

LABORATORY PROCEDURE

BD Phoenix™ PMIC/ID Panels BD Phoenix™ PMIC Panels BD Phoenix™ PID Panels

INTENDED USE

The BD Phoenix™ Automated Microbiology System is intended for the *in vitro* rapid identification (ID) of Gram Positive bacteria from pure culture belonging to the genera *Staphylococcus*, *Enterococcus*, and other Gram Positive cocci and Gram Positive bacilli. The BD Phoenix Automated Microbiology System is also intended for the quantitative determination of antimicrobial susceptibility by minimal inhibitory concentration (MIC) of most Gram Positive bacteria from pure culture belonging to the genera *Staphylococcus* and *Enterococcus*.

SUMMARY AND EXPLANATION OF THE TEST

Micromethods for the biochemical identification of microorganisms were reported as early as 1918¹. Several publications reported on the use of the reagent-impregnated paper discs and micro-tube methods for differentiating enteric bacteria¹⁻⁹. The interest in miniaturized identification systems led to the introduction of several commercial systems in the late 1960s, and they provided advantages in requiring little storage space, extended shelf life, standardized quality control, and ease of use.

Many of the tests used in the Phoenix ID panels are modifications of the classical methods. These include tests for fermentation, oxidation, degradation and hydrolysis of various substrates. In addition to these, the Phoenix system utilizes chromogenic and fluorogenic substrates as well as single carbon source substrates in the identification of organisms^{10,11}.

The modern broth microdilution test used today has origins in the tube dilution test used in 1942 by Rammelkamp and Maxon to determine *in vitro* antimicrobial susceptibility testing of bacterial isolates from clinical specimens¹². The broth dilution technique involves exposing bacteria to decreasing concentrations of antimicrobial agents in liquid media by serial two-fold dilutions. The lowest concentration of an antimicrobial agent in which no visible growth occurs is defined as the minimal inhibitory concentration (MIC).

The introduction in 1956 of a microtitrator system, using calibrated precision spiral wire loops and droppers for making accurate dilutions rapidly allowed Marymont and Wentz to develop a serial dilution antimicrobial susceptibility test (AST)¹³. The microtitrator system was accurate and allowed the reduction in volumes of antimicrobial agents. The term microdilution appeared in 1970 to describe the MIC tests performed in volumes of 0.1 mL or less of antimicrobial solution¹⁴.

The Phoenix AST test is a modified miniaturized version of the micro-broth doubling dilution technique. Susceptibility testing in the Phoenix system is performed through determination of bacterial growth in the presence of various concentrations of the antimicrobial agent tested.

PRINCIPLES OF THE PROCEDURE

A maximum of 100 identification and antimicrobial susceptibility tests can be performed in the Phoenix instrument at a time using Phoenix ID/AST combination panels. A sealed and self-inoculating molded polystyrene tray, with 136 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.

The Phoenix panel is comprised of a 51 well ID side and an 85 well AST side. The ID side contains 45 wells with dried biochemical substrates and 2 fluorescent control wells. The AST side contains 84 wells with dried antimicrobial agents and 1 growth control well. Panels are available as ID only (Phoenix™ NID Panels, Phoenix™ PID Panels), AST only (Phoenix™ NMIC Panels, Phoenix™ PMIC Panels), or ID/AST combination (Phoenix™ NMIC/ID Panels, Phoenix™ PMIC/ID Panels). Unused wells are reserved for future use.

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

Bacterial Identification: The ID portion of the Phoenix panel utilizes a series of conventional, chromogenic, and fluorogenic biochemical tests to determine the identification of the organism. Both growth-based and enzymatic substrates are employed to cover the different types of reactivity in the range of 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 that comprises the Phoenix ID Database is provided in Table A. Reactions employed by various substrates and the principles employed in the Phoenix ID reactions are described in Table B.

Antimicrobial Susceptibility Testing: The Phoenix AST method is a broth based microdilution test. The Phoenix system utilizes a redox indicator for the detection of organism growth in the presence of an antimicrobial agent¹⁵. 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. Organism identification is used in the interpretation of the MIC values of each antimicrobial agent producing Susceptible, Intermediate, or Resistant (SIR) result classifications.

A complete list of taxa for which the Phoenix system can provide AST results is provided in Table A. The list of antimicrobial agents and concentrations available for susceptibility testing in the Phoenix system is provided under Performance Characteristics.

There are antimicrobial agents for use with 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 both *in vitro* and in clinical infections refer to the individual pharmaceutical antimicrobial agent labeling. Alternatively, 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"¹⁶.

