait at least 10 minutes. A pink color developed in the whole cupule indicates a positive reaction. No color change is a negative reaction.
 - 8.6.3.3. **IND test:** This test must be performed last. Add 1 drop of Kovac's/James reagent to the IND tube. A positive test is indicated by a red ring within 2 minutes. A yellow ring is a negative reaction.

8.7. Interpretation:

- 8.7.1. Identification is determined by the numerical profile.
- 8.7.2. On the result sheet, the tests are separated into groups of 3 and a value of 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 20E strip. The oxidase reaction constitutes the 21st test and has a value of 4 if it is positive.
- 8.7.3. Identify the microorganism by entering the 7-digit numerical profile manually using the APIweb software.
- 8.7.4. Print out and record your results.
- 8.7.5. If the API 20E strip was incubated for 48 hours, and a 7-digit profile is not discriminatory enough perform the following supplementary tests.
 - 8.7.5.1. Reduction of nitrates to nitrites (NO₂) and N₂ gas: Add 1 drop each of NIT 1 and NIT2 to the GLU tube. Wait 2 to 5 minutes. A red color indicates a positive reaction (NO₂). A negative reaction (yellow) may be due to the reduction to nitrogen. Add 2 to 3 mg of Zn dust to the GLU tube. After 5 minutes, if the tube remains yellow this indicates a positive reaction (N₂). If the test turns orange-red, this is a negative reaction.
 - 8.7.5.2. Identify the microorganism by entering the 9-digit numerical profile manually using the APIweb software.

9. Attachments:

- 9.1. Figure 1: Reading Table

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10. History:

Name	Date	Amendment
Jason McMillan	18MAR14	Initial Release
Winsome Grenyion	05OCT18	Edited materials & procedure

11. Attachments:

Figure 1: Reading Table

TESTS	ACTIVE INGREDIENTS	QTY (mg/cup.)	REACTIONS/ENZYMES	RESULTS	
				NEGATIVE	POSITIVE
ONPG	2-nitrophenyl-βD-galactopyranoside	0.223	B-galactosidase (Ortho NitroPhenyl-βD-Galactopyranosidase)	colorless	yellow (1)
ADH	L-arginine	1.9	Arginine DiHydrolase	yellow	red/orange (2)
LDC	L-lysine	1.9	Lysine DeCarboxylase	yellow	red/orange (2)
ODC	L-ornithine	1.9	Ornithine DeCarboxylase	yellow	red/orange (2)
CIT	Trisodium citrate	0.756	CITrate utilization	pale green/yellow	blue-green/blue (3)
H ₂ S	sodium thiosulfate	0.075	H ₂ S production	colorless/greyish	black deposit/thin line
URE	urea	0.76	UREase	yellow	red/orange (2)
TDA	L-tryptophane	0.38	Tryptophane DeAnimase	TDA/immediate	
				yellow	reddish brown
IND	L-tryptophane	0.19	INDole Production	James/immediate	
				colorless pale green/yellow	pink
VP	sodium pyruvate	1.9	acetoin production (Vogues Proskauer)	VP1 + VP2/10 min	
				colorless	pink/red (5)
GEL	Gelatin (bovine origin)	0.6	GELatinase	no diffusion	diffusion of black pigment
GLU	D-glucose	1.9	fermentation/oxidation (GLUcose) (4)	blue/blue-green	yellow/greyish yellow
MAN	D-mannitol	1.9	fermentation/oxidation (MANnitol) (4)	blue/blue-green	yellow
INO	onositol	1.9	fermentation/oxidation (INOsitol) (4)	blue/blue-green	yellow
SOR	D-sorbitol	1.9	fermentation/oxidation (SORbitol) (4)	blue/blue-green	yellow
RHA	L-rhamnose	1.9	fermentation/oxidation (RHAmmose) (4)	blue/blue-green	yellow
SAC	D-sucrose	1.9	fermentation/oxidation (SACcharose) (4)	blue/blue-green	yellow
MEL	D-melibiose	1.9	fermentation/oxidation (MELibiose) (4)	blue/blue-green	yellow
AMY	amygdalin	0.57	fermentation/oxidation (AMYgdalin) (4)	blue/blue-green	yellow

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ARA	L-arabinose	1.9	fermentation/oxidation (ARAbinose) (4)	blue/blue-green	yellow
OX	(see oxidase test package insert)		cytochrome-Oxidase	(see oxidase test package insert)	

- (1) A very pale yellow should also be considered positive.
 - (2) An orange color after 36-48 hours incubation must be considered negative.
 - (3) Reading made in the cupule (aerobic).
 - (4) Fermentation begins in the lower portion of the tubes, oxidation begins in the cupule.
 - (5) A slightly pink color after 10 minutes should be considered negative.
- * The quantities indicated may be adjusted depending on the titer of the raw materials used.
* Certain cupules contain products of animal origin, notably peptones.