STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: E-Test	**Revision Date:** 20-April-2018	**Issue Date:** 20-April-2016
Document Number: MIC50800	**Status: Approved**	
Distribution: Microbiology Test Manual	**Page: 1 of 16**	
Approved by: S. Asmussen, Manager of Diagnostic Services	**Signed by:** 	

PURPOSE:

To standardize the preparation of samples and interpretation of results for MIC drug determination using Etest strips.

INTRODUCTION:

Occasionally, for certain pathogens, the Vitek 2 Compact cannot be used for AST. Manual methods such as E-strips must be substituted to provide reliable and accurate bacterial drug susceptibilities. E testing is a quantitative technique for determining the MIC (minimum Inhibitory Concentration) of antimicrobial agents against microorganisms.

SAMPLE INFORMATION:

Type	Well isolated colonies (QC organisms or patient samples) that are fresh ("overnight" colonies that are 18-24 hours old). **Rationale: colonies that are too young or colonies that are too old may give false susceptible results or false resistant results (lowered or elevated MIC's).
Inoculum	Direct colony suspension using the appropriate McFarland standard in 0.9% saline (isotonic NaCl).
Medium and Method	Agar plates (MHB, MHP or HTM). E-test method (validated against the standardized broth and agar dilution methods in CLSI).

REAGENTS and/or MEDIA:

Media	Information
Mueller Hinton Plain (MHP)	▪ Source: Oxoid ▪ Store at 2 – 8ºC away from direct light
Mueller Hinton Blood (MHB)	▪ Source: Oxoid ▪ Store at 2 – 8^0C away from direct light

Haemophilus Test Media (HTM)	▪ Source: Oxoid ▪ Store at 2 – 8^{0}C away from direct light
E-strips	▪ Source: Biomeriuex ▪ Source: Alere ▪ Store at -20^{0}C ▪ Calibrated with MIC scale in µg/mL ▪ The lab supplies the following E-strips: ❖ Amoxicillin (AC) ❖ Ampicillin (AMP ❖ Cefotaxime (CTX) ❖ Ceftriaxone (TX) ❖ Cefuroxime (XM) ❖ Penicillin (PG) ❖ Vancomycin (VA) **check supplies against Dynalife manual. Some isolates, such as invasive *Haemophilus*, requires E-testing for strips that the lab does not carry (must refer isolate out to reference lab).

SUPPLIES:

- Plastic test tubes and caps
- 35º ambient incubator
- 35º CO2 incubator
- Densicheck Plus
- Sterile forceps
- Sterile loops and wooden sticks
- Biosafety Cabinet
- 0.9% NaCl (Saline)
- Cotton-tipped swabs, sterile (wrapped in sets of two)

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:

E-strips are tested weekly in conjunction with the weekly KB testing and Vitek sensitivities.

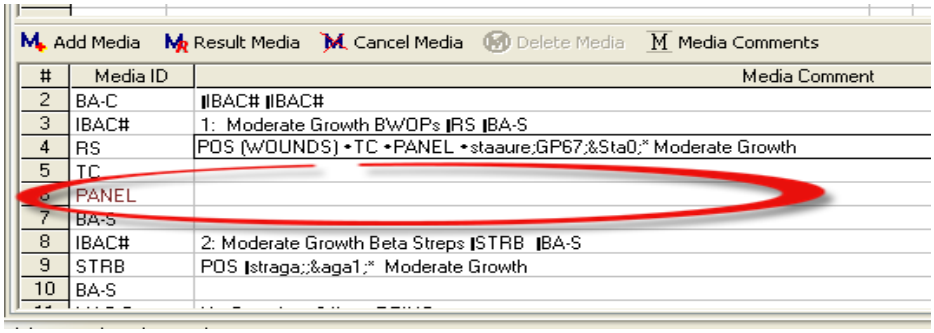
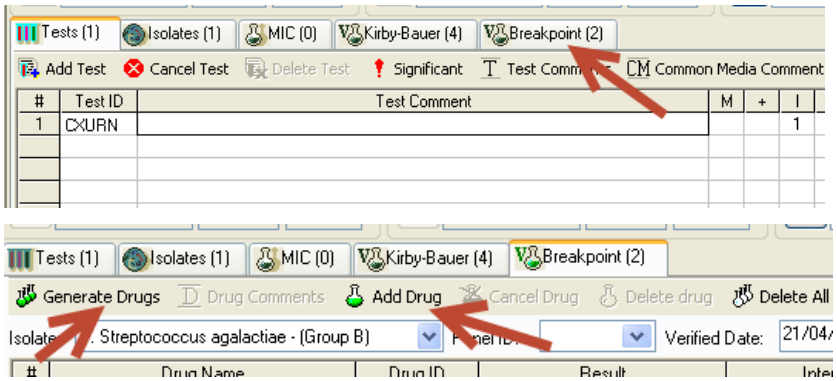
- *Strep pneumo* 49619 tests the complete repertoire of E-strips
- *Staph aureus* 29213 and *Enterococcus faecalis* 29212 test Vancomycin E-strip.
- *Haemophilus influenzae* 49247 and *Enterococcus faecalis* 29212 test Ampicillin
- *Haemophilus influenzae* 49247 tests Cefotaxime

