PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Phoenix Test Procedure

Department of Microbiology

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1.0 Principle

A maximum of 99 Phoenix™ panels for identification and/or antimicrobial susceptibility testing can be performed in each Phoenix™ instrument at a time. A sealed and self-inoculating molded polystyrene tray, with micro-wells containing dried reagents, serves as the Phoenix™ disposable. The combination panel includes an ID side with dried substrates for bacterial identification, an AST side with varying concentrations of antimicrobial agents, and growth and fluorescent controls at appropriate well locations. The Phoenix™ system utilizes an optimized colorimetric redox indicator for AST, and a variety of colorimetric and fluorometric indicators for ID. The AST Broth is cation-adjusted (e.g., Ca^{++} and Mg^{++}) to optimize susceptibility testing performance.

Panels are available as ID only (Phoenix™ NID and Phoenix™ Yeast ID), AST only panels (Phoenix™ NMIC, PMIC, and SMIC), or ID/AST combination panels (Phoenix™ NMIC/ID). Unused wells are reserved for future use.

Phoenix™ panels are inoculated with a standardized inoculum. Organism suspensions must be prepared only with the BD PhoenixSpec™ nephelometer or the BD Phoenix™ AP. Once inoculated, panels are placed into the instrument and continuously incubated at 35°C. The instrument tests panels every 20 min: on the hour; at 20 min past the hour; and again at 40 min past the hour up to 16 h, if necessary. Only the instrument reads Phoenix™ panels. Phoenix™ panels cannot be read manually.

Identification Testing: The ID portion of the Phoenix™ panel uses a series of conventional, chromogenic, and fluorogenic biochemical tests to determine the identification of the organism. Both growth-based and enzymatic substrates are used to cover the different types of reactivity in the range of included taxa. The tests are based on microbial utilization and degradation of specific substrates detected by various indicator systems. Acid production is indicated by a change in the phenol red indicator when an isolate is able to utilize a carbohydrate substrate. Chromogenic substrates produce a yellow color upon enzymatic hydrolysis of either p-nitrophenyl or p-nitroanilide compounds. Enzymatic hydrolysis of fluorogenic substrates results in the release of a fluorescent coumarin derivative. Organisms that utilize a specific carbon source reduce the resazurin-based indicator. In addition, there are other tests that detect the ability of an organism to hydrolyze, degrade, reduce, or otherwise utilize a substrate. A complete list of taxa included in the Phoenix™ ID Database is provided in the BD Phoenix™ System User's Manual, located on the main EpiCenter desktop and on the Providence Sacred Heart intranet Policies and Procedures folder. Reactions employed by various substrates and the principles of the Phoenix™ ID reactions are also described in the user's manual.

Antimicrobial Susceptibility Testing: The Phoenix™ AST method is a broth based microdilution test. The Phoenix™ system uses a redox indicator for the detection of organism growth in the presence of an antimicrobial agent. Continuous measurements of changes to the indicator as well as bacterial turbidity are used in the determination of bacterial growth. Each AST panel configuration contains several antimicrobial agents with a range of two-fold doubling dilution concentrations. The identification of the organism is used in the interpretation of the MIC

values of each antimicrobial agent. Endpoints for interpretation of Susceptible, Intermediate, or Resistant (SIR) result classifications are based on the CLSI M100 Performance Standard.

A complete list of taxa that the Phoenix™ system can provide AST results for is provided in the BD Phoenix™ System User's Manual. The package insert enclosed in the panel box provides a listing of the specific antimicrobial agents and concentrations found in the panel.

There are antimicrobial agents included in the Phoenix™ System that are not proven to be effective for treating infections for all organisms listed in the taxa. For interpreting and reporting results of antimicrobial agents that have been shown to be active against organism groups, refer to the most recent CLSI M100 Performance Standard, Table 1 "Suggested Groupings of US FDA-Approved Antimicrobial Agents That Should Be Considered for Routine Testing and Reporting on Organisms by Clinical Microbiological Laboratories."

Precautions: All microbial cultures are potentially infectious and should be treated with universal precautions. Panels, once inoculated, should be handled carefully until placed in the instrument.

2.0 Clinical Significance

The BD Phoenix™ Automated Microbiology System is intended for the rapid identification and antimicrobial susceptibility testing of clinically significant bacteria. The Phoenix™ system provides rapid results for most aerobic and facultative anaerobic Gram-positive bacteria as well as most aerobic and facultative anaerobic Gram-negative bacteria of human origin. The Phoenix™ system is also intended for the rapid identification of yeast and yeast-like organisms.

3.0 Scope

This procedure is classified under CLIA as high complexity. It should be carried out by technical personnel familiarized and trained on all levels of the operation of the BD Phoenix™ testing platform. Testing includes but is not limited to: instrument start up, shutdown, routine maintenance, performance checks, basic troubleshooting, QC checks, administrative tasks and record keeping of information vital to verification of instrument and technical proficiency in accordance with the department SOP. Records are to be kept within the employee's record in the department of continued competence and proficiency on the equipment. Performance reviews of technical personnel are to be carried out annually.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Microbiology Biohazards and Safety document. Follow proper handling, storage, and disposal of specimens and items that come into contact with specimens. Place contaminated materials in a biohazardous waste container.

The reagent(s) and/or chemical(s) that are used in this procedure may be hazardous to your health if handled incorrectly. A brief listing of precautions for each chemical hazard is included in the reagent section of this procedure.

More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Airborne pathogens
- Slightly hazardous reagents

To perform this procedure, you must use:

- Gloves
- Laboratory Coat

Disinfectant following procedure:

- Bleach dilution sprayers or wipes can be used for on demand disinfectant.

Reference for spill/decontamination

- MSDS
- Chemical hygiene plan

5.0 Specimen Requirements

Only pure culture isolates of aerobic and/or facultatively anaerobic organisms are acceptable for testing. The test isolate must be a pure culture. It is recommended that bacterial cultures be 18 to 24 h old, unless additional incubation is required to achieve sufficient growth. Yeast cultures should be 18 to 48 h old, unless additional incubation is required to achieve sufficient growth.

All significant isolates that are appropriate for automated testing should be tested on a Phoenix™ panel, even if subculture is required to obtain sufficient inoculum and isolation. The following are exceptions to this rule.

- Respiratory isolates of non-fermenting gram-negative bacilli from cystic fibrosis patients may be placed on Phoenix™ for identification with conventional back-up testing as needed. Susceptibility testing should be performed by Kirby-Bauer.
- Isolates that fail to yield AST results on the Phoenix™ should be retested by Kirby-Bauer.
- When specific drug results are not available from Phoenix™.
- Beta-strep should be tested by Kirby-Bauer for D-test results.

The appropriate panel type should be selected based on gram stain, colony morphology, etc. (e.g., NMIC/ID panel for use with Gram Negative organisms). Selection of the incorrect panel type could lead to incorrect results.

For AST testing in the Phoenix™ system, isolates recovered from non-selective media are recommended. It is recommended that media containing antibiotics not be used for organisms to be tested in the Phoenix™ system.

For ID and AST testing, refer to Table 1 below for recommended media. Only cotton-tipped swabs should be used to prepare the inoculum suspensions. Some polyester swabs may cause problems with inoculation of the panels.

Inoculum for use on the Phoenix™ system is prepared by the CLSI-recommended direct colony suspension method. Due to variations in inoculum concentrations prepared with McFarland standards, use of the BD PhoenixSpec™ nephelometer or the BD Phoenix™ AP is required for adjusting the test inoculum prior to use in the Phoenix™ system.

The purity of the inoculum should be checked by preparing a purity plate for each test isolate. See “Purity Check” below.

6.0 Materials

6.1 Equipment and Testing System

- BD Phoenix™
- Epicenter™ software
- Incubator
- BD PhoenixSpec™ nephelometer
- Inoculation Station
- Phoenix™ Panel Transport Caddy

6.2 Reagents & Supplies

- Epicenter Barcode Labels (Catalog Number 441503), 12 rolls

- Sterile cotton swabs
- **Panels**: (NID, NMIC, NMIC/ID, PMIC, SMIC, and YEAST ID)

  Panels are individually packaged and must be stored unopened at room temperature (15 – 25 °C). Do not refrigerate or freeze. Visually inspect the package for holes or cracks in the foil package. Do not use if the packaging or panel appears to be damaged. If stored as recommended, the panels will retain expected reactivity until the date of expiration.
- **Phoenix™ ID Broth** (Catalog Number 246001) 100 tubes, store at 2 – 25 °C

  Visually inspect the tubes for cracks, leaks, etc. Do not use if there appears to be a leak, tube or cap damage or visual evidence of contamination (i.e., haziness, turbidity).
- AST Broth (Catalog Number 246003) 100 tubes, store at 2 – 25 °C

  Visually inspect the tubes for cracks, leaks, etc. Do not use if there appears to be a leak, tube or cap damage or visual evidence of contamination (i.e., haziness, turbidity).
- **Manual Phoenix™ Panel Inoculation Components (Strep and Yeast)**
  - 25 or 50 µL pipette and sterile tips
  - AST-S Indicator Solution for Strep (246009) 10 bottles, store at 2 – 8 °C