The components required for testing using the Phoenix system include: 1) Phoenix panels with panel closures, 2) Phoenix ID Broth, 3) Phoenix AST Broth, 4) Phoenix AST Indicator solution 5) Phoenix Inoculation Station, 6) Phoenix Transport Caddy, 7) BBL CrystalSpec or BD PhoenixSpec Nephelometer, 8) 25 µL pipettor and sterile tips, and 9) Miscellaneous lab supplies (listed under Materials Required But Not Provided).

Prior to inoculation, the Phoenix panel is placed on the Inoculation Station with the inoculation ports at the top for filling. Separate inocula are added manually to the ID and AST ports. The inocula flow down the panel in serpentine fashion, filling the panel wells as the liquid front progresses toward the pad. The pad absorbs excess inoculum. Closures are manually inserted in the fill ports. An air admittance port is located in the divider area of the panel lid to ensure adequate oxygen tension in the panel for the duration of the test.

INGREDIENTS:

For a listing of biochemical substrates used in the Phoenix panel refer to Table B. The package insert enclosed in the panel box provides a listing of the specific antimicrobial agents and concentrations found in the panel.

PRECAUTIONS

For *in vitro* Diagnostic Use

All patient specimens and microbial cultures are potentially infectious and should be treated with universal precautions. Please refer to CDC manual *Bio-safety in Microbiological and Biomedical Laboratories*, 4th Edition, 1999, as well as other recommended literature.

Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

Panels, once inoculated, should be handled carefully until placed in the instrument.

STORAGE AND HANDLING

Phoenix Panels: 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 panel or packaging appears to be damaged. If stored as recommended, the panels will retain expected reactivity until the date of expiration.

Phoenix ID Broth: Tubes are packaged as 100 tube packs. 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). Store Phoenix ID Broth tubes at 2-25°C. Expiration dating is shown on the tube label.

Phoenix AST Broth: Tubes are packaged as 100 tube packs. 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). Store Phoenix AST Broth tubes at 2-25°C. Expiration dating is shown on the tube label.

Phoenix AST Indicator Solution: The indicator solution is individually pouched and packaged as a package of 10 dropper bottles. 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. Store Phoenix AST Indicator Solution at 2-8°C. 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. **Be sure the bottle is held vertically when dispensing the AST Indicator Solution.**

SPECIMEN COLLECTION AND PROCESSING

The Phoenix system is not for use directly with clinical specimens. Only pure culture isolates of Gram Positive organisms are acceptable for testing. The test isolate must be a pure culture. It is recommended that cultures be no more than 24 hours old unless additional incubation is required to achieve sufficient growth.

Isolates must be tested with a Gram stain test to assure the appropriate selection of Phoenix panel type. Once the Gram stain reaction is confirmed, select the appropriate Phoenix panel for inoculation (e.g., PMIC/ID panel for use with Gram Positive 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. Selective media may inhibit some strains of bacteria; therefore, caution must be used when selecting isolated colonies from these media.

For ID and AST testing, refer to Table C for recommended media.

For ID only testing of Gram Positive organisms, isolates from one of the following media may be used: Trypticase™ Soy Agar without blood, Columbia Colistin Nalidixic Acid (CNA) Agar with 5% sheep blood and Phenylethanol Agar (PEA).

When swabs are used, only cotton-tipped applicators should be used to prepare the inoculum suspensions. Some polyester swabs may cause problems with inoculation of the panels.

The usefulness of the Phoenix system or any other diagnostic procedure performed on clinical specimens is directly influenced by the quality of the specimens themselves. It is strongly recommended that laboratories employ methods discussed in the *Manual of Clinical Microbiology*¹⁷ for specimen collection, transport, and placement on primary isolation media.

Inoculum for use on the Phoenix system is prepared by the CLSI recommended direct colony suspension method¹⁸. Due to variations in inoculum concentrations prepared with McFarland standards, use of the BBL CrystalSpec or BD PhoenixSpec nephelometer is required for adjusting the test inoculum prior to use in the Phoenix system.

It is highly recommended that the purity of the inoculum be checked by preparing a purity plate. See "Purity Check" below.

MATERIALS REQUIRED

Materials Provided:

- Phoenix Panels
- Phoenix ID Broth
- Phoenix AST Broth
- Phoenix AST Indicator Solution
- Phoenix Inoculation Station
- Phoenix Transport Caddy
- BBL™ CrystalSpec™ or BD PhoenixSpec™ Nephelometer and Standards
- 25 µL pipettor and sterile tips
- 50 µL pipettor and sterile tips
- 2 Pipette stands

Materials Required But Not Provided:

- Gram stain reagents
- Sterile cotton swabs

- Non-selective culture plated media (e.g., Trypticase™ Soy Agar with 5% Sheep Blood)
- Incubators
- Biohazard disposable container
- Markers, etc

PHOENIX TEST PROCEDURE

Note: The Phoenix instrument should always be powered on. If it is not, power on the instrument and allow 2 hours for the instrument to warm up before loading panels. Prepare the Phoenix instrument to receive new panels as described in the BD Phoenix System User's Manual ("Operation, Daily System Maintenance").