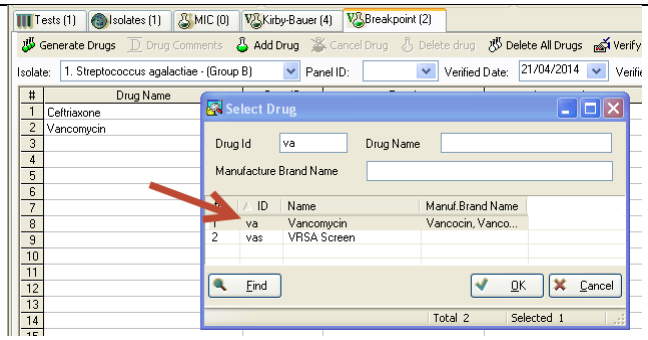
What is the purpose of E-test QC?

1. Batch to batch media/strip performance
2. Correct handling, storage and use of Etest strips
3. Tech competency → correct selection of MIC end points.

Refer to **Procedure MIC60300 – Weekly KB and E-Test QC** (In the Quality Control Binder, or Section 60000 in the Microbiology Manual).

PROCEDURE INSTRUCTIONS:

Step	Action:
Follow the steps in the table below to set-up E-tests	
1	**If performing an E-Test for Quality Control:** • The test should be automatically generated in TQC and can be resulted in the microbiology TQC resulting worklist (see the Reporting Results section below). **If performing an Etest on a patient sample:** • Add **"^PANEL"** in plate log • This adds a Vitek label to the Vitek worklist and adds a line in plate log for recording purity.  Add the appropriate antibiotics added under the Breakpoint tab:  Either click on Generate Drugs if the E-tests have been pre-programmed into the LIS, or manually add the individual drugs by clicking on the Add Drug button.

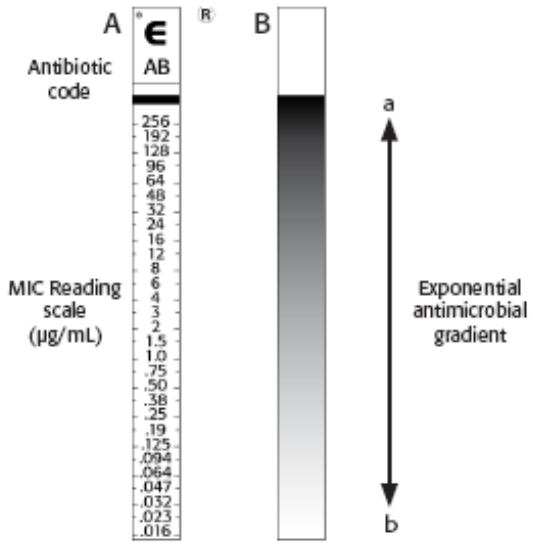
	 Select the drug by searching in the drug ID box, the drug name, or the brand name. Select your drug by clicking on it and pressing OK.
2	Consult the Dynalife AST manual for specific applications. • Note the media required, the antibiotics, and any beta lactam charts that require additional E-strips to be set-up. • Note the McFarland standard required (mucoid strains often require 1.0 McF), and the incubation atmosphere.
3	**Perform E-testing on pure, fresh isolates only (16-24 hour incubation).** • If not enough colonies on plate to make the required McF standard, subculture the organism and set up testing the following day after an overnight incubation. Record in plate log.
4	**Obtain all supplies that are stored below room temperature first.** • Allow them to warm to room temperature while you perform other tasks.
5	**Label plates using the PANEL (Vitek) labels.** • Vitek labels can be printed by selecting the appropriate order number in the Vitek/Miscellaneous worklist and printing the LABEL_IQ labels. Or, the labels can be printed from Result Entry.
6	Sterilize the forceps located inside the black box by cleaning the tips with an alcohol pad. Flaming the forceps can be done by placing the tip into an incinerator but please use caution if doing this. Metal conducts heat quickly, so the handle may be scorching hot and cause burns if the forceps are left in the incinerator to too long. Alcohol sterilization is recommended.