    Visually inspect the bottle for cracks, leaks, etc. Do not use if there appears to be a leak, bottle or cap damage or any change from a dark blue color. Each bottle contains enough solution to test up to 100 panels. Expiration dating is shown on the box, pouch, and bottle label and is for unopened bottles. An opened bottle is stable for up to 14 days if stored at 2 - 8°C. When a new bottle is removed from its pouch it should be labeled with the date opened **and** the 14-day expiration date.
- **Phoenix™ AP Consumables and Storage Requirements**
  - **AP ID Broth** (448012) 5 bags, store at 2 – 25 °C
  - AP AST Indicator (246006) 10 bottles, store at 2 – 8 °C
  - **AP Pipette Tips** (448038) 960 tips
  - **Waste Liquid Bottle** (448014) 10 bottles
  - **Waste Tip Bin** (448013) 25 bins
  - **AP Pump Dispense Tubing** (448015) 5 sets
- **Phoenix™ Calibrator Tube Set** 15 - 30°C
- **Trypticase™ Soy Agar with 5% Sheep Blood**
- **Biohazard disposable container**
- **Markers, etc.**

6.3 Controls and Calibrators

- **Controls**: refer to the Quality Control section for a list of control organisms
- **Calibrators**: Phoenix™ Calibrator Tube Set (441951) 1 set. Calibrators are used for daily QC and calibration.

7.0 Procedure

For additional information regarding Phoenix™ testing and instrument operation, refer to the BD Phoenix™ System User's Manual and the BD Phoenix™ AP Instrument User's Manual located on the intranet. Phoenix™ testing may be performed by manual preparation of ID/AST broths or with the aid or the Phoenix™ AP instrument. The Phoenix™ AP instrument cannot be used to prepare AST tubes for the inoculation of Phoenix™ Strep panels but may be used to adjust the turbidity of the ID Broth tube. The Phoenix™ AP instrument cannot be used to prepare ID Broth inoculum for YEAST ID.

7.1 Isolate Selection

Test organisms must be from a pure culture. Aerobic and/or facultative anaerobic Gram negative and Gram positive bacteria are acceptable for testing. Bacterial cultures that are 18-24 h old are recommended. Yeast isolates should be 18-48 h old and grown at 25-35°C.

7.2 Recommended Media

Isolates recovered from non-selective media are recommended for AST testing in the Phoenix™ system. Media containing antibiotics should not be used for organisms to be tested in the Phoenix™ system. Table 1 below lists media recommended for Phoenix™ testing.

Table 1 – Recommended Media for Phoenix™ Testing

Recommended Media for Bacteria	Approved Use			
	ID	AST	Strep AST	YEAST
CHROMagar MRSA II	N/A	Yes	N/A	N/A
CHROMagar O157	Yes	N/A	N/A	N/A
CHROMagar Orientation	Yes	Yes[1]	No	No
CHROMagar Salmonella	Yes	Yes	N/A	N/A
Chocolate Agar	Yes	Yes	No	Yes
Columbia CNA Agar with 5% Sheep Blood	Yes	Yes	No	No
MacConkey Agar	Yes	Yes	N/A	N/A
Mannitol Salt Agar	N/A	Yes	N/A	N/A
Sabouraud Dextrose Agar	N/A	N/A	N/A	Yes
Trypticase Soy Agar with 5% Sheep Blood	Yes	Yes	Yes	Yes
Xylose Lysine Desoxycholate Agar	Yes	Yes	N/A	N/A

[1] The use of CHROMagar Orientation may produce false AST results when testing erythromycin with Gram positive organisms. However, erythromycin is not reported for urinary isolates.

7.3 Panel Selection

The table below provides guidelines for selecting appropriate panels. Using MIC only panels whenever possible helps to reduce waste and keep operating costs lower.

Isolate	**Panel**
E. coli & Swarming *Proteus*	NMIC
Other Coliforms	NMIC/ID
Pseudomonas aeruginosa	NMIC
Other Non-fermenters	NMIC/ID
Pasteurella or *Eikenella*	NID
Staphylococcus	PMIC
Enterococcus	PMIC
S. pneumoniae & Viridans	SMIC
Yeast	Yeast ID

7.4 Broth Preparation

1. Using a sterile cotton swab, pick colonies of the same morphology and aseptically transfer to a tube of ID broth.
2. Make a suspension that is visually at or above desired inoculum density of 0.25 or 0.50 McFarland for bacteria or 2 McFarland for yeast. Minimize fingerprints on the ID Broth tube.
3. Wring out swab thoroughly.

4. Inoculate a TSA blood agar plate for purity. **Only one isolate should be placed on each TSA blood agar plate to allow adequate space to streak for isolation. The validity of the test results is dependent on verifying that the inoculum used is pure.** Incubate the plate for 18-48 h at 35°C.
5. Cap tube and label before placing in holding rack. **Note: Unstandardized suspensions in ID Broth must be processed at Phoenix™ AP instrument within 2.5 h.**

7.5 Batch Login for Test Selection

The Batch Login module in EpiCenter is used to login pending orders. Batch Login is accessible from an icon on the EpiCenter toolbar.

Using the barcode scanner and the Phoenix™ Menu located at the bench, scan in the following information:

1. **Accession number** from culture plate.
2. **Test code** on Phoenix™ Menu. This selects panel type. Selection of the incorrect panel type could lead to incorrect results.
3. **Isolate number** on Phoenix™ Menu. The default is 1. If the isolate will report in LIS on a different line, enter appropriate number.
4. **Organism name** on Phoenix™ Menu. If a MIC panel was ordered, a name must be associated with the panel. Scan the appropriate ID or select organism from dropdown list.
5. **Add Order** on the Phoenix™ Menu. If Batch Login is in Single Entry Mode, scanning "Add Order" will initiate LIS query and printing of barcode labels. The barcode labels should be used to label the ID Broth tube and the purity plate.

7.6 Phoenix™ AP Instrument Operation

Bacterial suspensions must be processed on the AP instrument within 2.5 h of preparation.

1. Vortex each ID Broth tube and place in the front row of a Sample Rack.
2. If the barcode label indicates AST has been ordered, place an AST Broth tube in the space directly behind the ID Broth tube.
3. Remove all caps from broth tubes.
4. Place Sample Rack on the Input Queue with rack position barcodes facing forward.
5. Tap the Run button on the AP touch screen menu.
6. The Phoenix™ AP instrument will add AST indicator to the AST tube, read ID Broth density and dilute to standardized 0.25 or 0.5 McFarland density, and transfer and mix ID Broth to the AST tube.
7. When the instrument moves the Sample Rack to the Output Queue the tubes are ready for panel inoculation. Refer to the Phoenix™ User's Manual or Quick Reference Guide to troubleshoot any errors or problems that may occur.

   Note: ID and AST broth must be used within 30 min of being processed by the AP instrument. The AP instrument provides aging information for each completed rack.
8. Transfer the Sample Rack with prepared tubes to the AP Inoculation Station.

7.7 AP Login at the Inoculation Station

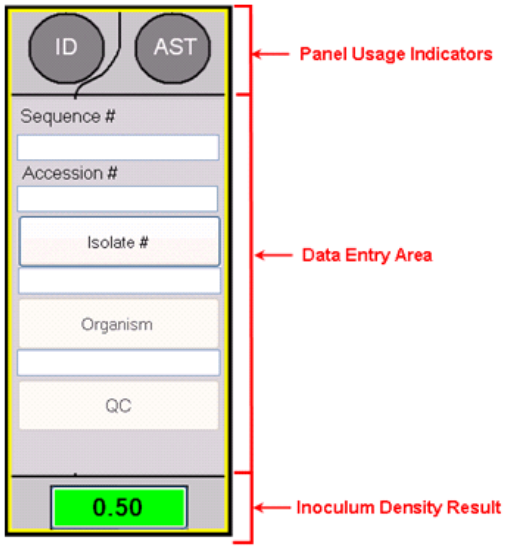
Phoenix™ AP Login is activated by clicking or taping the AP Login icon on the EpiCenter toolbar.

1. When the AP Login opens the initial screen is blank, waiting for a Rack to be recognized via a radio frequency tag in the Sample Rack.
2. When the Sample Rack is inserted into the AP Inoculation Station, a message is displayed stating "Reading Rack Information." When the Sample Rack data is retrieved, the AP Login screen opens and displays information corresponding to the tubes in the Sample Rack. The inoculum density is displayed in a green box for each isolate.

If the AP instrument encountered a problem with any of the suspensions an error will be displayed in a red box. These suspensions may need to be prepared again. Refer to the AP Instrument User's Manual for specific information.

3. Open appropriate panels and place on the inoculation station behind the corresponding tubes. The appropriate panel type for each isolate can be found on the barcode on each ID Broth tube. Examine the pouch. Do not use panels if the pouch is punctured or open or if the desiccant is missing. Care should be exercised when handling Phoenix™ panels. You should handle panels by the sides only to avoid marking, smudging or obscuring the front or back of the panel in any way. Place panel on the inoculation station so that the panel sequence barcode is facing upward. **Note: Panels must be used within 2 h of being removed from the pouch.**
4. For each test isolate, scan:
    1.) Sample Rack position barcode
    2.) Barcode on the ID Broth
    3.) Panel sequence number barcode

    The barcode data should transfer into the appropriate locations on the AP Login screen.



