PURPOSE: The API 20 E system is used for the identification of Enterobacteriaceae as well as other Gram-negative bacteria. The strip consists of 21 microtubes containing a variety of different dehydrated substrates. The reactions are read and recorded into the APIweb electronic database. The software interprets the reactions and provides identification of the organism.

SAMPLE INFORMATION:

Type	One, well isolated colony

REAGENTS and/or MEDIA:

Type	BioMerieux API 20 E reagent strip, tray and lid
Stability and Storage Requirements	• Store strips at 2°C to 8°C. • Strips are supplied in an aluminum pouch with desiccant sachets. • Strips are stable for 10 months after opening the foil pouch or until the expiration date indicated on the package. • Once opened, the pouch should be re-sealed using the clip seal included in the kit.

SUPPLIES:

- Sterile water
- 0.9% sterile saline
- Plastic Vitek tubes and caps
- Disposable inoculation needles
- Sterile pipettes
- Mineral oil
- Blood agar
- 35° ambient air incubator
- Ferric chloride reagent
- Kovacs reagent
- Alpha naphthol reagent
- 40% Potassium hydroxide reagent
- Nitrate reagent 1 and 2
- Zinc powder
- Oxidase reagent

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Quality control is performed on new shipments/new lot numbers:
  - *Proteus mirabilis* ATCC 35659
- A TQC order is automatically generated when a new kit is received to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the API 20 E	
1	**Preparation of the strip:** a. Dispense 5 mL of sterile water into the honey-combed wells of the tray to create a humid atmosphere. b. Record the specimen accession number and date and time on the elongated flap of the tray. Do not record on the lid as it may be misplaced during the procedure. c. Remove the strip from its foil packaging. d. Place the strip in the incubation box.
2	**Preparation of the inoculum:** a. Add 5 mL of 0.9% sterile saline to a test tube. b. Using a disposable inoculation needle, remove a single, well-isolated colony and emulsify in the saline creating a homogenous bacterial suspension. c. This suspension must be used immediately after preparation.
3	**Inoculation of the strip:** a. Tilt the incubation tray and fill the tube section of the microtubes by placing the pipette tip against the side of the cupule to minimize bubble formation. **NOTE:** The ADH, LDC, ODC, H_2S and URE reactions are interpreted best if these microtubes are slightly under filled. b. Fill the tube and cupule of the tests [CIT], [VP] and [GEL] with the bacterial suspension. c. Using the same pipette, place one drop of the organism suspension on a Blood agar plate and use the pipette to streak for purity check. d. After inoculation, completely fill the cupule section of the ADH, LDC, ODC, H_2S and URE tubes with mineral oil to create an anaerobic environment. e. Close the incubation box and incubate at 36°C ± 2°C for 18 to 24 hours in the O_2 incubator.

4	**<u>Reading the strip</u>:** a. After 18 hours and before 24 hours, read the strip by referring to the Reading Table in the appendix of this procedure. b. Inspect the purity plate. If not pure, repeat using a pure culture. c. Take note of the positive tests: if the number of positive tests including GLU is less than 3, do not add reagents. Re-incubate for an additional 24 hours before adding any reagents. d. If 3 or more tests (GLU test + or -) are positive, read the spontaneous reactions and record as + or – on the results sheet. e. Add the following reagents: **TDA test:** add 1 drop of Ferric Chloride reagent. **IND test:** add 1 drop of Kovacs reagent. **VP test:** add 1 drop of each VP1 and VP2 reagents. Wait at least 10 minutes. f. Read the reactions by referring to the Reading Table.
5	**<u>Interpretation of the strip:</u>** a. Identification is obtained with the numerical profile of the organism. b. The tests are separated into groups of three. c. The following numerical value is assigned to each reaction recorded: 1 – Positive reaction in the first test of the group 2 – Positive reaction in the second test of the group 4 – Positive reaction in the third test of the group 0 – Negative reaction in any test d. 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. e. The oxidase reaction constitutes the 21st test and has a value of 4 if it is positive. f. In some cases, the 7 digit profile is not discriminatory enough and the following supplementary tests need to be carried out: • Reduction of nitrates to nitrites (NO_2) and N_2 gas: Add 1 drop of each Nitrate 1 and Nitrate 2 reagent to the GLU tube. Wait 2 minutes. If reaction is negative, add 2-3 mg of Zn dust to the GLU tube. Read after 5 minutes. • This reaction is useful when testing Gram-negative, oxidase positive rods.

6	**Identification:** a. Log into APIweb: https://apiweb.biomerieux.com. • At login, type: **laura_steven@gov.nt.ca** • At password, type: **YKNIFE** • Select **"Go"** • Select **"API 20 E"** from the list b. Enter the profile number results and select "Confirm". Record the profile number and result in the LIS.