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.

Accession barcode labels affixed to a Phoenix panel should:

- Not be of fluorescent material
- Not cover any Phoenix panel reaction wells
- Not cover the Phoenix panel sequence number barcode

Broth and Panel Preparation:

1. Confirm the Gram stain reaction of the isolate before proceeding with the inoculum preparation for use in the Phoenix instrument. Once the Gram stain reaction is confirmed, select the appropriate Phoenix panel for inoculation. Selection of the incorrect panel type could lead to incorrect results.
2. Examine the pouch, and do not use the panel if the pouch is punctured or opened. 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 hours of being removed from the pouch.**
3. Place the panel on the Inoculation Station with ports at the top and pad on the bottom.
4. Label a Phoenix ID Broth tube with the patient's specimen number. Using aseptic technique, pick colonies of the same morphology with the tip of a sterile cotton swab (do not use a polyester swab) or a wooden applicator stick from one of the recommended media. See Table C.
5. Suspend the colonies in the Phoenix ID Broth (4.5 mL).
6. Cap the tube and vortex for 5 seconds.
7. Allow approximately ten seconds for air bubbles to surface. Tap the tube gently to aid in eliminating bubbles.
8. Confirm default settings for inoculum density before inoculating panels. Insert the tube into the BBL CrystalSpec or BD Phoenix Spec Nephelometer. Make sure the tube is inserted as far as it will go. **Note: Only the BD PhoenixSpec Nephelometer can be used to make inoculum densities of 0.25 McFarland.** (Refer to the BBL CrystalSpec Nephelometer or BD PhoenixSpec product insert for correct usage instructions and calibration verification.)
9. If the inoculum density is set to 0.5 for the panel type being run, then a range of 0.50-0.60 is acceptable. If the inoculum density is set to 0.25 for the panel type being run, then a range

- of 0.20-0.30 is acceptable. If the density of organisms is 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 of organisms exceeds 0.6 McFarland, follow the steps below to dilute the broth. It is very important to accurately indicate the level of the liquid in the tube since this volume is needed to adequately fill the wells in the panel. **Note: The standardized bacterial suspension in ID broth must be used within 60 minutes of preparation.**
- a Using a marker, mark the broth level in the over-inoculated Phoenix ID Broth tube.
 - b Using a sterile pipette, aseptically add fresh Phoenix ID Broth to the inoculum. Only Phoenix ID Broth may be used to dilute the inoculum.
 - c Vortex the tube and allow to sit for 10 seconds.
 - d Place the tube in the nephelometer and remeasure the turbidity of the suspension.
 - If the reading is greater than 0.6, repeat Steps b-d.
 - If the reading is 0.5 – 0.6, go to Step e.
 - e Using a sterile pipette, aseptically remove excess broth to the original level indicated by the mark on the tube created in Step a.

Remove excess broth to avoid overfilling the panel. Also, do not remove too much broth, as there may be insufficient broth to adequately fill the panel.
 - f Broth may now be used to inoculate the Phoenix AST Broth and/or the Phoenix Panel.
10. If you are performing identification only, proceed to Step 15 and continue the procedure.
 11. Label a Phoenix AST Broth tube (8.0 mL) with the patient's specimen number. Holding the AST Indicator Solution bottle vertically, add one free-falling drop of AST indicator solution to the AST broth tube. Invert to mix. **DO NOT VORTEX. Note: Allow AST Indicator Solution to warm to room temperature before dispensing into AST broth. The unused portion of the indicator should be returned to 2°- 8°C as soon as possible. Do not store at room temperature for more than 2 hours. Opened bottles should be discarded after 14 days from initial opening. If volume other than one drop is added inadvertently, discard the tube and use a fresh tube of AST broth. After the addition of the Indicator to AST broth, the mixed solution can be stored in the dark, at room temperature, for as long as 8 hours. Tubes must be used within 2 hours after the addition of the indicator solution if exposed to light.**
 12. If an inoculum density of 0.50 – 0.60 was used, transfer 25 µL of the bacterial suspension from the ID tube into the AST broth tube. If an inoculum density of 0.20 – 0.30 was used, transfer 50 µL (use 2 shots if utilizing a 25 µL pipettor) of the bacterial suspension from the ID tube into the AST broth tube. **Note: Panels must be inoculated within 30 minutes of the time that the AST inoculum is prepared.**
 13. Cap the AST tube and invert several times to mix. **Do not vortex.**
 14. Wait a few seconds for air bubbles to surface. Tap the tube gently to aid in eliminating bubbles.
 15. Pour the ID tube inoculum into the fill port on the ID side of the panel (51-well side). Allow the fluid to traverse down the tracks before moving the panel. If using an AST (only) panel, **DO NOT** inoculate the ID side of the panel. Retain the ID tube for a purity check.