5. Save the data entered.
6. Turn panels over so that the inoculation ports face upward.
7. Pour ID broth into fill port on ID side of the panel (left). Pour AST broth into the fill port on the AST side of the panel (right).
8. Insert panel closures into both fill ports.
9. Inspect panels to ensure each well is filled.
10. Carefully place panels upright into Panel Transport caddy. Transfer and load into a Phoenix™ instrument being careful not to bump or jar the panels.

    Note: Panels must be loaded in the BD Phoenix™ Instrument within 30 min of inoculation.

7.8 Manual Broth and Panel Preparation for Strep

1. Order SMIC test in Batch Login as outlined above and use the barcode labels to label the ID Broth tube and a TSA blood agar plate for purity.
2. Using a sterile cotton swab, pick colonies of the same morphology and aseptically transfer to a tube of ID broth.
3. Make a suspension that is visually at or above desired inoculum density. Minimize fingerprints on the ID Broth tube.
4. Wring out swab thoroughly.

5. Cap the tube and vortex for 5 s.
6. Allow approximately 10 s for air bubbles to surface.
7. Insert the tube into the BD PhoenixSpec™ Nephelometer and take density reading. If the density of organisms is too low, you can add colonies from the isolate. Re-vortex the sample and reread to confirm that the correct density has been achieved. If the density exceeds 0.6 McFarland, additional ID Broth may be added to dilute the sample. The initial level of fluid should be marked on the tube. Cap the tube and vortex the suspension. When the target density is achieved, use a sterile transfer pipette to draw down the fluid level to the original level marked on the tube. **Alternatively, the suspension of ID Broth may be diluted by the AP instrument. Do not add an AST tube to the Sample Rack. When the rack has processed through to the output queue, transfer the rack to the Inoculation Station. Verify the final density of the sample. Touch the Inoculation Density button to Skip the sample and then press Save. The ID Broth can then be used to manually prepare the AST-S tube.**
8. Uncap a Phoenix™ AST-S Broth tube (8.0 mL) and place it in the Manual Inoculation Station. Holding the AST-S Indicator Solution bottle vertically, add one free-falling drop of AST-S indicator solution to the AST-S broth tube. Cap the AST-S tube and invert to mix. DO NOT VORTEX. **Note: Allow AST-S Indicator Solution to warm to room temperature before dispensing into AST broth. The unused portion of the indicator should be returned to 2-8ºC as soon as possible. Do not store at room temperature for more than 2 h. Opened bottles should be discarded after 14 d from initial opening. If volume other than one drop is added inadvertently, discard the tube and use a fresh tube of AST-S broth. After the addition of the Indicator to AST-S broth, the mixed solution can be stored in the dark, at room temperature, for as long as 8 h. Tubes must be used within 2 h after the addition of the indicator solution if exposed to light.**
9. If the final density of the bacterial suspension was 0.5 -0.6 McFarland, transfer 25 µL of the suspension from the ID broth tube into the AST-S broth tube. If the final density of the bacterial suspension was 0.2 – 0.3 McFarland, transfer 50 µL of the suspension from the ID Broth tube into the AST-S broth tube. **Note: Panels must be inoculated within 30 min of the time that the AST inoculum is prepared.**
10. Cap the AST-S tube and invert several times to mix. **Do not vortex**.
11. Wait a few seconds for air bubbles to surface.
12. Select Phoenix™ SMIC panel for inoculation. Examine the pouch, and do not use the panel if the pouch is punctured or opened.
13. Remove the panel from the pouch. Discard the desiccant. Do not use the panel if there is no desiccant or if the desiccant pouch is torn. **Note: Panels must be used within 2 h of being removed from the pouch.**
14. Place the panel on the Manual Inoculation Station with ports at the top and pad on the bottom.
15. Pour the AST-S tube inoculum into the fill port on the AST side of the panel (85-well side). Allow the fluid to traverse down the tracks before moving the panel.
16. Snap on the panel closures. Make sure that the closures are fully seated.
17. Visually inspect panels to be sure each of the wells is full. Make certain that the wells are not overfilled. If any of the wells are unfilled or overfilled, inoculate a new panel. **Note: Panels must be loaded into the instrument within 30 min of inoculation. Panels must be kept in the inoculation station after inoculation until the excess fluid has been completely absorbed by the pad. Panels should stay vertical in the transport caddy until loaded into the instrument. Inoculated panels should be handled with care. Avoid knocking or jarring the panel.**
18. Login the panel at the Phoenix™ instrument. Save the information and load the panel into the Phoenix™ instrument.

7.9 Manual Broth and Panel Preparation for Yeast

1. Order Yeast ID test in Batch Login as outlined above, and use the barcode labels to label the ID Broth tube and a TSA blood agar plate for purity.

2. Using a sterile cotton swab, pick colonies of the same morphology, and aseptically transfer to a tube of ID broth.
3. Make a suspension that is visually at or above desired inoculum density (2.0 – 2.4 McFarland). The BD Phoenix™ AP cannot be used to achieve this concentration. Minimize fingerprints on the ID Broth tube.
4. Wring out swab thoroughly.
5. Cap the tube and vortex for 5 s.
6. Allow approximately 10 s for air bubbles to surface.
7. Insert the tube into the BD PhoenixSpec™ Nephelometer and take density reading. If the density of organisms is too low, you can add colonies of the isolate. Re-vortex the sample and reread to confirm that the correct density has been achieved. If the density exceeds 2.4 McFarland, additional ID Broth may be added to dilute the sample. The initial level of fluid should be marked on the tube. Cap the tube and vortex the suspension. When the target density is achieved, use a sterile transfer pipette to draw down the fluid level to the original level marked on the tube.
8. Select Phoenix™ Yeast ID panel for inoculation. Examine the pouch, and do not use the panel if the pouch is punctured or opened.
9. Remove the panel from the pouch. Discard the desiccant. Do not use the panel if there is no desiccant or if the desiccant pouch is torn. **Note: Panels must be used within 2 h of being removed from the pouch.**
10. Place the panel on the Manual Inoculation Station with ports at the top and pad on the bottom.
11. Pour the ID tube inoculum into the fill port on the ID side of the panel. Allow the fluid to traverse down the tracks before moving the panel.
12. Snap on the panel closures. Make sure that the closures are fully seated.
13. Visually inspect panels to be sure each of the wells is full. Make certain that the wells are not overfilled. If any of the wells are unfilled or overfilled, inoculate a new panel. **Note: Panels must be loaded into the instrument within 30 min of inoculation. Panels must be kept in the inoculation station after inoculation until the excess fluid has been completely absorbed by the pad. Panels should stay vertical in the transport caddy until loaded into the instrument. Inoculated panels should be handled with care. Avoid knocking or jarring the panel.**
14. Login the panel at the Phoenix™ instrument. You must select the type of media that the test isolate was grown on. Save the information and load the panel into the Phoenix™ instrument.

7.10 Purity Check

A purity check should be performed by inoculating a TSA blood agar plate using the same inoculum suspension used to inoculate the Phoenix panel. Do not over inoculate the purity check plate. It is essential to streak the plate for isolation to determine the purity of the inoculum. The purity check plate should be incubated for 18 – 24 h under appropriate isolate conditions and examined to ensure that only the test isolate is present. If the purity plate appears mixed, the Phoenix results should be discarded and testing repeated using isolated colonies.

8.0 Results

Organism identification will appear in EpiCenter with a probability percentage from the Phoenix™ database based on the substrate reaction profile. Results from each substrate will appear as +, -, V or X for each reaction. The MIC results and Interpretive Categorical Results (SIR) will be shown for the appropriate organism/antimicrobial agent combinations.

Special messages will be shown when the BDXpert System detects results that are of particular clinical interest.

Further information concerning results obtained from the Phoenix™ system can be found in the BD Phoenix™ System User's Manual ("Obtaining Results").

Messages

Error messages may appear if the system detects unexpected reactivity due to inappropriate procedure or instrument malfunction. For a complete listing of error codes and their meaning refer to the BD Phoenix™ System User's Manual ("System Alerts", "Needs Attention" and "Troubleshooting").

9.0 Repeat Testing

Testing should be repeated if the purity check plate reveals a mixed or contaminated inoculum was used. Repeat testing may also be performed to confirm unusual susceptibility results. Repeat Phoenix™ testing should not be performed to confirm identifications. If Phoenix™ testing produces no identification, or if the identification is unusual, consult Rounds and pursue offline testing.

10.0 Quality Control & Quality Assurance

10.1 Panel Quality Control

10.1.1 Frequency of Testing

Each new lot or shipment of Phoenix™ panels should undergo quality control testing upon receipt to ensure that they function expected. Weekly testing should be performed on all MIC or MIC/ID lots currently in use. Testing is typically performed with fresh subcultures every Tuesday.