7	Ensure the Densicheck has been QC'd. Refer to Procedure **MIC70100 DensicheckPlus Use and Maintenance**. Follow the **Daily Maintenance** instructions or Monthly QC if applicable.
8	Label a plastic test tube with the sample number or QC isolate. Label near the top of the tube. The Densicheck reads through the tube at the bottom so do not place any writing on the bottom half on the test tube. Dispense approximately 2 mL of 0.45% saline into the tubes
9	Using a wooden applicator stick (located on top of Vitek bench), select several colonies from culture. Or, use a sterile cotton swab to pick up colonies. Place the colonies into the saline within the test tube.
10	Cap the plastic test tube with a plastic cap (located on Vitek bench), and vortex for 2-3 seconds.
11	Insert saline suspension into the DensicheckPlus and turn 360°
12	Adjust the turbidity of the suspension by adding more colonies or additional saline until the appropriate McFarland is reached.
13	**Inoculate media** • Using a sterile cotton-tipped applicator stick dip the cotton end into the suspension. Remove and "wring out" excess moisture by pressing cotton tip against the inside side walls of the test tube. Streak agar plates by gently rubbing the cotton tip stick back and forth over the media, like colouring in a circle. Rotate the media 60° and re-streak. Rotate media again 60° and re-streak. Rim the edges of the plates with the swab and discard swab into the yellow disinfectant bucket. **Inoculate media within 15 minutes to prevent bacterial overgrowth in the suspension.
14	**Allow moisture to absorb into the media.** • Wait 3 minutes to a maximum of 15 minutes before adding the E-strips onto the agar. If inoculating multiple plates and performing multiple E-tests on the same isolate, inoculate ALL the plates first, and then place E-strips.

15	**Ensure the black antimicrobial box is at room temperature before opening** (touch the top or the side of the box to evaluate temperature and ensure the box is DRY). • A desiccant inside removes moisture from the disks and E-strips as the antimicrobials warm up. Opening the box before it equilibrates to RT prevents the desiccant from doing its job, so the antimicrobials retain moisture. This affects their performance.
16	**Open Estrip.** • Using the sterile forceps, peel back the foil on the appropriate E-strip and carefully separate the E-strips so that ONE Strip is removed from the package. If several strips stick together, twist the TOP of the strip (where the drug abbreviation letters are) to separate the strips.
17	**Place the base of the E-strip onto the agar** • Touch the bottom of the strip firmly to the agar surface and slowly "roll" the length of the strip onto the surface. Move slowly and gently to prevent bubbles forming beneath the strip and the agar. Gently tap out any bubbles using the forceps. Once the E-strip has touched the agar, do not move it, as the antibiotic diffuses out almost immediately. **Please note: The E-strips are impregnated with an antibiotic on one side, so the printed side needs to be facing UP when placed onto the agar surface.**
18	Sterilize forceps and place into box.
19	If necessary, two E-strips may be placed onto one plate. Just ensure that the plate is rotated 180 ° and the MIC's of the E-strips are facing opposite directions.
20	Incubate plates within 15 minutes of E-strip application. Ensure the plates are incubated in the correct atmosphere by consulting the CLSI guidelines or the Dynalife AST manual.
21	Put away all supplies in the appropriate storage.