16. Pour the AST 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.
17. Before placing panel closure, check for residual droplets of inoculum on the edge of the fill ports. If a droplet is present, remove the droplet with absorbent material. The used absorbent material must be discarded along with your biohazard waste.
18. Snap on the panel closure. **Make sure that the closure is fully seated.**
Visually inspect panels to be sure each of the wells is full. Look at both sides of the panel. 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 minutes 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.**

Purity Check

1. Using a sterile loop, recover a small drop from the inoculum fluid either before or after inoculation of the panel.
2. Inoculate an agar plate (any appropriate medium) for a purity check.
3. Discard inoculum fluid tube and cap in a biohazard disposal container.
4. Incubate the plate for 24-48 hours at 35°C under appropriate conditions.

ID Inoculum Density Flexibility

You may run the ID portion of a panel in the opposite mode from what is configured by darkening well A17 on the back of a panel before placing the panel in the instrument. This allows you to run a panel at an inoculum density of 0.20 – 0.30 even if you are configured for a density of 0.5 for that particular panel type. Likewise, you can run the panel at an inoculum density of 0.50 – 0.60 if you are configured for a density of 0.25.

There is no way to alter the density setting during Panel Login. To use a panel in the opposite density mode, using a black Sharpie™ (permanent marker), blacken the A17 well entirely. See the BD Phoenix System User’s Manual (Operation, ID Inoculum Density Flexibility) for position of well A17.

For instructions for panel login and loading, refer to the BD Phoenix System User’s Manual (“Panel Login” and “Inserting Panels in the Instrument”).

Current Instrument Inoculum Density Configuration	Inoculum Concentration Desired for Test Panel	Amount of ID Inoculum to Add to AST Broth**	Well A-17
0.50	0.25	50 µL	Blackened
0.25	0.50	25 µL	Blackened
** If also running AST			

USER QUALITY CONTROL

In order to ensure appropriate set up procedure and acceptable performance of the system, the following organisms are recommended to be tested. The user is advised to review the individual AST panel formats to determine if all test strains need to be tested for routine laboratory Quality Control. Refer to the Package Insert that accompanies the Phoenix panels for expected ID results and AST results for QC organisms.

For instructions for QC panel login and loading, refer to the BD Phoenix System User's Manual ("Panel Login" and "Inserting Panels in the Instrument").

ID (PMIC/ID and PID panels):

Staphylococcus aureus ATCC™ 29213

Enterococcus faecalis ATCC™ 29212

AST (PMIC/ID, PMIC panels):

Staphylococcus aureus ATCC™ 29213

Enterococcus faecalis ATCC™ 29212

Staphylococcus aureus ATCC™ 25923 (PMIC/ID panels only. QC for Nitrocefin.)

Enterococcus faecalis ATCC™ 51299

For the most reliable results, it is recommended that the QC organisms be subcultured at least twice on two consecutive days onto TSA II with 5% Sheep Blood Agar before use in the Phoenix system.

Compare recorded results to those listed in the Package Insert. If discrepant results are obtained, review test procedures as well as confirm purity of the quality control strain used before contacting BD Diagnostics Technical Services Department. Unacceptable QC results are documented as "Fail" and acceptable QC results are documented as "Pass" on the QC Report.

RESULTS

Organism identification will appear on the Phoenix Report Form 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").

Special Notes

In general, the Phoenix System provides a MIC for all organisms at any of the concentrations defined on a specific panel. For certain antimicrobial/organism combinations a specific minimum or maximum MIC is reported even if there is a lower or higher concentration on the panel. These MIC values are applied by the software and are reported out as less than or equal to (</=) for the minimum MIC or greater than (>) for the maximum MIC. The table below provides the range for these special antimicrobial/organism combinations.