10.1.2 Quality Control Strains

Gram-Negative Panel for ID - NID

- *Escherichia coli* ATCC™ 25922
- *Pseudomonas aeruginosa* ATCC™ 27853

Gram-Negative Panels with AST - NMIC/ID & NMIC-133

- *Escherichia coli* ATCC™ 25922
- *Pseudomonas aeruginosa* ATCC™ 27853
- *Escherichia coli* ATCC™ 35218
- *Klebsiella pneumoniae* ATCC™ 700603

Gram-Positive Panel for AST - PMIC-108

- *Staphylococcus aureus* ATCC™ 29213
- *Staphylococcus aureus* ATCC™ BAA-976
- *Staphylococcus aureus* ATCC™ BAA-977
- *Enterococcus faecalis* ATCC™ 29212
- *Enterococcus faecalis* ATCC™ 51299

Strep Panel for AST - SMIC-101

- *Streptococcus pneumoniae* ATCC™ 49619

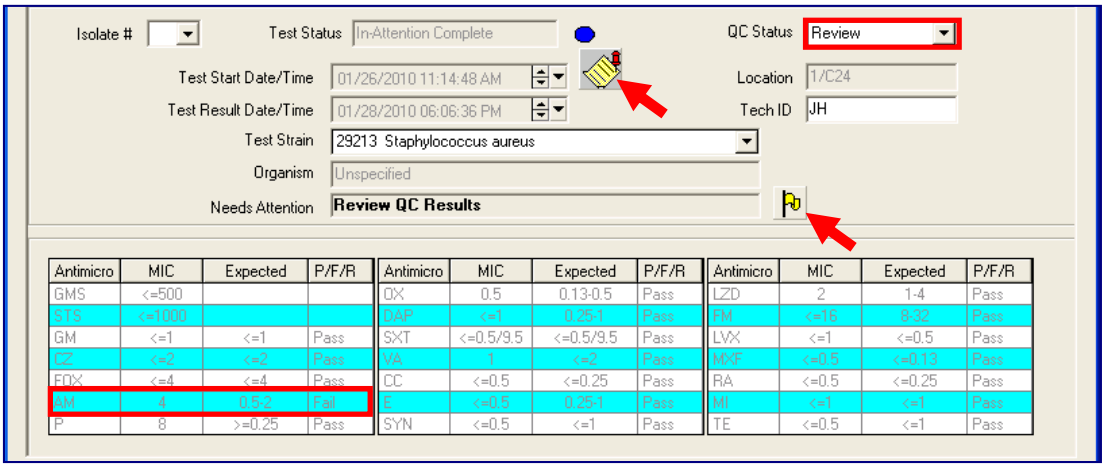
Yeast ID Panel

- *Candida albicans* ATCC™ 24433
- *Candida parapsilosis* ATCC™ 22019

10.1.3 QC Panel Results

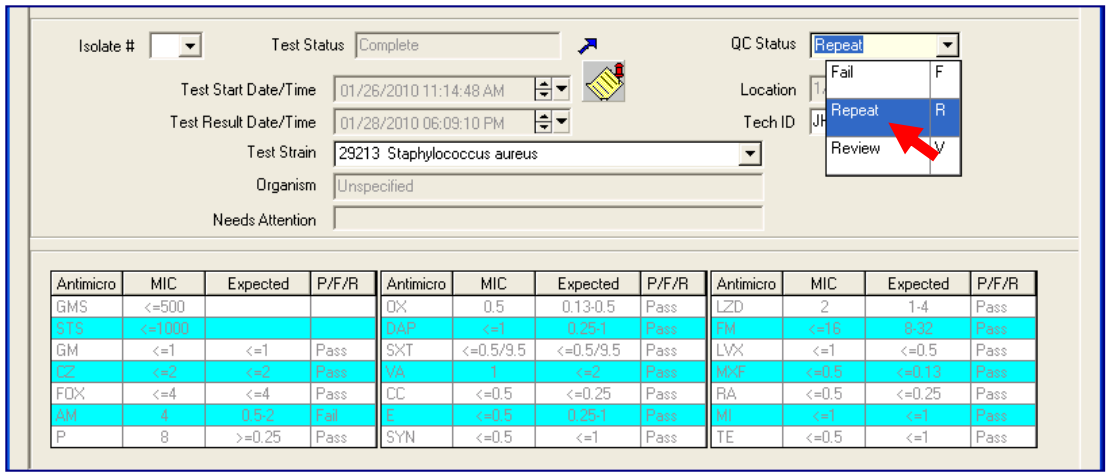
On the following day the QC test results must be verified. QC results can be reviewed in Epicenter by using the Cumulative QC Test Results filter. If all panels passed no further action is required. If discrepant results are obtained, review test procedures as well as confirm purity of the quality control strain used. Any panels that fail QC will automatically show up in the Needs Attention filter (yellow flag icon). From this filter the test results may be reviewed by right clicking to access the options menu and then selecting "Show Test Details." Check the P/F column to determine which AST results are out of range. QC results for ESBL and iMLSb can be viewed

under Special Messages (yellow note). Acknowledge the Needs Attention alert by clicking on the yellow flag, selecting “Review QC Results,” and clicking OK.



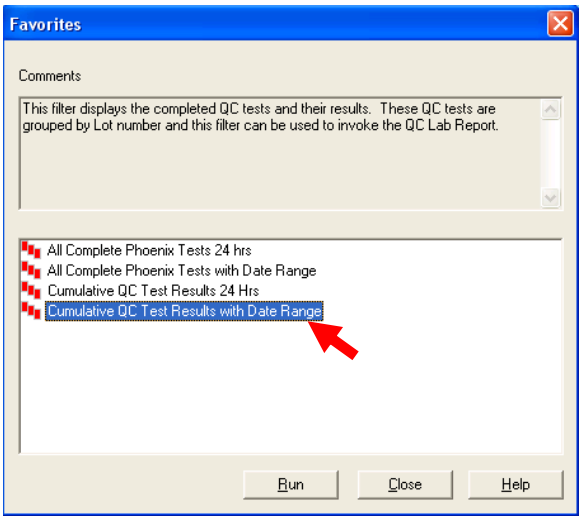
Antimicro	MIC	Expected	P/F/R	Antimicro	MIC	Expected	P/F/R	Antimicro	MIC	Expected	P/F/R
GMS	<=500			OX	0.5	0.13-0.5	Pass	LZD	2	1-4	Pass
STS	<=1000			DAP	<=1	0.25-1	Pass	FM	<=16	8-32	Pass
GM	<=1	<=1	Pass	SXT	<=0.5/9.5	<=0.5/9.5	Pass	LVX	<=1	<=0.5	Pass
CZ	<=2	<=2	Pass	VA	1	<=2	Pass	MXF	<=0.5	<=0.13	Pass
FOX	<=4	<=4	Pass	CC	<=0.5	<=0.25	Pass	RA	<=0.5	<=0.25	Pass
AM	4	0.5-2	Fail	E	<=0.5	0.25-1	Pass	MI	<=1	<=1	Pass
P	8	>=0.25	Pass	SYN	<=0.5	<=1	Pass	TE	<=0.5	<=1	Pass

The QC Status will be held in “Review” until you determine a designation of either “Repeat” or “Fail.” Typically, QC results that are out of range are random occurrences and the results should be repeated. The “Fail” designation would be used only if repeat testing fails to yield results within the expected QC limits. Click on the drop down list for QC Status and select the appropriate designation.