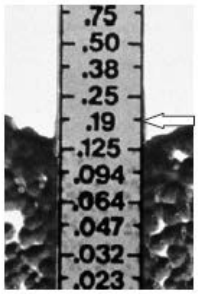
INTERPRETATION OF RESULTS:

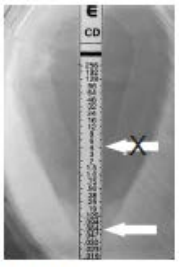
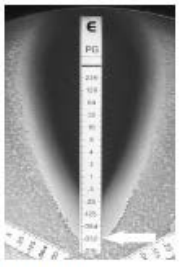
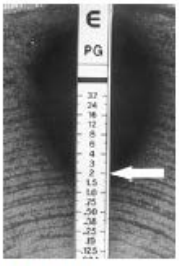
Refer to cardboard AB BIODISK charts for a visual Etest Reading Guide (posted on ambient air incubator.



Interpretation of E-test: MIC scale and antimicrobial gradient.

Step	Action:
Follow the steps in the table below to interpret the MIC:	
1	After 18-24 hour incubation the plates should show good growth with clear zones of inhibition. Inhibition area should resemble an asymmetrical ellipse.
2	Read the MIC's in a well lit area. Tilt the plate to view small translucent colonies.
3	Read the MIC where the ellipse intersects the breakpoints on the strips. Typical E-strip test result gives a crisp asymmetrical zone of inhibition around the strip. Example on right: MIC = 0.064 ug/mL 

4	End points must be read where there is complete inhibition of all growth Incorporate pinpoint colonies and/or hazes into the MIC breakpoint. Example on right: MIC = 1.0 ug/mL	
5	MICs must be interpreted using CLSI guidelines. The Etests represent a continuous gradient, so MIC values can be observed between two points. Always round up these values to the next highest point before interpretation. Example on right: MIC = 0.19 ug/mL	

Troubleshooting Etest Reading				
Organism related issues:				
Effect & Action	 **Figure 7.** Ignore swarming. MIC 0.064 µg/mL	 **Figure 8.** Ignore haemolysis; read the inhibition of growth. MIC 0.032 µg/mL.	 **Figure 9.** Tilt plate or use a magnifying glass to see pin-point colonies and hazes, e.g. enterococci, pneumococci, fusobacteria, and *Stenotrophomonas* spp. MIC 1 µg/mL.	 **Figure 10.** Scrutinise ß-lactam endpoints for pneumococci for hazes and microcolonies. MIC 2 µg/mL.

Technical issues - Drug related Effects:

Effect & Action

Figure 11. Bactericidal agents give sharp MIC endpoints. MIC 0.064 µg/mL.

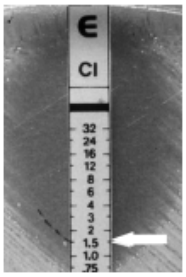


Figure 12. Bactericidal agents; read at complete inhibition of hazes and microcolonies. MIC 1.5 µg/mL.

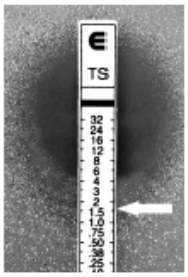


Figure 13. Bacteriostatic agents; read at 80% inhibition. MIC 1.5 µg/mL.

Figure 14. Linezolid; read at 90% inhibition (ignore finer hazes and pinpoint colonies). MIC 0.75 µg/mL.

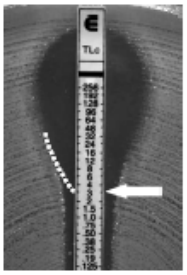


Figure 15. ß-lactamase inhibitors e.g. tazobactam; extrapolate the upper curvature to the strip. MIC 3 µg/mL.

Figure 16. Trim/sulfa; read at 80% inhibition (ignore lawn of haze within the ellipse). *Stenotrophomonas* spp. MIC 0.19 µg/mL.

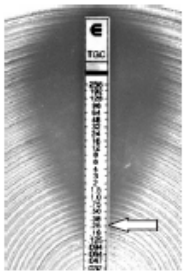


Figure 17. Tigecycline; read at 80% inhibition (ignore trailing microcolonies or hazes). MIC 0.25 µg/mL.

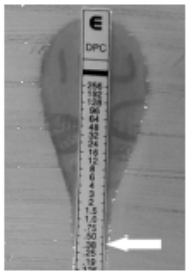


Figure 18. Polypeptides; read at the bottom of the "dip" if colonies are absent. MIC 0.38 µg/mL.