Antimicrobial Agent	Organism(s)	Applied Range (µg/mL)
Oxacillin	Coagulase negative staphylococci	0.0625-1.0
Penicillin	<i>Staphylococcus</i> spp.	0.0625-1.0
	<i>Enterococcus</i> spp.	1.0-32
Gentamicin	<i>Staphylococcus epidermidis</i>	<4 and >16*
Moxifloxacin	<i>Enterococcus</i> spp. other than <i>E. faecium</i>	0.25-8

* MICs of 4, 8, 16 not reported

LIMITATIONS OF THE PROCEDURE

See the package insert shipped with the panel for specific organism/antimicrobial limitations.

General

A Gram stain test is 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 C. Media containing esculin should not be used. Use of mixed colonies could result in inaccurate identification and/or AST interpretations.

If the instrument inoculum density is configured to 0.5 (for the panel type being used), an inoculum density of 0.50 – 0.60 must be met. Only the BBL CrystalSpec or BD Phoenix Spec Nephelometer can be used to measure the inoculum density.

If the instrument inoculum density is configured to 0.25 (for the panel type being used), an inoculum density of 0.20 – 0.30 McFarland must be met. Only the BD PhoenixSpec Nephelometer can be used to measure inoculum density for this range.

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.

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.

Antimicrobial Susceptibility Testing

After the addition of the 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.

PERFORMANCE CHARACTERISTICS

Gram Positive Identification

In two internal studies, the performance of the Phoenix Gram Positive identification was evaluated. The 0.5 inoculum density configuration and the 0.25 inoculum density configuration were tested with 696 strains (0.5) and 755 strains (0.25), respectively. Results were evaluated against commercial and non-commercial methods.

The Phoenix Gram Positive identification performance is outlined below:

	McFarland	Agreement	No Agreement	No ID
Species Level	0.5	95.4%	3.9%	0.7%
	0.25	98.0%	1.6%	0.4%

An internal study was performed to simulate inter-site reproducibility. The identification results obtained using the Phoenix system were compared with expected results. This performance testing demonstrated intra-site and inter-site reproducibility of at least 95% or greater.

Gram Positive Susceptibility

Clinical, stock, and challenge isolates were tested across multiple clinical sites to determine Essential Agreement (EA) and Category Agreement (CA) of the Phoenix system to the CLSI broth microdilution reference method. Essential Agreement occurs when the MIC of the Phoenix system and the reference method agree exactly or is within ± 1 dilution of each other. Category Agreement occurs when the Phoenix system results agree with the reference method with respect to the CLSI categorical interpretative criteria (susceptible, intermediate, resistant). The table below summarizes the data from these studies.

Additionally, testing performed at multiple clinical sites demonstrated at least 95% reproducibility or greater within ± 1 doubling dilution for all antimicrobial agents listed in the table below.

DRUG CLASS	DRUG NAME	DRUG CODE	DRUG RANGE ($\mu\text{g/mL}$)	EA N	EA %	CA N	CA %
5-Fluoroquinolone	Gatifloxacin	GAT	0.25-8	1180	98.6	1180	90.1
5-Fluoroquinolone	Levofloxacin	LVX	0.25-8	1878	96.8	1878	95.1

5-Fluoroquinolone	Moxifloxacin	MXF	0.125-8	1777	96.0	1777	90.1
5-Fluoroquinolone	Norfloxacin	NOR	0.25-16	1252	96.9	1252	97.4
5-Fluoroquinolone	Ofloxacin	OFX	0.25-8	1184	98.7	1184	98.2
Aminoglycoside	Gentamicin	GM	0.25-16	1223	91.9	1223	95.2
Aminoglycoside	Gentamicin Synergy	GMS	500	—	—	763	98.6
Aminoglycoside	Streptomycin Synergy	STS	1000	—	—	756	97.8
Aminoglycoside	Tobramycin	NN	0.5-16	953	93.5	797	98.5
Ansamycin	Rifampin	RA	0.25-32	1261	98.3	1261	98.2
B-Lac/B-Lac. Inh	Ampicillin/Clavulanate	AMC	0.25/0.12-32/16	871	94.1	871	96.7
B-Lactam Pen.	Ampicillin	AM	0.06-32	475	93.3	475	98.5
B-Lactam Pen Inh	Ampicillin/sulbactam	SAM	2/1-32/16	1240	97.2	1240	97.3
B-Lactam Pen.	Oxacillin	OX	0.06-4	1231	95.4	1231	96.6
B-Lactam Pen.	Penicillin	P	0.06-32	1256	93.6	1256	97.5
Carbapenem	Meropenem	MEM	0.5-16	620	98.4	1198	96.6
Cephem	Cefazolin	CZ	2-32	597	99.5	597	99.7
Cephem	Cefoxitin	FOX	1-32	1164	96.3	1164	90.1
Cephem	Cephalothin	CF	0.5-64	904	96.2	904	98.0
Folate Antagonist	Trimethoprim-Sulfamethoxazole	SXT	0.5/9.5- 16/304	634	96.4	634	97.9
Glycopeptide	Vancomycin	VA	0.5-32	1981	97.7	1981	99.4
Macrolide	Erythromycin	E	0.06-8	1395	95.0	1395	94.6
Lincosamide	Clindamycin	CC	0.12-8	1242	98.2	1242	98.7
Lipopeptide	Daptomycin*	DAP	0.125-32	1568	97.4	1568	98.8
Nitrofurantoin	Nitrofurantoin*	FM	16-512	1757	99.8	1757	99.8
Oxazolidinone	Linezolid	LZD	0.25-32	1454	91.1	1454	95.3
Phenicol	Chloramphenicol	C	1-32	1447	93.4	1447	93.4
Streptogramin	Quinupristin/Dalfopristin	SYN	0.25-4	2019	94.5	1500	95.5
Tetracycline	Tetracycline	TE	0.5-16	2040	96.9	2040	96.5