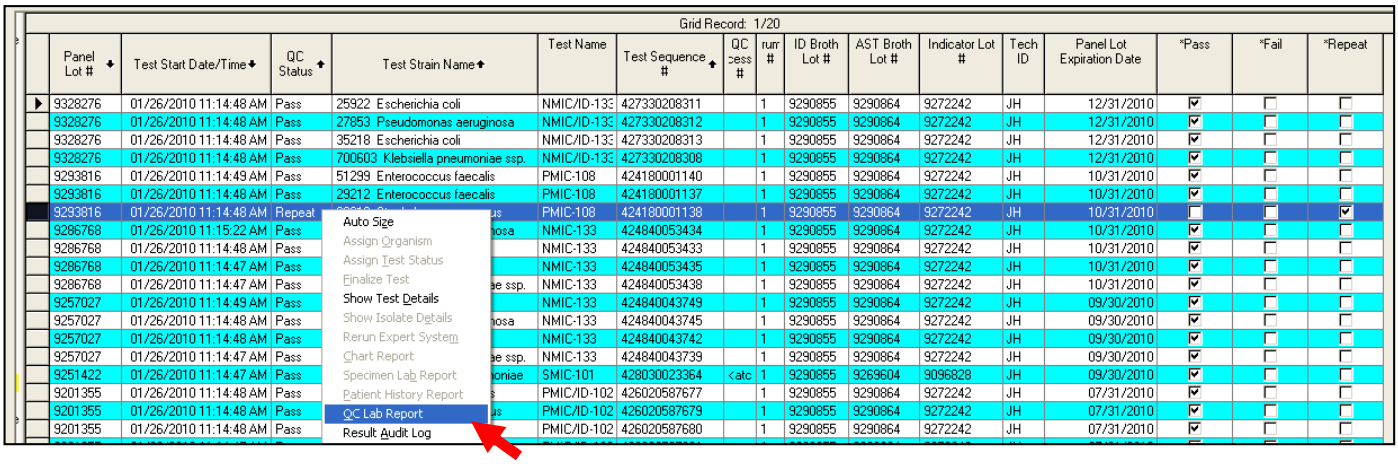


Antimicro	MIC	Expected	P/F/R	Antimicro	MIC	Expected	P/F/R	Antimicro	MIC	Expected	P/F/R
GMS	<=500			OX	0.5	0.13-0.5	Pass	LZD	2	1-4	Pass
STS	<=1000			DAP	<=1	0.25-1	Pass	FM	<=16	8-32	Pass
GM	<=1	<=1	Pass	SXT	<=0.5/9.5	<=0.5/9.5	Pass	LVX	<=1	<=0.5	Pass
CZ	<=2	<=2	Pass	VA	1	<=2	Pass	MXF	<=0.5	<=0.13	Pass
FOX	<=4	<=4	Pass	CC	<=0.5	<=0.25	Pass	RA	<=0.5	<=0.25	Pass
AM	4	0.5-2	Fail	E	<=0.5	0.25-1	Pass	MI	<=1	<=1	Pass
P	8	>=0.25	Pass	SYN	<=0.5	<=1	Pass	TE	<=0.5	<=1	Pass

A **QC Lab Report** should be printed for any unacceptable QC results. This can be performed from the Cumulative QC Test Results filter.



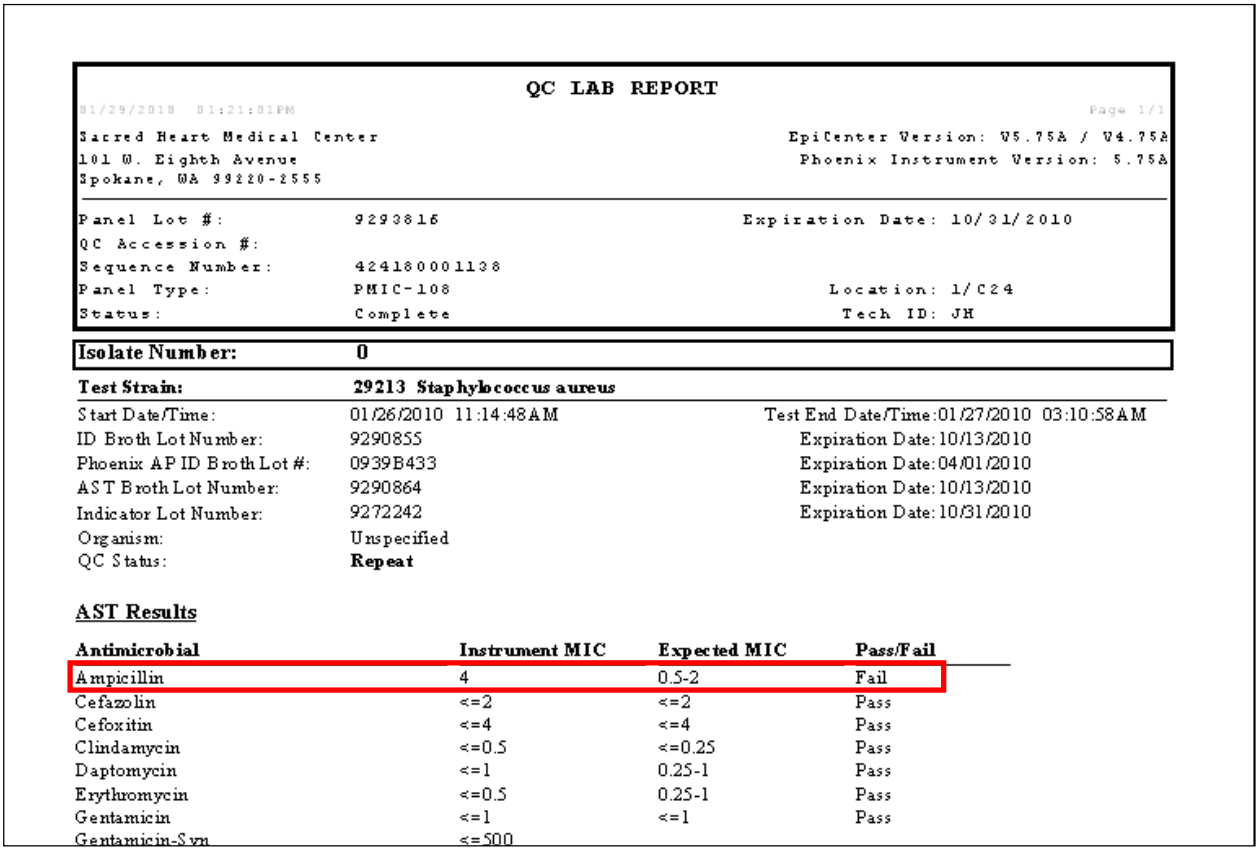
After running the filter select the panel with the unacceptable result(s) from the list. Right click on the row to access the options menu (see example below). Choose QC Lab Report and then print



Grid Record: 1/20

Panel Lot #	Test Start Date/Time	QC Status	Test Strain Name	Test Name	Test Sequence #	QC Access #	run #	ID Broth Lot #	AST Broth Lot #	Indicator Lot #	Tech ID	Panel Lot Expiration Date	*Pass	*Fail	*Repeat
9328276	01/26/2010 11:14:48 AM	Pass	25922 Escherichia coli	NMIC/ID-133	427330208311		1	9290855	9290864	9272242	JH	12/31/2010	☑	☐	☐
9328276	01/26/2010 11:14:48 AM	Pass	27853 Pseudomonas aeruginosa	NMIC/ID-133	427330208312		1	9290855	9290864	9272242	JH	12/31/2010	☑	☐	☐
9328276	01/26/2010 11:14:48 AM	Pass	35218 Escherichia coli	NMIC/ID-133	427330208313		1	9290855	9290864	9272242	JH	12/31/2010	☑	☐	☐
9328276	01/26/2010 11:14:48 AM	Pass	700603 Klebsiella pneumoniae ssp.	NMIC/ID-133	427330208308		1	9290855	9290864	9272242	JH	12/31/2010	☑	☐	☐
9293816	01/26/2010 11:14:49 AM	Pass	51299 Enterococcus faecalis	PMIC-108	424180001140		1	9290855	9290864	9272242	JH	10/31/2010	☑	☐	☐
9293816	01/26/2010 11:14:48 AM	Pass	29212 Enterococcus faecalis	PMIC-108	424180001137		1	9290855	9290864	9272242	JH	10/31/2010	☑	☐	☐
9293816	01/26/2010 11:14:48 AM	Repeat	[illegible]	PMIC-108	424180001138		1	9290855	9290864	9272242	JH	10/31/2010	☐	☐	☑
9286768	01/26/2010 11:15:22 AM	Pass	[illegible]	NMIC-133	424840053434		1	9290855	9290864	9272242	JH	10/31/2010	☑	☐	☐
9286768	01/26/2010 11:14:48 AM	Pass	[illegible]	NMIC-133	424840053433		1	9290855	9290864	9272242	JH	10/31/2010	☑	☐	☐
9286768	01/26/2010 11:14:47 AM	Pass	[illegible]	NMIC-133	424840053435		1	9290855	9290864	9272242	JH	10/31/2010	☑	☐	☐
9286768	01/26/2010 11:14:47 AM	Pass	[illegible]	NMIC-133	424840053438		1	9290855	9290864	9272242	JH	10/31/2010	☑	☐	☐
9257027	01/26/2010 11:14:49 AM	Pass	[illegible]	NMIC-133	424840043749		1	9290855	9290864	9272242	JH	09/30/2010	☑	☐	☐
9257027	01/26/2010 11:14:48 AM	Pass	[illegible]	NMIC-133	424840043745		1	9290855	9290864	9272242	JH	09/30/2010	☑	☐	☐
9257027	01/26/2010 11:14:48 AM	Pass	[illegible]	NMIC-133	424840043742		1	9290855	9290864	9272242	JH	09/30/2010	☑	☐	☐
9257027	01/26/2010 11:14:47 AM	Pass	[illegible]	NMIC-133	424840043739		1	9290855	9290864	9272242	JH	09/30/2010	☑	☐	☐
9251422	01/26/2010 11:14:47 AM	Pass	[illegible]	SMIC-101	428030023364	<atc	1	9290855	9269604	9096828	JH	09/30/2010	☑	☐	☐
9201355	01/26/2010 11:14:48 AM	Pass	[illegible]	PMIC/ID-102	426020587677		1	9290855	9290864	9272242	JH	07/31/2010	☑	☐	☐
9201355	01/26/2010 11:14:48 AM	Pass	[illegible]	PMIC/ID-102	426020587679		1	9290855	9290864	9272242	JH	07/31/2010	☑	☐	☐
9201355	01/26/2010 11:14:48 AM	Pass	[illegible]	PMIC/ID-102	426020587680		1	9290855	9290864	9272242	JH	07/31/2010	☑	☐	☐

