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| REAGENT QUALITY CONTROL FOR ID PANELS AND BIO TUBES |
| **Purpose** | This procedure provides instruction for REAGENT QUALITY CONTROL FOR ID PANELS AND BIO TUBESThe API 20E Reagent QC test is a standardized and abbreviated version of the API 20E strip used to quality control reagents used during ID panel testing and tube biochemicals. Each strip contains substrates for tryptophan deaminase, indole, nitrate reduction, and Voges-Proskauer. The substrates are incorporated into microtubes and provide a positive and negative control set on each strip. After reconstitution with a bacterial suspension and incubation, reagents are added to each microtube. Results are read based on a color change. Quality control is performed on each new lot or shipment of reagents and weekly thereafter. |
| **Policy Statements** | This procedure applies to laboratory personnel who perform culture set-up and/or plate reading. |
| **Materials** | **Reagents** | **Supplies** | **Equipment** |
|  | • Kovac’s indole (IND). Dade Behring MicroScan® product number B1010-41A.• 40% potassium hydroxide (VP1). Dade Behring MicroScan® product number B1010-43A.• α-napthol (VPA), Becton, Dickinson and Company product number 261192.• 0.8% sulfanilic acid (NIT1). Dade Behring MicroScan® product number B1010-44A.• 0.5% N, N-dimethylalphanapthylamine (NIT2). Dade Behring MicroScan® product number B1010-45A.• 10% ferric chloride (TDA). Dade Behring MicroScan® product number B1010-48A.• Granular zinc dust. Baker Chemical Company product number 1-4244 | • API 20E Reagent QC Strips. bioMérieux product number 20240.• API 20E Reagent QC inoculating trays and lids. Included in API20E Reagent QC kit.• 5-ml sterile, normal saline. Cardinal Health product number 4321819.• Sterile, cotton tipped applicator swabs. Warehouse product number 112.• Sterile, wooden applicator sticks. Cardinal Health product number A500-1.• Sterile, disposable plastic transfer pipettes. Cardinal Health product number P5214-1S.• MicroScan Reagent Bottle Dropper Caps. Dade Behring MicroScan® product number B1013-12A.• Deionized water. | • 35-37° C ambient air, non-CO2 incubator• 2-8° C refrigerator• Vortex |
| Sample | Quality Control Organisms |
|  | 1. *Enterobacter cloacae* ATCC 13047\*
2. *Proteus vulgaris* ATCC 13315\*
3. *Myroides (Flavobacterium) oderatus* ATCC 4651\*

\*Fresh, 18-24 hour old pure cultures of the recommended organisms isolated on non-selective agar should be used. |
| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policie*s***:**1. *Biohazard Containment*
2. *Safety in the Microbiology/Virology Laboratory*
* *Biohazardous Spills*
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| **Quality Control** | 1. Perform QC with each new lot or shipment before put into service. Record results in API QC manual.
2. Once kit is in use, Reagent QC is performed weekly.
3. If there is a QC failure; document observation, notify supervisor and call bioMérieux technical service at 1-800-682-2666.
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| **Procedure** | Inoculum Preparation:Using a sterile applicator stick or cotton swab, gently touch the center of a well-isolated colony 2-3 millimeters in diameter.1. Insert the inoculated applicator stick into a labeled tube of normal saline and mix well in a vortex-like fashion.
2. Repeat the previous steps for each organism to be tested.

Strip Preparation:1. Label the elongated flap of incubation tray with date testing is performed.
2. Distribute approximately five milliliters of deionized water into incubation tray to provide a moist environment.
3. Remove one API 20E Reagent QC Strip and place it into moistened incubation tray. Reseal pouch with clip seal.

Strip Inoculation1. API 20E Reagent QC strips contain eight labeled microtubes, each consisting of a tube and cupule portion. Fill only the lower, tube portion for TDA, IND, and NIT tests. Fill both the tube and cupule portions for the VP test.
2. Using a separate sterile pipette for each organism:
	1. Fill the first set of microtubes (1-4) with the suspension of *M. oderatus*.
	2. Fill the TDA, IND and NIT portions of the second set of microtubes (6-8) with the suspension of *P. vulgaris*.
	3. Fill the VP microtube (10) with the suspension of *E. cloacae*.
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| **Interpretation** | 1. After incubating 18-24 hours, add reagents to appropriate cupules on the strip. Quantities of reagents and reaction interpretation appear below.

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| **TEST:** | **REAGENT:** | **INTERPRETATION:** |
| **positive:** | **negative:** |
| TDA | 1 drop of TDA reagent | brown – red | yellow |
| IND | 1 drop of Kovac’s reagent | Red ring | Yellow |
| NIT | 2 drops of NIT1 reagent, and 2 drops NIT2 reagent; then zinc dust in tubes with negative reactions | NO2: redN2 gas: yellow with zinc | NO2: yellowN2 gas: red-pink with zinc |
| VP | 1 drop of VP1 reagent, then 1 drop VPA reagent | pink-red | colorless |

1. Interpretation of test results:
2. Expected reactions for each quality control organism appear below.

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|  | **TDA:** | **IND:** | **NIT:** | **VP:** |
| *M. oderatus* ATCC 4651 | - | - | - | - |
| *P. vulgaris* ATCC 13315 | + | + | + |  |
| *E. cloacae* ATCC 13047 |  |  |  | + |

1. If observed reactions match those in above table, reagents are considered satisfactory for use.
2. If any observed reaction differs from above table, the reagent is considered unsatisfactory and should be removed from use until the discrepancy is resolved.
3. The TDA and IND reactions produce an immediate color change.
4. When testing reagents for VP test, wait 10 minutes before considering the reaction negative. A pale pink color developing after 10 minutes should be considered negative. An immediate pale pink color changing to dark pink or red after 10 minutes should be considered positive.
5. A positive NIT reaction may take 2-5 minutes for color development. A red color change after the addition of zinc dust confirms a negative reaction.
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| **Limitations** | 1. The API 20E Reagent QC kit is designed for quality control of reagents used in the TDA, IND, NIT, and VP tests.
2. The kit is not designed as an identification scheme and should not be used as such.
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| **References** | bioMérieux. (2003). API 20E Reagent QC product insert. Revised November 2003. Durham, North Carolina. |
| **Training Plan/** **Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Pat Ackerman | 02/02/1992 | Initial Version |
| 1.1 | Kristen Renner | 11/20/2003 | Reformat to PC version |
| 2 | Becky Carlson  | 4/4/2015 | Re-format to CMS template and Re-numbered from MC 813 |
|  | 2 | Becky Carlson | 5/1/2017 | Updated logo, Updated references, and bioMérieux technical Services contact information. |  |  |
| 3 | Susan DeMeyere | 2/26/2018 | Biennial review 2/5/2018 SD |
| **Archived by:** |  | **Archived Date:** |  |