^aThe ability of the system to detect resistance for this antimicrobial with *Enterococcus* species and *Staphylococcus* species is unknown because resistant organisms were not available at the time of comparative clinical testing.

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Manufactured by
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Made in USA

TECHNICAL INFORMATION

Approved by: _____
Date Effective: _____
Supervisor: _____ Date: _____
Director: _____ Date: _____
Reviewed: _____
9/2006

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Table A Taxa for ID/AST Determination

There are antimicrobial agents for use with the Phoenix system that are not proven to be effective for treating infections for all organisms listed in this section. For interpreting and reporting results of antimicrobial agents that have shown to be active against organism groups both *in vitro* and in clinical infections refer to the individual pharmaceutical antimicrobial agent labeling. Alternatively, 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."

Gram Positive (0.5 McFarland)

Gram Positive Taxa ¹	ID, AST, ID/AST
<i>Aerococcus urinae</i>	ID
<i>Aerococcus viridans</i>	ID
<i>Alloiococcus otitidis</i>	ID
<i>Arcanobacterium haemolyticum</i>	ID
<i>Arcanobacterium pyogenes</i>	ID
<i>Bacillus cereus</i>	ID
<i>Bacillus circulans</i>	ID
<i>Bacillus coagulans</i>	ID
<i>Bacillus licheniformis</i>	ID
<i>Bacillus megaterium</i>	ID
<i>Bacillus pumilus</i>	ID
<i>Bacillus sphaericus</i>	ID
<i>Bacillus subtilis</i>	ID
<i>Bacillus thuringiensis</i>	ID

<i>Brevibacillus brevis</i>	ID
<i>Brevibacterium</i> species	ID
<i>Cellulomonas turbata</i>	ID
<i>Corynebacterium amycolatum</i>	ID
<i>Corynebacterium bovis</i>	ID
<i>Corynebacterium diphtheriae</i>	ID
<i>Corynebacterium jeikeium</i>	ID
<i>Corynebacterium kutscheri</i>	ID
<i>Corynebacterium matruchotii</i>	ID
<i>Corynebacterium minutissimum</i>	ID
<i>Corynebacterium propinquum</i>	ID
<i>Corynebacterium pseudodiphtheriticum</i>	ID
<i>Corynebacterium pseudotuberculosis</i>	ID
<i>Corynebacterium renale</i>	ID
<i>Corynebacterium striatum</i>	ID
<i>Corynebacterium ulcerans</i>	ID
<i>Corynebacterium urealyticum</i>	ID
<i>Corynebacterium xerosis</i>	ID
<i>Dermabacter hominis</i>	ID
<i>Dermacoccus nishinomiyaensis</i>	ID
<i>Enterococcus asini</i>	AST
<i>Enterococcus avium</i>	ID/AST
<i>Enterococcus casseliflavus</i>	ID/AST
<i>Enterococcus cecorum</i>	AST
<i>Enterococcus columbae</i>	AST
<i>Enterococcus dispar</i>	AST
<i>Enterococcus durans</i>	ID/AST
<i>Enterococcus faecalis</i>	ID/AST
<i>Enterococcus faecium</i>	ID/AST
<i>Enterococcus flavescens</i>	AST
<i>Enterococcus gallinarum</i>	ID/AST
<i>Enterococcus gilvus</i>	AST
<i>Enterococcus haemoperoxidus</i>	AST
<i>Enterococcus hirae</i>	ID/AST
<i>Enterococcus malodoratus</i>	AST