Auto Size
Assign Organism
Assign Test Status
Finalize Test
Show Test Details
Show Isolate Details
Rerun Expert System
Chart Report
Specimen Lab Report
Patient History Report
QC Lab Report
Result Audit Log



QC LAB REPORT

01/29/2010 01:21:01PM Page 1/1

Sacred Heart Medical Center
101 W. Eighth Avenue
Spokane, WA 99220-2555

EpiCenter Version: V5.75A / V4.75A
Phoenix Instrument Version: 5.75A

Panel Lot #: 9293816 Expiration Date: 10/31/2010
QC Accession #:
Sequence Number: 424180001138
Panel Type: PMIC-108 Location: 1/C24
Status: Complete Tech ID: JH

Isolate Number: 0

Test Strain: 29213 Staphylococcus aureus

Start Date/Time:	01/26/2010 11:14:48AM	Test End Date/Time:	01/27/2010 03:10:58AM
ID Broth Lot Number:	9290855	Expiration Date:	10/13/2010
Phoenix AP ID Broth Lot #:	0939B433	Expiration Date:	04/01/2010
AST Broth Lot Number:	9290864	Expiration Date:	10/13/2010
Indicator Lot Number:	9272242	Expiration Date:	10/31/2010
Organism:	Unspecified		
QC Status:	**Repeat**		

AST Results

Antimicrobial	Instrument MIC	Expected MIC	Pass/Fail
Ampicillin	4	0.5-2	Fail
Cefazolin	<=2	<=2	Pass
Cefoxitin	<=4	<=4	Pass
Clindamycin	<=0.5	<=0.25	Pass
Daptomycin	<=1	0.25-1	Pass
Erythromycin	<=0.5	0.25-1	Pass
Gentamicin	<=1	<=1	Pass
Gentamicin-Syn	<=500		

Document the following information on the QC Lab Report:

- ✓ Known explanations for the QC failure (such as contamination)
- ✓ Corrective actions that have been initiated
- ✓ Your initials

Give this report to the Microbiology Technical/QA specialist.

10.2 AP Instrument & PhoenixSpec™ QC Testing & Maintenance

Refer to the BD Phoenix™/AP User's Manuals for detailed instruction on performing instrument maintenance and QC testing.

1. Nephelometer Calibration - Daily

   This function calibrates the nephelometers as part of routine maintenance or to correct an error condition. There are four calibration standards used for the AP instruments. The results are transferred into Epicenter when the sample rack is placed on the inoculation station. Three of the standards are used for the PhoenixSpec™ calibration. Refer to the user's manuals for instructions.

2. Nephelometer Check - Daily

   The nephelometers on the AP instrument and in the PhoenixSpec™ must be verified daily for accuracy. There are four calibration tubes used to verify the AP instrument nephelometer. The results are transferred into Epicenter when the sample rack is placed on the inoculation station. Two of the calibration tubes are used to verify the PhoenixSpec™. These values should be entered into the Phoenix™ Maintenance Log which is accessible on the Epicenter server desktop. If the daily check fails for either instrument despite calibration, notify the technical specialist, supervisor, or lead tech. Do not use the instrument for clinical samples until the issue has been resolved.

3. Dispense Pump Calibration - Daily

   This function verifies the volume dispensed and adjusts the dispense pump based on the results. Calibration should be performed daily or after the dispense tubing set is replaced. . If the daily calibration fails for either instrument, notify the technical specialist, supervisor, or lead tech. Do not use the instrument for clinical samples until the issue has been resolved.

4. Pipettor Verification (annual)

   This function is used to verify the accuracy of the pipettor. Detailed instructions are outlined in the instrument user's manual. Results should be entered into the Phoenix™ Maintenance Log.

10.3 Phoenix™ Instrument QC & Maintenance

1. Temperature Daily Check
   Record the temperature on the LCD display and on the temperature standard panel in the Phoenix™ Maintenance Log. Note that the temperature panel can be brought into view by selecting one of the instrument LED check functions on the Maintenance menu.

2. Normalizer Panel Check

   Each day the Phoenix™ instrument resets its optical parameters against normalizer panels. The user should verify that the instrument check passed on the Daily Instrument Report (refer to user's manual for details).

3. Verification of LEDs, System Alert Indicator, and Audible Alarm

   These components should be checked daily. Record results in the Phoenix™ Maintenance Log.

11.0 Limitations

11.1 General

- A Gram stain test may be required for the selection of the appropriate Phoenix™ panel types. Accurate identification and/or AST results may not be made without this test.
- Use only well-isolated bacterial colonies from one of the recommended primary isolation media (see Table 1). Media containing esculin should not be used. Use of mixed colonies could result in inaccurate identification and/or AST interpretations.

- Only the BD PhoenixSpec™ Nephelometer or the BD Phoenix™ AP can be used to measure the inoculum density.
- Phoenix™ panels can be read only by the Phoenix™ instrument. Visual interpretation of the Phoenix™ panels is not possible. Any attempt to manually interpret results from the panel may lead to misidentification and/or inaccurate AST interpretations.

11.2 Identification

- The unique panel environment combined with the shortened incubation time may result in Phoenix™ panel reactions varying from those obtained using conventional biochemical media.

11.3 Antimicrobial Susceptibility Testing

- Refer to package insert for each panel for a list of specific AST limitations.
- After the addition of Phoenix™ AST Indicator Solution to the AST broth tubes, mix by inversion. DO NOT VORTEX. Vortexing may cause air bubbles to form in the AST broth, which can result in inappropriate filling of the Phoenix™ panel during inoculation.
- Because of the low probability of occurrence or special growth requirements, some organisms included in the ID taxa are not included in the AST database. These organisms will display the message “Organism not included in the AST database, perform alternate method.”
- For some organism/antimicrobial combinations, the absence of resistant strains precludes defining any result categories other than “susceptible.” For strains yielding results suggestive of a “nonsusceptible” category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory that will confirm the result using the CLSI reference dilution method.

12.0 Verification of the Test Method

In order to verify the performance of the BD Phoenix™ automated microbial identification and susceptibility testing system, numerous clinical isolates were tested. Results from Phoenix™ were compared to those obtained by current methods. These methods included identification by spot tests, conventional biochemical testing, and manual commercial systems. Antimicrobial susceptibility testing (AST) was performed by Kirby-Bauer disk diffusion and/or by E-test. Phoenix™ testing was performed using the NMIC/ID-133 panel for gram-negative bacilli, PMIC/ID-102 for gram-positive cocci, and SMIC/ID-100 for strep. The following tables provide a summary of the results for each group of organisms tested. Abbreviations: N = # of isolates tested, CA = categorical agreement, mE = minor errors, ME = major errors, VME = very major errors.

12.1 Gram-Negative Bacilli

Enterobacteriaceae	N	% CA	(%) of isolates associated with: mE	ME	VME
Overall ID to genus Level	139	99			
Citrobacter spp.	7	100			
E. coli	55	100			
Edwardsiella tarda	1	100			
Enterobacter spp.	12	100			
Hafnia alvei	3	100			
Klebsiella spp.	16	100			
Morganella morganii	2	100			
Proteus spp.	13	100			
Providencia spp.	2	100			
Salmonella spp.	13	92*			
Serratia spp.	7	100			
Shigella spp.	8	100			
Amoxicillin/Clavulanate	118	89	11	0	0
Ampicillin	139	99	1	0	0
Cefazolin	118	92	8	0	0
Ceftazidime	118	94	6	0	1**
Ceftriaxone	118	92	8	0	0
Ciprofloxacin	118	99	3	0	0
Gentamicin	117	99	1	0	0
Impipenem	28	96	4	0	0
Levofloxacin	139	98	2	0	0
Nitrofurantoin	105	92	8	0	0
Piperacillin/Tazobactam	118	97	3	0	0
Trimethoprim/Sulfamethoxazole	132	99	1	0	0

*1 *Salmonella arizonae* misidentified as *E. coli*

**1 ESBL not detected by Phoenix

Since the completion of the validation study, another *S. arizonae* isolate was misidentified by Phoenix™ as an *E. coli*. Both isolates were verified by the WA State lab as *S. arizonae*. Both isolates were submitted to BD for review.

The initial validation included 20 ESBL-producing isolates, including both *E. coli and Klebsiella* spp. Phoenix™ failed to identify 1 of these isolates as ESBL. An additional 70 isolates that were positive for ESBL by disk approximation were tested on Phoenix™. All 70 were correctly identified as ESBL. The overall sensitivity for detecting ESBL in this study was 99%. Other studies published on detection of ESBL by Phoenix™ have reported the specificity to range between 33.3 and 72.2%, depending on the specific genus/species tested. Because of this, we confirm all isolates identified by Phoenix™ as ESBL by perform the CLSI disk approximation test. A 3-month review of 66 isolates that tested ESBL-positive on Phoenix™ revealed that 37 isolates were also positive by disk diffusion, yielding a specificity of 56%.