Technical issues - Resistance Mechanism related Effects:

Effect & Action

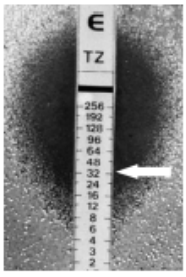


Figure 19. Small colony variants and bactericidal agents; read at complete inhibition. MIC 32 µg/mL.

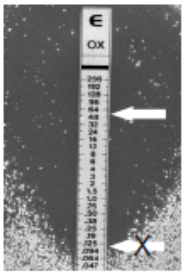


Figure 20. Isolated colonies for oxacillin represent heteroresistant subpopulations i.e. ORSA. MIC 48 µg/mL.

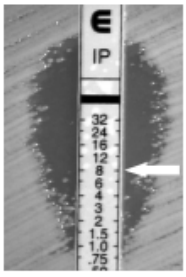


Figure 21. Isolated colonies for carbapenems may represent resistant subpopulations e.g. KPC. MIC 8 µg/mL.

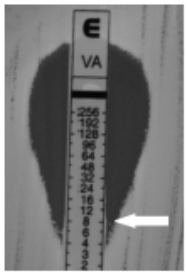
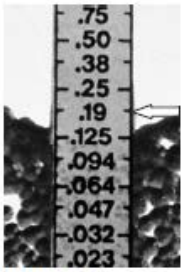
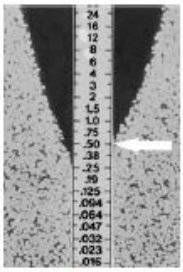
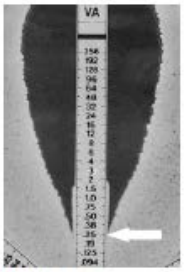
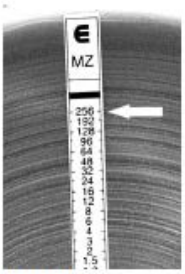
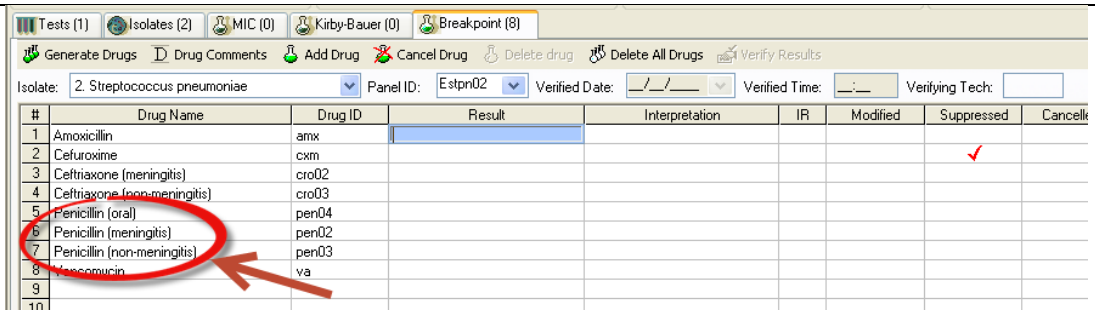


Figure 22. Trailing growth (hazes, microcolonies, macrocolonies) represent VISA/hVISA. MIC 8 µg/mL.

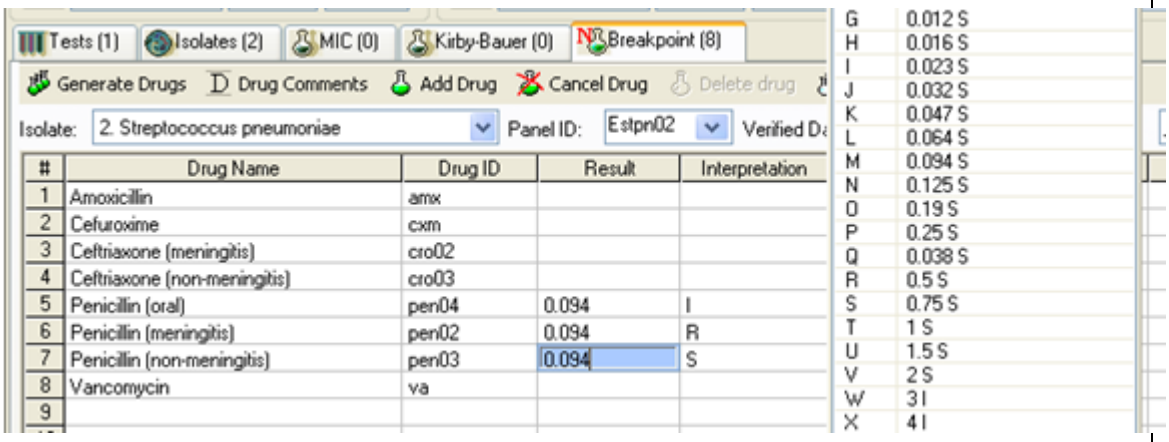
Technical issues - Technical and Handling related Effects:	
Effect & Action	 **Figure 23.** Intersection in-between markings, read the upper value. MIC 0.19 µg/mL.  **Figure 24.** Uneven intersections; read the higher value. If >1 dilution, repeat the test. MIC 0.5 µg/mL.  **Figure 25.** Ignore a thin line of growth alongside the strip. MIC 0.25 µg/mL.  **Figure 26.** Complete growth around the strip. MIC ≥ 256 µg/mL.