<i>Enterococcus moraviensis</i>	AST
<i>Enterococcus mundtii</i>	AST
<i>Enterococcus pallens</i>	AST
<i>Enterococcus pseudoavium</i>	AST
<i>Enterococcus raffinosus</i>	ID/AST
<i>Enterococcus ratti</i>	AST
<i>Enterococcus saccharolyticus</i>	AST
<i>Enterococcus solitarius</i>	AST
<i>Enterococcus sulfureus</i>	AST
<i>Erysipelothrix rhusiopathiae</i>	ID
<i>Gardnerella vaginalis</i>	ID
<i>Gemella haemolysans</i>	ID
<i>Gemella morbillorum</i>	ID
<i>Globicatella sanguinis</i>	ID
<i>Helcococcus kunzii</i>	ID
<i>Kocuria kristinae</i>	ID
<i>Kocuria rosea</i>	ID
<i>Kocuria varians</i>	ID
<i>Kytococcus sendentarius</i>	ID
<i>Lactococcus garvieae</i>	ID
<i>Lactococcus lactis</i> ssp. <i>cremoris</i>	ID
<i>Lactococcus lactis</i> ssp. <i>hordniae</i>	ID
<i>Lactococcus lactis</i> ssp. <i>lactis</i>	ID
<i>Lactococcus plantarum</i>	ID
<i>Lactococcus raffinolactis</i>	ID
<i>Leifsonia aquatica</i>	ID
<i>Leuconostoc citreum</i>	ID
<i>Leuconostoc lactis</i>	ID
<i>Leuconostoc mesenteroides</i> ssp. <i>cremoris</i>	ID
<i>Leuconostoc mesenteroides</i> ssp. <i>mesenteroides</i>	ID
<i>Leuconostoc pseudomesenteroides</i>	ID
<i>Listeria grayi</i>	ID
<i>Listeria innocua</i>	ID
<i>Listeria ivanovii</i>	ID
<i>Listeria monocytogenes</i>	ID

<i>Listeria welshimeri</i>	ID
<i>Macrococcus caseolyticus</i>	ID
<i>Micrococcus luteus</i>	ID
<i>Micrococcus lylae</i>	ID
<i>Oerskovia xanthineolytica</i>	ID
<i>Paenibacillus alvei</i>	ID
<i>Paenibacillus macerans</i>	ID
<i>Pediococcus acidilactici</i>	ID
<i>Pediococcus damnosus</i>	ID
<i>Pediococcus dextrinicus</i>	ID
<i>Pediococcus parvulus</i>	ID
<i>Pediococcus pentosaceus</i>	ID
<i>Rhodococcus equi</i>	ID
<i>Rothia dentocariosa</i>	ID
<i>Rothia mucilaginoso</i>	ID
<i>Staphylococcus arlettae</i>	AST
<i>Staphylococcus aureus</i>	ID/AST
<i>Staphylococcus auricularis</i>	ID/AST
<i>Staphylococcus capitis</i>	ID/AST
<i>Staphylococcus capitis</i> ssp. <i>capitis</i>	ID/AST
<i>Staphylococcus capitis</i> ssp. <i>ureolyticus</i>	ID/AST
<i>Staphylococcus caprae</i>	ID/AST
<i>Staphylococcus carnosus</i>	ID/AST
<i>Staphylococcus chromogenes</i>	ID/AST
<i>Staphylococcus cohnii</i>	ID/AST
<i>Staphylococcus cohnii</i> ssp. <i>cohnii</i>	ID/AST
<i>Staphylococcus cohnii</i> ssp. <i>urealyticum</i>	ID/AST
<i>Staphylococcus condimentii</i>	AST
<i>Staphylococcus delphini</i>	AST
<i>Staphylococcus epidermidis</i>	ID/AST
<i>Staphylococcus equorum</i>	ID/AST
<i>Staphylococcus felis</i>	ID/AST
<i>Staphylococcus fleurettii</i>	AST
<i>Staphylococcus gallinarum</i>	ID/AST
<i>Staphylococcus haemolyticus</i>	ID/AST