BD recommends using XLD agar for ID but not for antimicrobial susceptibility testing. To validate the use of XLD agar for susceptibility testing, 10 *Salmonella* and 10 *Shigella* isolates were tested on TSA 5% sheep blood agar and XLD agar in parallel. No AST discrepancies were encountered.

To validate the use of CHROMagar O157 for use for identification testing, a total of 10 confirmed *E. coli* O157 isolates were tested. All of the isolates were grown overnight on CHROMagar O157 and then used to inoculate NID panels. All 10 (100%) of the isolates produced an identification of *E. coli.*

To validate the use of CHROMagar Salmonella for identification testing, a total of 11 confirmed *Salmonella* isolates were tested. These isolates were confirmed by the WA state public health laboratory. All of the isolates were grown overnight on CHROMagar Salmonella and then used to inoculate Phoenix™ panels. All 11 (100%) of the isolates produced an identification of *Salmonella* species. An additional 10 *Salmonella* isolates that had been previously been tested from XLD for susceptibility testing were subcultured to CHROMagar Salmonella. AST was repeated using growth from the CHROMagar medium. There was 100% concordance between the AST results obtained from the XLD inoculum and the results obtained from the CHROMagar inoculum.

			(%) of isolates associated with:		
P. aeruginosa	N	% CA	mE	ME	VME
ID to genus and species	15	100			
Ceftazidime	15	93	7	0	0
Ciprofloxacin	15	93	7	0	0
Gentamicin	15	93	7	0	0
Impipenem	15	100	0	0	0
Levofloxacin	15	93	7	0	0
Piperacillin/Tazobactam	15	93	7	0	0
Tobramycin	15	100	0	0	0
			(%) of isolates associated with:		
Acinetobacter spp.	N	% CA	mE	ME	VME
ID to genus	9	100			
Ceftazidime	9	78	22	0	0
Gentamicin	9	89	11	0	0
Impipenem	9	100	0	0	0
Levofloxacin	9	100	0	0	0
Trimethoprim/Sulfamethoxazole	9	100	0	0	0
			(%) of isolates associated with:		
S. maltophilia	N	% CA	mE	ME	VME
ID to genus and species					
Levofloxacin	5	80	20	0	0
Trimethoprim/Sulfamethoxazole	5	100	0	0	0

A variety of other non-fermenting gram-negative bacilli were tested and correctly identified by Phoenix™. These included 1 *Alcaligenes* faecalis, 1 *Achromobacter* spp., 4 *B. cepacia*, 3 *Chryseobacterium* spp., 1 *Delftia* acidivorans, 2 *Methylobacterium* spp., 1 *Myroides odoratum*, 8 *Pseudomonas* spp. (not aeruginosa), 1 *Shewanella putrifaciens*, 2, *Rhizobium* radiobacter, and 1 *Sphingomonas paucimobilius.* Isolates that were misidentified by Phoenix™ included 1 *Achromobacter* spp., 1 *Acidovorax* temperans, 1 *Alcaligenes* faecalis, 1 *Flavimonas* spp., 1 *Moraxella* spp., and 2 *Roseomonas* spp. Identifications of these less frequently encountered non-fermenters should be made with Rounds consultation and backed up by conventional testing when necessary.

Other gram-negative bacilli that were tested on Phoenix™ for identification include 9 *Eikenella corrodens* (100% agreement to species level), 9 *Pasteurella* spp. (100% agreement to genus level), 5 *Aeromonas* spp. (100% agreement to genus level), and 5 *Vibrio* spp. (100% agreement to species level)

12.2 Gram-Positive Cocci

			(%) of isolates associated with:		
Staphylococcus aureus	N	% CA	mE	ME	VME
ID to genus and species (included 13 MRSA)	26	100			
Cefoxitin	26	100	0	0	0
Clindamycin	26	100	0	0	0
Erythromycin	26	100	0	0	0
Gentamicin	26	100	0	0	0
Levofloxacin	26	100	0	0	0
Nitrofurantoin	4	100	0	0	0
Oxacillin	26	100	0	0	0
Trimethoprim/Sulfamethoxazole	26	100	0	0	0
Vancomycin	26	100	0	0	0

D-tests are performed offline for staph isolates that test resistant to erythromycin and susceptible to clindamycin. D-test results are entered into Epicenter for correct interpretation of clindamycin.

BD recommends using CNA agar for ID but not for antimicrobial susceptibility testing. To validate the use of CNA agar for susceptibility testing, 20 *S. aureus* isolates were tested on TSA 5% sheep blood agar and CNA agar in parallel. 3 (10%) minor errors were encountered with gatifloxacin and 1 (5%) minor error with moxifloxacin. Results were “S” on blood agar and “I” on CNA agar. Neither of these drugs are routinely reported.

Mannitol Salt Agar (MSA) is routinely used to isolate *S. aureus* from Cystic Fibrosis respiratory samples. Since BD does not list this medium as a recommended medium for Phoenix™ testing, an evaluation was performed using 20 *S. aureus* isolates. This included 10 MSSA and 10 MRSA. Phoenix™ susceptibility testing was performed on each isolate grown on TSA w/5% sheep blood and MSA. There was 100% concordance between AST results obtained from the isolates grown on MSA as compared to TSA w/5% sheep blood.

CHROMagar MRSA II medium (BD) was put into use for MRSA screen cultures in 3/2011. A study was performed to validate this medium for the use with Phoenix™ PMIC panels. A total of 10 specimens that yielded MRSA in routine culture were retrieved and used to inoculate CHROMagar. These plates were incubated aerobically overnight. All 10 (100%) of the specimens yielded mauve colonies consistent with MRSA. Inoculum from the CHROMagar plates was used to perform AST on Phoenix™ PMIC-108 panels. These results were compared to those obtained from the routine cultures. No minor or major discrepancies were encountered on isolates tested from CHROMagar as compared to routine media.

			(%) of isolates associated with:		
Coagulase-negative Staph	N	% CA	mE	ME	VME
All IDs were spp. of CoNS	22	100			
S. lugdunensis	6	100			
Clindamycin	22	100	0	0	0
Erythromycin	22	95	5	0	0
Gentamicin	22	100	0	0	0
Levofloxacin	22	100	0	0	0
Nitrofurantoin	8	88	13	0	0
Vancomycin	22	100	0	0	0

There are currently no CLSI MIC breakpoints for coagulase-negative staph and cefoxitin. Cefoxitin testing is currently performed offline by disk diffusion. Cefoxitin disk diffusion results are entered into Epicenter for correct interpretation of oxacillin and cefazolin.

To validate the use of CNA agar for susceptibility testing, 20 coagulase-negative staph isolates were tested on TSA 5% sheep blood agar and CNA agar in parallel. No AST discrepancies were encountered.

In 12/2009, an additional validation study was performed to evaluate the newly released PMIC-108 panel. The manufacturer claimed that this panel is able to detect Staphylococcus isolates with inducible Macrolide-Lincosamide-Streptogramin type B (iMLSb) phenotype. Detection of such isolates has been previously accomplished by performing the disk diffusion D-test. In this validation study, a total of 41 staph isolates were tested. This included 21 *S. aureus* and 20 coagulase-negative staph. Only 1 (2%) unresolved discrepancy was encountered in which the Phoenix™ was unable to detect iMLSb phenotype for a coagulase-negative staph that was D-test positive. The tables below summarize the data from this study.

Phoenix™ PMIC-108 Panel vs. D-test

S. aureus	Positive D-Test	Negative D-Test	Total
iMLSb Positive	10	0	10
iMLSb Negative	0	11	11
Total	10	11	21

% Agreement = 100% (84.5 to 100%)
Positive Agreement = 100%
Negative Agreement = 100%

CoNS	Positive D-Test	Negative D-Test	Total
iMLSb Positive	9	0	9
iMLSb Negative	1	10	11
Total	10	10	20

% Agreement = 95.0% (76.4 to 99.1%)
Positive Agreement = 90%
Negative Agreement = 100%

			(%) of isolates associated with:		
Enterococcus spp.	N	% CA	mE	ME	VME
E. faecalis	15	100			
E. faecium	5	100			
Ampicillin	19	100	0	0	0
Erythromycin	19	100	0	0	0
Gentamicin (HL)	3	100	0	0	0
Levofloxacin	19	89	11	0	0
Nitrofurantoin	15	100	0	0	0
Vancomycin	19	100	0	0	0

4 (100%) of the isolates were correctly identified as VRE. Speciation of *E. faecalis* and *E. faecium* were confirmed using arabinose, melibiose, and sorbitol CTA sugars. There was 100% correlation between Phoenix™ identification and results obtained from CTA sugars. Identifications other than these two species have not been validated at Sacred Heart and should be reported as *Enterococcus* species.
To validate the use of CNA agar for susceptibility testing, 20 *Enterococcus* isolates were tested on TSA 5% sheep blood agar and CNA agar in parallel. The results are summarized in the table below.

