| TITLE: API-20E | Revision Date: 20-April-2018 | Issue Date: 20-April-2016 |
|---|---|---|
| Document Number: MIC50200 | Status: Approved | |
| Distribution: Microbiology Test Manual | Page: 1 of 5 | |
| Approved by: S. Asmussen, Manager of Diagnostic Services | Signed by: | |

## PURPOSE:

The API-20E is a useful tool for the identification of *Enterobacteriaceae* and other non-fastidious Gram-negative bacilli. It uses 21 miniaturized biochemical tests and an online database for identification. Organisms tested should be Gram-negative, oxidase negative bacilli. A few oxidase positive non-fastidious organisms can be identified as well.

## SAMPLE INFORMATION:

| | |
|---|---|
| **Storage Requirements** | Store at 2-7°C, stable for 10 months after opening foil pouch. |

## REAGENTS and/or MEDIA:

- API 20E strip and incubation box (bioMerieux Inc, REF 20 100)
- Blood Agar Plate (BAP)
- 5 mL of 0.85% NaCl
- ~5 mL sterile water
- Ferric Chloride Reagent
- James or Kovacs Reagent
- VP1 and VP2 Reagent
- NIT1 and KOH Reagent
- Zn Powder
- Mineral Oil

## SUPPLIES:

- Sterile Pipette

## 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 where 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.

## QUALITY CONTROL:

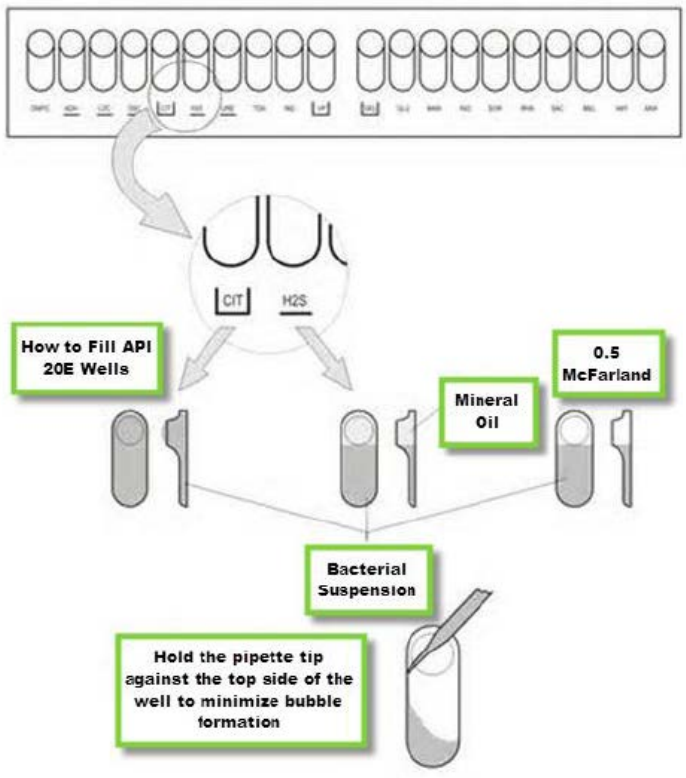
QC is to be performed on each shipment or new lot # received.

- **QC organism**: *Proteus mirabilis* ATCC# 35659 - supplied on Culti-Loops from Remel/Thermo Scientific.
- **Method:** Streamlined QC. Tests for degradation of the most delicate reagents on the strip:

| ONPG | ADH | LDC | ODC | CIT | H2S | URE | TDA | IND | VP | GEL | GLU | MAN | INO | SOR | RHA | SAC | MEL | AMY | ARA | NO2 | N2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | - | + | + | - | - | - | - | + | - | - | + | + | - | + | + | - | + | - | + | + | - |

Generate TQC order via TQC Order Entry – result QC results in TQC.

## PROCEDURE INSTRUCTIONS:

| Step | Action |
|---|---|
| **Setting Up an API-20E** | |
| 1 | In your plate log – Order **^20E.** |
| 2 | Prepare the incubation tray by adding 5 mL of sterile water – Filling up the honeycomb wells. |
| 3 | Remove the strip from its packaging and place it in the incubation tray. |
| 4 | Write the specimen number on the flap attached to the tray and date. |
| 5 | Aliquot approximately 4 mLs of 0.85% saline into a plastic test tube. |
| 6 | Prepare a 0.5 McFarland suspension of the organism – the culture should be pure and 18-24 hours old. |
| 7 | Tilt the API strip and, using a sterile pipette; slowly distribute the bacterial suspension into the tubes.<br>• Hold the pipette tip against the top side of the well to minimize bubble formation.<br>  |
| 8 | For the ICITI, IVPI, and IGELI – Fill both the tube and the cupule. |

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| **9** | For all other tests, fill only the tube. |
| **10** | For all underlined tests: ADH, LDC, ODC, H2S, and URE – overlay with mineral oil to create an anaerobic environment. |
| **11** | Incubate at 36°C +/- 2°C for 18-24 hours. |
| **12** | Using 1 drop of the suspension, streak out a BAP for purity. |
| **13** | If **less than 3** tests are positive, re-incubate the strip for an additional 24 hours **BEFORE** adding any reagents. |
| **14** | Inspect the purity plate – if not pure, repeat API using a pure culture. |
| **15** | The following wells require the addition of reagent:<br>**TDA:** Add 1 drop of ferric chloride<br>**IND:** Add 1 drop of James (Kovacs) reagent ***Perform test last as the gas produced interferes with other tests.<br>**VP:** Add 1 drop each of VP1 and VP2 reagent – wait 10 minutes. |
| **16** | Refer to the Reference Table for colour reactions in the supplied package insert. Package inserts are located in the MIC D3 drawer to the right of the wound bench. |
| **17** | Log in to the apiweb: https://apiweb.biomerieux.com |
| **18** | Login name: **NSTANTONTERRITORIALHOSPITAL**<br>Password: **YKNIFE**<br>Hit **Go**. |
| **19** | Select the appropriate API item (ie. API20E). |
| **20** | Input reactions and hit "**CONFIRM**". |
| **21** | Print out ID sheet and evaluate the outcome. |

## REFERENCES:


- bioMerieux. (2006, 02). api 20E.

**REVISION HISTORY:**

| REVISION | DATE | Description of Change | REQUESTED BY |
|---|---|---|---|
| 1.0 | 31Jul13 | Initial Release | A. Darrach |
| 1.1 | 06Mar14 | Document control number changed from MTE10200 to MIC50200 | C. Russell |
| 1.2 | 08May14 | Revised QC Isolate to Manufacturer recommended isolate | L. Driedger |
| 2.0 | 31Mar16 | Update of “Special Safety Precautions” to reflect risk assessment recommendations. | C. Russell |
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