REPORTING RESULTS:

Step	Action:
Reporting E-test results in patient samples:	
1	Interpret the MIC for the isolate using above instructions and troubleshooting guide.
2	Log into SoftMIC LIS. In Result Entry, scan in or type the patient sample order #. Locate the Breakpoint tab in the sample screen.
3	If the antibiotics have not already been generated, add them to the Breakpoint list by following the steps above (ie. Generate Drugs, or Add Drugs in the Breakpoint tab).
4	Click “CTRL+K” to open the Keypad. Select the correct MIC that was interpreted from the E-test. The Interpretation of Sensitive, Intermediate and Resistant should automatically be filled out by the LIS. If it is not, consult the CLSI guidelines and manually type in the interpretation. Please note that some breakpoint reporting requires meningitis and non-meningitis BP interpretations to be reported for one antibiotic. (see below).

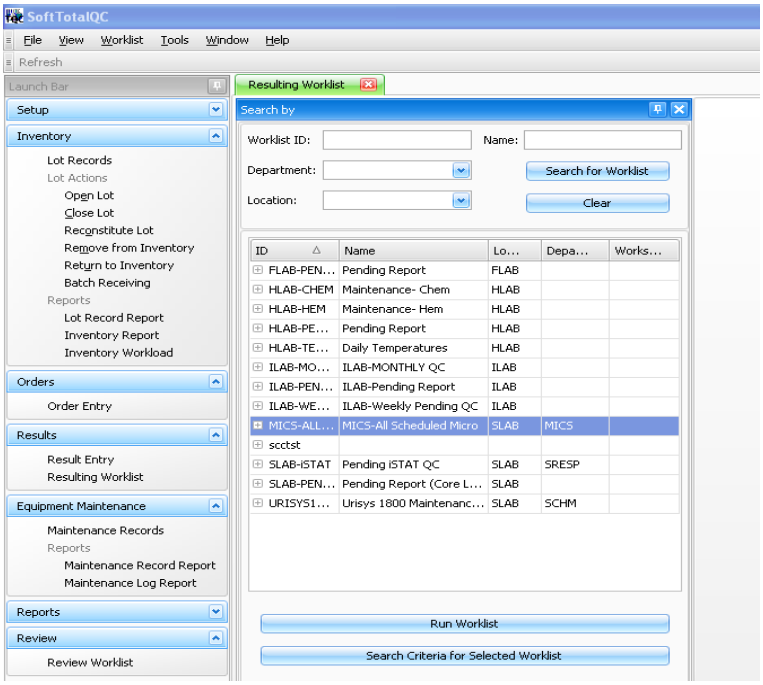


Example. *Strep pneumo* in a Blood culture requires oral, meningitis and non-meningitis MIC's to be reported on the final report (all 3 must be reported to the physician). Some MIC's, depending on how the antibiotic is administered, give different interpretations.



Example: MIC of 0.094 ug/mL for Penicillin for *Strep pneumo* in blood culture. **Although the MIC result does not change**, **the interpretation differs depending if the antibiotic is administered orally or parenteral and if the case is meningitis or not**. Refer to the Dynalife AST manual and the CLSI guidelines.

