PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC53100 – API 20E Test	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
The API 20E test 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.

PURPOSE/RATIONALE:
This standard operating procedure describes how to perform the API 20E test.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) performing the API 20E test.

SAMPLE INFORMATION:

Type	One, well isolated colony

REAGENTS and/or MEDIA:

Type	bioMerieux API 20E strip
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
- Plastic Vitek tubes and caps
- Sterile saline
- Disposable inoculation needles
- Sterile pipettes
- Mineral oil
- Blood agar
- Ferric chloride reagent
- Kovacs reagent
- Alpha naphthol reagent
- 40% Potassium hydroxide
- Nitrate reagent 1 and 2
- Zinc powder
- Oxidase reagent
- Blood agar

EQUIPMENT

- 35° ambient air incubator

SPECIAL SAFETY PRECAUTIONS:

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

- Ensure that appropriate hang hygiene practices be used.
- 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 of 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. Routine Practices 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 20E test	
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 3 mL of 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 **NOTE:** 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 **NOTE:** The quality of filling is very important e. Close the incubation box and incubate at 36°C ± 2°C for 18 to 24 hours in the O_2 incubator
4	**Reading the strip:** a. After 18 hours and before 24 hours, read the strip 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 VP1 and VP2 reagents. Wait at 10 minutes f. Read the reactions by referring to the Reading Table on APIweb

5	**Interpretation of the strip:** 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 20E 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

INTERPRETATION OF RESULTS:

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

LIMITATIONS:

1. The API 20E 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.

REFERENCES:

1. bioMérieux. (2016-12*). API 20E* package insert

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	23 Mar 19	Initial Release	L. Steven
2.0	30 Jun 21	Procedure reviewed and added to NTHSSA policy template	L. Steven