LIMITATIONS/PRECAUTIONS:

1. The API 20 E system is intended for the identification of those non-fastidious, Gram-negative bacilli included in the database. It cannot be used to identify other organisms or exclude their presence.
2. Test only pure cultures of a single organism.
3. A slight pink colour in the VP test after 10 minutes should be considered negative.
4. The biochemical reactions should be read after 18 to 24 hours incubation. If the test cannot be read at 24 hours incubation, remove strips from incubator and store in refrigerator until reactions can be read.
5. The indole production test and nitrate reduction test must be performed last since this reaction releases gaseous products which interfere with the interpretation of other tests on the strip. The plastic incubation lid should not be replaced after the addition of the reagent.
6. To complete the identification, it may be necessary to perform supplementary tests.

REFERENCES:

- BioMerieux, API 20 E package insert and API 20 E reagents package insert, 2016/12

APPENDIX (APPENDICIES):

I. Reading Table:

TUBE	INCUBATION	POSITIVE	NEGATIVE	COMMENTS
ONPG		Yellow	Colourless	(1) Any shade of yellow is a positive reaction. (2) VP tube, before the addition of reagents, can be used a negative control.
ADH	18-24 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	Orange reactions occurring at 36-48 hours should be interpreted as negative.
LDC	18-24 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	Any shade of orange within 18-24 hours is a positive reaction. At 36-48 hours, orange decarboxylase reactions should be interpreted as negative.
ODC	18-34 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	Orange reactions occurring at 36-48 hours should be interpreted as negative.
CIT		Turquoise or Dark Blue	Light Green Or Yellow	(1) Both the tube and cupule should be filled. (2) Reaction is read in the aerobic (cupule) area.
H_2S		Black Deposit	No Black Deposit	(1) H_2S production may range from a heavy black deposit to a very thin black line around the tube bottom. Carefully examine the bottom of the tube before considering the reaction negative. (2) A "browning" of the medium is a negative reaction unless a black deposit is present. "Browning" occurs with TDA positive organisms.
URE	18-24 hr 36-48 hr	Red or Orange Red	Yellow Yellow or Orange	A method of lower sensitivity has been chosen. *Klebsiella*, *Proteus* and *Yersinia* routinely give positive reactions.
TDA	Add 1 drop 10% Ferric chloride.	Brown-Red	Yellow	(1) Immediate reaction. (2) Indole positive organisms may produce a golden orange colour due to indole production. This is a negative reaction.
IND	Add 1 drop Kovacs Reagent	Red Ring	Yellow	(1) The reaction should read within 2 minutes after the addition of the Kovacs reagents and the results recorded. (2) After several minutes, the HCl present in Kovacs reagent may react with the plastic of the cupule resulting in a change from a negative (yellow) colour to a brownish-red. This is a negative reaction.

I. Reading Table cont.:

TUBE	INCUBATION	POSITIVE	NEGATIVE	COMMENTS
VP	Add 1 drop of 40% Potassium Hydroxide, then 1 drop of alpha-napthol.	Red	Colourless	(1) Wait 10 minutes before considering the reaction negative. (2) A pale pink colour which appears immediately after the addition of reagents but which turns dark `pink or red after 10 minutes should be interpreted as positive. Motility may be observed by hanging drop or wet mount preparation.
GEL		Diffusion of the pigment	No diffusion	(1) The solid gelatin particles may spread throughout the tube after inoculation. Unless diffusion occurs, the reaction is negative. (2) Any degree of diffusion is a positive reaction.
GLU		Yellow Or Gray	Blue or Blue-Green	COMMENTS FOR ALL CARBOHYDRATES Fermentation (*Enterobacteriaceae, Aeromonas, Vibrio*) (1) Fermentation of the carbohydrates begins in the most anaerobic portion (bottom) of the tube. Therefore, these reactions should be read from the bottom of the tube to the top. (2) A yellow colour at the bottom of the tube only indicates a weak or delayed positive reaction.
MAN INO SOR RHA SAC MEL AMY ARA		Yellow	Blue or Blue-Green	Oxidation (Other Gram-negatives) (1) Oxidative utilization of the carbohydrates begins in the most aerobic portion (top) of the tube. Therefore, these reactions should be read from the top to the bottom of the tube. (2) A yellow colour in the upper portion of the tube and blue in the bottom of the tube indicate oxidative utilization of the sugar. This reaction should be considered positive only for non-*Enterobacteriaceae* gram negative rods. This is a negative reaction for fermentative organisms such as *Enterobacteriaceae*.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	23 MAR 19	Initial Release	L. Steven