KCMC Biotechnology Laboratory, Microbiology	STANDARD OPERATING PROCEDURE	Effective Date 9 March 07	SOP-Number MIC.022.01
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Title: API 20E TEST

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Annual Review	
By	**Date**

Title: **API 20E TEST**

Document History:

Version Number	Reason for Changes	Date
MIC.022.01	Addition of APIWeb access instructions	9 March 2007

Copies distributed to:

Name	Date

Title: # API 20E TEST

PURPOSE

For the identification of enterobacteriaceae and some nonfermenting Gram negative bacilli.

PRINCIPLE

Each test strip consists of 20 cupules containing dehydrated substrates of biochemicals. A saline suspension of bacteria is added and the test strip is incubated overnight. Indicator reagents are added to appropriate cupules and all the reactions assessed positive or negative. An octal code is calculated from the reaction results. The APIWeb data base is then consulted for an interpretation of the octal code and identification of the isolate.

SCOPE

This Standard Operating Procedure applies to the use of the API 20E test strip by technical staff in the microbiology laboratory that have been trained and are competent in performing this procedure.

MATERIALS/STORAGE

api20E Test kit (25 tests/kit)

- Test strips (1 per isolate)
- Plastic trays -
- Result forms

Sterile saline – 5 ml/Room temperature
Wooden applicator stick
Pasteur pipets
Tap water
Mineral oil/Room temperature
James Reagent / 2-10^{o} C in a closed container to protect from light
Ferric chloride (TDA)/ 2-10^{o} C
KOH 4% (VP-1)/ 2-10^{o} C
alpha-naphthylamine (VP-2)/ 2-10^{o} C
Fresh (18-24 hr) culture of test organism.

QUALITY CONTROL

Frequency: Each lot/shipment of kits before use.

Control: QC Organism: *E. coli* ATCC 25922

Acceptable Results: Appropriate reactions in all biochemicals (see API20E QC sheet)

Corrective Actions for Unacceptable QC Results:

1. Repeat test with fresh subculture of frozen organism stock, passed twice.
2. If still unacceptable, contact manufacturer for replacement. Do not use for patient testing.

Documentation:

1. Record results on API20E QC sheet.
2. Document corrective actions of all unacceptable results on QC DEVIATION FORM and submit to supervisor.
3. Supervisor will review QC results monthly.

PREPARATION OF BACTERIAL SUSPENSION

1. Using a sterile wooden applicator stick, touch the center of one well- isolated colony and add to a tube of 5 ml saline.
2. Twirl applicator stick on bottom of tube, close cap and invert several times to mix well.

PREPARATION OF TEST STRIPS

1. Set up a plastic incubation tray and lid.
2. Record the lab number on the elongated flap of the tray.
3. Dispense approximately 5 ml tap water into the incubation tray.
4. Remove the API strips from the sealed envelope and place a strip in the incubation tray.

INOCULATION OF THE STRIPS

1. Each 20E strip contains 20 microtubules each of which consists of a tubule at the bottom and cupule section at the top.
2. Slightly tilt strip to fill.
3. Using a sterile capillary pipette, fill the microtubules as follows:
    a. Fill only the tubule sections on all <u>except</u> CIT VP and GEL - these should be completely filled (tubule and cupule sections).
    b. <u>Slightly</u> underfill ADH, LDC, ODC and URE.
    c. Fill the cupule section of ADH, LDC, ODC, UR, H2S with sterile mineral oil.
3. Streak a loopful of bacterial suspension to a BAP for purity check. Incubate plate at 35^{o}C overnight.
4. Place strip in plastic container to prevent drying.
5. Incubate strip at 35^{o}C for 18-24 hours.

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READING/INTERPRETATION

1. Examine purity plate. If culture is mixed or does not grow, API test cannot be interpreted. Repeat test using fresh culture.
2. Add appropriate reagents to the cupules (see Interpretation table Appendix.
3. Enter the specimen number and the date on an API result form.
4. Assess the outcome of the biochemical reaction in each cupule and record the result on the API form (see Interpretation table Appendix A).
5. Calculate the 7 digit profile number by adding up the numbers of the cupule values of positive reactions in each triplet of tests.
6. Look up identification in the APIWeb data base. Enter the specimen number on the result page and print a copy.

   Accessing APIWeb:

   a. Software is loaded on supervisor's and ISAAC computer in technician's office.
   b. Load the CD into the CD drive.
   c. Click on the APIWeb icon to connect to website.
   d. Select API 20E.
   e. Enter the study (REF) number.
   f. Enter the octal code in spaces provided.
   g. Select CONFIRM.
   h. Print a copy of the APIWeb report and attach to the POSITIVE CULTURE form.

REPORTING RESULTS

1. If EXCELLENT IDENTIFICATION OR VERY GOOD IDENTIFICATION - Report results.
2. If ACCEPTABLE IDENTIFICATION OR GOOD LIKELIHOOD, LOW SELECTIVITY - Perform additional tests when possible and consult supervisor if at least "Very Good" identification is not achieved.
3. Organisms that cannot be identified will be reexamined at the Duke Medical Center Microbiology Laboratory. Consult supervisor for appropriate reporting verbiage.
4. Enter the code number and organism name on the POSITIVE CULTURE REPORT FORM.
5. If from blood culture enter organism name and organism code number on BLOOD CULTURE result form.

6. Freeze isolate at -80° C. Refer to MIC.003 FREEZING ISOLATES for procedure.

REFERENCES

Package Insert, 2004, api 20E, bioMerieux, Inc., Durham, NC.

Smith, P.B., et al. 1972. API System: A Multitube Method for Identification of Enterobacteriaceae. Appl. Microbiol. 24:449-452.

APPENDIX A – API 20E Interpretation Table

APPENDIX B – API 20E QC sheet (see copy on separate Page)

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APPENDIX A

Api20E INTERPRETATION TABLE

TEST	REACTION	REAGENT	NEG	POS
ONPG	b-galactosidase	None	colorless	yellow
ADH	Arginine Decarboxylase	None	yellow	red/orange
LDC	Lysine Decarboxylase	None	yellow	red/orange
ODC	Ornithine decarboxylase	None	yellow	red/orange
CIT	Citrate	None	green/yellow	blue
H2S	H2S production	None	colorless/greyish	black
URE	Urease production	None	yellow	red/orange
TDA	Tryptophane deaminase	Ferric chloride (TDA reagent)	yellow	reddish brown
IND	Indol production	James reagent	colorless	pink
VP	Acetoin production	VP-1 (4% KOH), VP-2 (alpha-naphthalamine)	colorless	Pink/red
GEL	Gelatinase production	None	No diffusion	Diffusion of black pigment
GLU	Glucose ferm/oxid	None	blue/blue green	yellow
MAN	Mannitol ferm/oxid	None	blue/blue green	yellow
INO	Inositol ferm/oxid	None	blue/blue green	yellow
SOR	Sorbitol ferm/oxid	None	blue/blue green	yellow
RHA	Rhamnose ferm/oxid	None	blue/blue green	yellow
SAC	Glucose ferm/oxid	None	blue/blue green	yellow
MEL	Mellibiose ferm/oxid	None	blue/blue green	yellow
AMY	Amydalin ferm/oxid	None	blue/blue green	yellow
ARA	Arabinose ferm/oxid	None	blue/blue green	yellow