5	Double check the suppression rules against the Dynalife AST manual. Verify results; view the Instant Report to preview the reported sensitivities. Resolve any errors by double-checking suppression rules, correct Test line being resulted, etc and preview the report once to check is errors have been resolved. Status the report if required, and save the report to exit.

Step	Action:
Follow the steps below for Reporting E-test results in TQC:	
1	Interpret the MIC for the isolate using transmitted light and Troubleshooting guide above.
2	Log into TQC LIS. Go to the pending micro worklist following the steps below:  Figure 4: Log into TQC → Click on “Resulting Worklist” on the left side of screen → Click on “MICS – All Scheduled Micro” → click on “Run Worklist” to generate a list of all Bacteriology pending TQC tests.

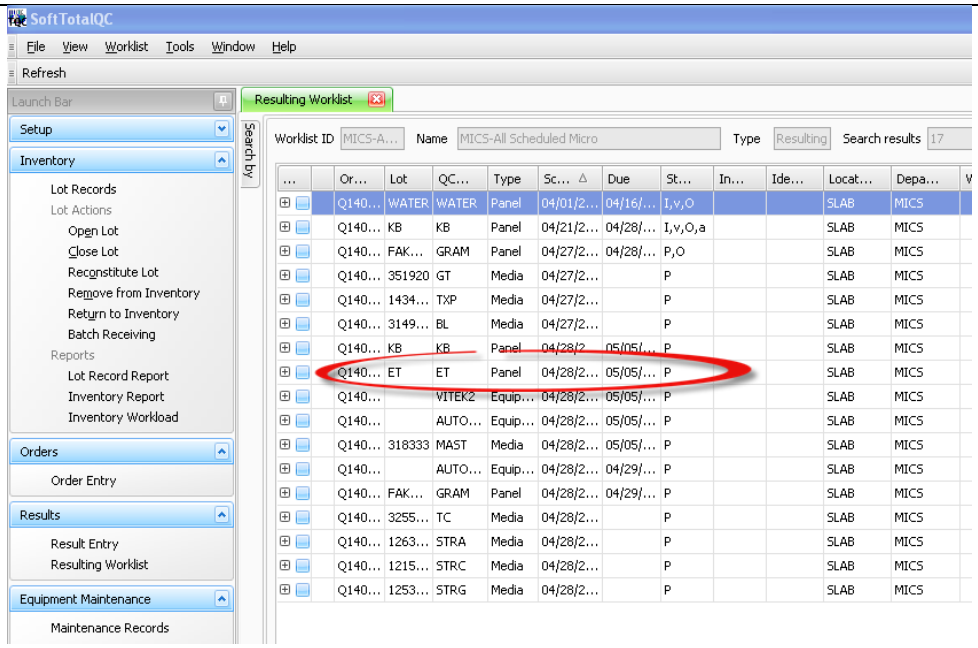


Figure 5: After running the worklist, a list of pending tests should generate. Find the "ET" QC item in the list and double-click on it to open a list of pending ET tests.

3

Type in the result in the Result column. Press the "tab" key to enter the result and move to the next Etest item.