Testing *Enterococcus* on CNA Agar vs. TSA w/5% Sheep Blood

Enterococcus spp.	N	% CA	(%) of isolates associated with: mE	ME	VME
E. faecalis	16	100			
E. faecium	4	100			
Ampicillin	20	100	0	0	0
Erythromycin	20	95	5	0	0
Gentamicin (HL)	20	100	0	0	0
Levofloxacin	20	85	15	0	0
Linezolid	20	70	30	0	0
Nitrofurantoin	20	100	0	0	0
Synercid	4	100	0	0	0
Tetracycline	20	100	0	0	0
Vancomycin	20	100	0	0	0

S. pneumoniae	N	% CA	(%) of isolates associated with: mE	ME	VME
ID to genus and species*	24	100			
Ceftriaxone (E-test vs. PHX)	15	67	33	0	0
Clindamycin	17	100	0	0	0
Erythromycin	23	100	0	0	0
Levofloxacin	15	100	0	0	0
Penicillin (OX or E-test vs. PHX)	24	79	21	0	0
Trimethoprim sulfa	24	96	4	0	0
Vancomycin	23	100	0	0	0

*1 (4%) isolate was identified as *S. mitis/pneumoniae* and required performance of bile solubility test offline to confirm Phoenix™ identification.

Viridans Strep	N	% CA	(%) of isolates associated with: mE	ME	VME
All IDs were spp. of Viridans group	17	100			
Clindamycin	17	100	0	0	0
Penicillin (E-test vs. PHX)	15	80	0	0	0
Vancomycin	17	100	0	0	0

Beta-hemolytic Strep	N	% CA	(%) of isolates associated with: mE	ME	VME
***S. agalactiae* (Group B) ID**	15	100			
Clindamycin	15	100	0	0	0
Erythromycin	15	100	0	0	0
Penicillin	14	100	0	0	0
Vancomycin	12	100	0	0	0
***S. pyogenes* (Group A) ID**	3	100			
Clindamycin	3	100	0	0	0
Erythromycin	3	100	0	0	0
Penicillin	2	100	0	0	0
Vancomycin	3	100	0	0	0
Beta Strep (non-A, non-B)	3	100			
(PHX ID = *S. dysagalactiae*)	3				
Clindamycin	3	100	0	0	0
Erythromycin	3	67	0	33	0
Penicillin	2	100	0	0	0
Vancomycin	3	100	0	0	0

1 (33%) Beta-hemolytic Strep, non-A/B was susceptible to erythromycin by disk diffusion and resistant by Phoenix™. Repeat testing did not resolve the discrepancy.
5 of the beta-hemolytic strep isolates were D-test positive and required offline testing for the correct interpretation of clindamycin.

12.3 Yeast

In September, 2011, the Phoenix™ YEAST ID panel was evaluated with a total of 104 yeast isolates. This included newly isolated clinical strains, previously identified frozen isolates, ATCC strains, and strains from past CAP surveys. Testing with the Phoenix™ YEAST ID panel was performed as per manufacturer's protocol. All isolates were grown on TSA with 5% sheep blood at 35 ± 2°C unless growth was insufficient. Some of the *Cryptococcus* isolates grew poorly on blood agar and were alternatively grown on Sabouraud Dextrose agar for testing. Inoculation preparation was performed manually with the use of a BD PhoenixSpec™ nephelometer to adjust the turbidity to a 2 to 2.4 McFarland. For manual identification, isolates were identified with a variety of methods including microscopic morphology, colony morphology on BBL™ CHROMagar Candida, germ tube production, rapid trehalose assimilation, colony morphology on bird seed agar, urease activity, and growth on CGB agar, or by one of two manual commercial systems, the API 20 C AUX (bioMérieux, Durham, NC) or the Uni-Yeast-Tek® (Remel, Lenexa, KS). Phoenix™ identifications were compared to and verified with the offline identifications. Discrepant results were resolved by submitting isolates to specialized reference laboratories for identification.

A total of 104 yeast isolates were used in the evaluation. Of those, 94 were identified to the species level. These included 80 *Candida* and 14 *Cryptococcus* isolates. Overall, the Phoenix™ YEAST ID panel correctly identified 93 (99%) out of 94 of the isolates to the species level. One *Cryptococcus laurentii* isolate was designated with the correct genus but not the correct species. Manual methods correctly identified 89 (95%) of the isolates. Four (5%) of the *Candida* isolates were designated with the incorrect species and no identification was achieved with 1 *Cryptococcus uniguttulatus* isolate. The other 10 yeast isolates evaluated included 8 *Trichosporon* and 2 *Rhodotorula* isolates. These genera are routinely identified only to the genus level and comparisons of species identifications were not determined. The Phoenix™ YEAST ID and manual identification methods correctly identified 9 (90%) of these isolates. The Phoenix™ YEAST ID produced no identification for 1 (10%) of the *Trichosporon* isolates while manual methods produced an erroneous identification for another. The table on the following page summarizes the results of the evaluation. Note that other genera such as *Geotrichum* and *Saccaromyces* were not available for this evaluation. The performance of the Phoenix™ system with other yeast will need to be evaluated prospectively.

Organism	Isolates Tested	No. (%) of isolates							
		Correct species		Correct genus only		Incorrect ID		No identification	
		Phoenix YEAST ID	Manual Systems	Phoenix YEAST ID	Manual Systems	Phoenix YEAST ID	Manual Systems	Phoenix YEAST ID	Manual Systems
***Candida* spp.**									
C. albicans	27	27	27	0	0	0	0	0	0
C. dubliniensis	2	2	0	0	2	0	0	0	0
C. glabrata	13	13	13	0	0	0	0	0	0
C. guilliermondii	1	1	1	0	0	0	0	0	0
C. lipolytica	1	1	0	0	1	0	0	0	0
C. lusitaniae	4	4	3	0	1	0	0	0	0
C. parapsilosis	12	12	12	0	0	0	0	0	0
C. pelliculosa	1	1	1	0	0	0	0	0	0
C. krusei	6	6	6	0	0	0	0	0	0
C. tropicalis	13	13	13	0	0	0	0	0	0
Subtotal	**80**	**80 (100)**	**76 (95)**	**0 (0)**	**4 (5)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**
***Cryptococcus* spp.**									
Cr. albidus	1	1	1	0	0	0	0	0	0
Cr. neoformans/gattii	11	11	11	0	0	0	0	0	0
Cr. laurentii	1	0	1	1	0	0	0	0	0
Cr. uniguttulatus	1	1	0	0	0	0	0	0	1
Subtotal	**14**	**13 (93)**	**13 (93)**	**1 (7)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**1 (7)**
Combined Totals	**94**	**93 (99)**	**89 (95)**	**1 (1)**	**4 (4)**	**0 (0)**	**0 (0)**	**0 (0)**	**1 (1)**
Other Genera									
Rhodotorula spp.	2	ND	ND	2	2	0	0	0	0
Trichosporon spp.	8	ND	ND	7	7	0	1	1	0
Subtotal	**10**	**ND**	**ND**	**9 (90)**	**9 (90)**	**0 (0)**	**1 (10)**	**1 (10)**	**0 (0)**

13.0 References

1. BD Phoenix™ System User's Manual (Document number 8012724 (F)).
2. BD Phoenix™ V5.15A and BD Epicenter™ V5.10A User's Training Manual.
3. BD Phoenix™ AP Instrument User's Manual (Document number 8085581).
4. BD Phoenix™ NMIC/ID-133 package insert. 2008/03.
5. BD Phoenix™ PMIC/ID-102 package insert. 2008/03.
6. BD Phoenix™ SMIC-101 package insert. 2007/08.
7. BD Phoenix™ YEAST ID package insert. 2011/06.
8. Leverstein-van Hall, M., Fluit, A., Paauw, A., Box, A., Brisse, S., Verhoef, J. (2002). Evaluation of the Etest **ESBL** and the BD Phoenix™, VITEK 1, and VITEK 2 Automated Instruments for Detection of Extended-Spectrum Beta-Lactamases in Multiresistant *Escherichia coli* and *Klebsiella* spp. *J. Clin. Microbiol.* 40: 3703-3711.

14.0 Document Control History

Adopted/Approved by director (AR) 06/22/2009, 02/01/2012

Approved by J. Schappert 03/10/2010

Reviewed by supervisor (JC) 06/15/2009, 06/2011, 02/01/2012, 06/2013 (MM), (JA) 05/19/2015

03/15/2011 Changed from Remel Spectra MRSA agar to CHROMagar MRSA II as an acceptable medium for performing AST and added verification data.

06/24/2011 Added Mannitol Salt Agar as an acceptable medium for *S. aureus* AST and added verification data.

02/01/2012 Added instructions and verification data for Yeast ID panel.

11/21/2012 Added CHROMagar Salmonella as an acceptable medium for ID/AST and CHROMagar 0157 as an acceptable medium for ID.

06/12/2013 Clarified that beta-strep should be tested by Kirby-Bauer for D-test results.

05/19/2015 Added to the procedure section: "For additional information regarding Phoenix™ testing and instrument operation, refer to the BD Phoenix™ System User's Manual and the BD Phoenix™ AP Instrument User's Manual located on the intranet." Added purity check details under the procedure section. Added section for repeat testing.