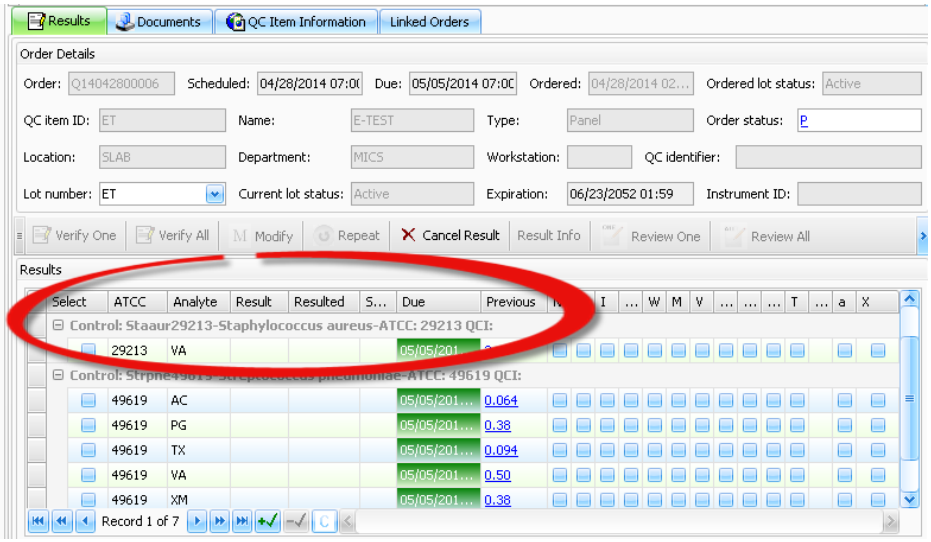
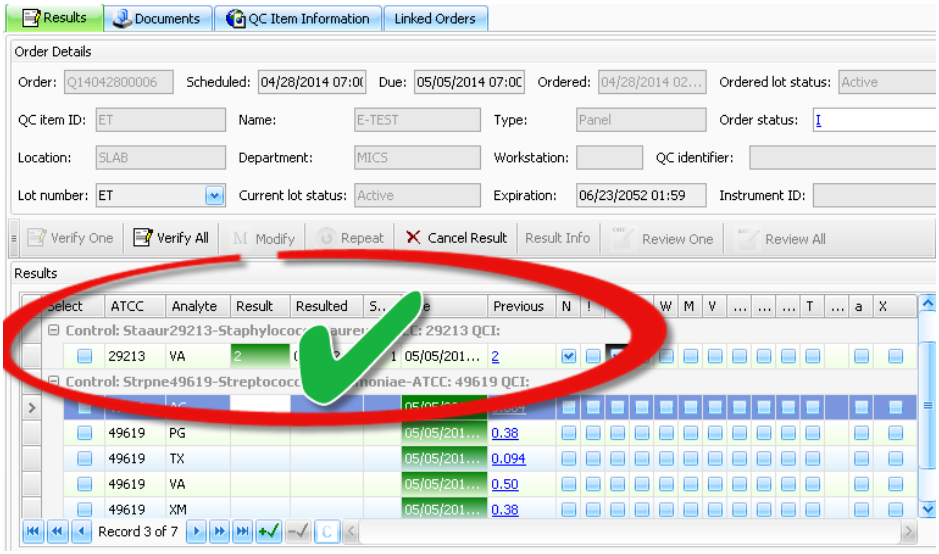
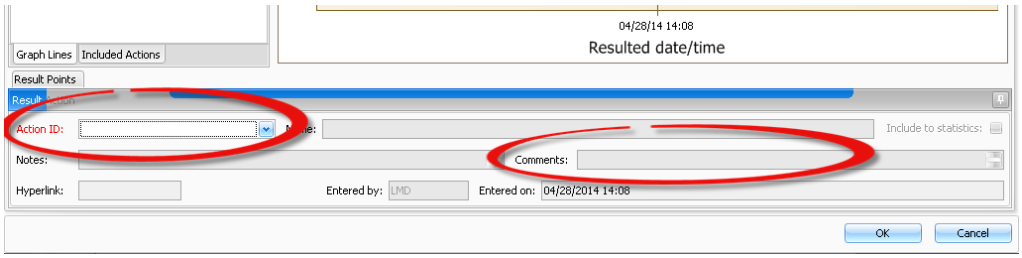


Figure 6. ET QC items are organized in a list of multiple ATCC strains with sub-lists of pending ETests. Circled is the pending E-test (Vancomysiin) for *S.aureus* ATCC#

	29213.  If QC passes, the column turns green.
4	If QC is out-of-range, a Corrective Action window pops up.  Fill out two lines in the bottom portion of the screen: 1. **Action ID** (in the drop-down menu, choose “REDO – repeat testing”), and 2. **Comments** (a descriptive comment explaining why the QC failed). Click OK and a repeat TQC test is automatically generated for that antibiotic.
5	Click on the Resulting Worklist tab to get back into the worklist or exit the program.

RELATED DOCUMENTS:

Procedure MIC60300 – Weekly KB and E-Test QC

Procedure MIC70100 DensicheckPlus Use and Maintenance

REFERENCES:

- Etest Reading Guide cardboard posters. AB BioDisk. Dalvagen, Sweden.
- Etest product insert. Biomerieux.
- Soft Total QC manuals can be found on SharePoint.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	17APR2014	Initial Release	Driedger (L)
1.1	05Feb2015	Review	S. Webber
2.0	31Mar2016	Update of “Special Safety Precautions” to reflect risk assessment recommendations.	C. Russell