<i>Staphylococcus hominis</i>	ID/AST
<i>Staphylococcus hyicus</i>	ID/AST
<i>Staphylococcus intermedius</i>	ID/AST
<i>Staphylococcus kloosii</i>	ID/AST
<i>Staphylococcus lentus</i>	ID/AST
<i>Staphylococcus lugdunensis</i>	ID/AST
<i>Staphylococcus lutrae</i>	AST
<i>Staphylococcus muscae</i>	AST
<i>Staphylococcus pasteurii</i>	ID/AST
<i>Staphylococcus piscifermentans</i>	AST
<i>Staphylococcus pulvereri</i>	AST
<i>Staphylococcus saccharolyticus</i>	AST
<i>Staphylococcus saprophyticus</i>	ID/AST
<i>Staphylococcus schleiferi</i>	ID/AST
<i>Staphylococcus schleiferi</i> ssp. <i>coagulans</i>	ID/AST
<i>Staphylococcus schleiferi</i> ssp. <i>schleiferi</i>	ID/AST
<i>Staphylococcus sciuri</i>	ID/AST
<i>Staphylococcus simulans</i>	ID/AST
<i>Staphylococcus succinus</i> ssp. <i>casei</i>	AST
<i>Staphylococcus succinus</i> ssp. <i>succinus</i>	AST
<i>Staphylococcus vitulinus</i>	ID/AST
<i>Staphylococcus warneri</i>	ID/AST
<i>Staphylococcus xylosus</i>	ID/AST
<i>Streptococcus acidominimus</i>	ID
<i>Streptococcus agalactiae</i> (<i>Strep.</i> group B)	ID
<i>Streptococcus anginosus</i>	ID
<i>Streptococcus bovis</i> I (<i>Strep.</i> group D)	ID
<i>Streptococcus bovis</i> II (<i>Strep.</i> group D)	ID
<i>Streptococcus canis</i>	ID
<i>Streptococcus constellatus</i>	ID
<i>Streptococcus cristatus</i>	ID
<i>Streptococcus dysgalactiae</i> ssp. <i>dysgalactiae</i>	ID
<i>Streptococcus dysgalactiae</i> ssp. <i>equisimilis</i>	ID
<i>Streptococcus equi</i>	ID
<i>Streptococcus equi</i> ssp. <i>equi</i>	ID

<i>Streptococcus equi</i> ssp. <i>zooepidemicus</i>	ID
<i>Streptococcus equines</i>	ID
<i>Streptococcus gordonii</i>	ID
<i>Streptococcus intermedius</i>	ID
<i>Streptococcus mitis</i>	ID
<i>Streptococcus mitis/pneumoniae</i>	ID
<i>Streptococcus mutans</i>	ID
<i>Streptococcus oralis</i>	ID
<i>Streptococcus parasanguinis</i>	ID
<i>Streptococcus pneumoniae</i>	ID
<i>Streptococcus porcinus</i>	ID
<i>Streptococcus pyogenes</i> (Strep. group A)	ID
<i>Streptococcus salivarius</i>	ID
<i>Streptococcus sanguinis</i>	ID
<i>Streptococcus sobrinus</i>	ID
<i>Streptococcus uberis</i>	ID
<i>Streptococcus vestibularis</i>	ID

¹ Not all species encountered during clinical performance evaluations.

Gram Positive (0.25 McFarland)

Gram Positive Taxa ¹	ID, AST, ID/AST
<i>Aerococcus urinae</i>	ID
<i>Aerococcus viridans</i>	ID
<i>Alloiococcus otitidis</i>	ID
<i>Dermacoccus nishinomiyaensis</i>	ID
<i>Enterococcus avium</i>	ID/AST
<i>Enterococcus casseliflavus</i>	ID/AST
<i>Enterococcus durans</i>	ID/AST
<i>Enterococcus faecalis</i>	ID/AST
<i>Enterococcus faecium</i>	ID/AST
<i>Enterococcus gallinarum</i>	ID/AST
<i>Enterococcus hirae</i>	ID/AST
<i>Enterococcus raffinosus</i>	ID/AST
<i>Gemella haemolysans</i>	ID

<i>Gemella morbillorum</i>	ID
<i>Globicatella sanguinis</i>	ID
<i>Helcococcus kunzii</i>	ID
<i>Kocuria kristinae</i>	ID
<i>Kocuria rosea</i>	ID
<i>Kocuria varians</i>	ID
<i>Kytococcus sendentarius</i>	ID
<i>Lactococcus lactis</i> ssp. <i>cremoris</i>	ID
<i>Lactococcus lactis</i> ssp. <i>hordniae</i>	ID
<i>Lactococcus plantarum</i>	ID
<i>Leuconostoc citreum</i>	ID
<i>Leuconostoc lactis</i>	ID
<i>Leuconostoc mesenteroides</i> ssp. <i>mesenteroides</i>	ID
<i>Listeria innocua</i>	ID
<i>Listeria monocytogenes</i>	ID
<i>Macrococcus caseolyticus</i>	ID
<i>Micrococcus luteus</i>	ID
<i>Micrococcus lylae</i>	ID
<i>Pediococcus acidilactici</i>	ID
<i>Pedicococcus damnosus</i>	ID
<i>Pediococcus dextrinicus</i>	ID
<i>Pediococcus parvulus</i>	ID
<i>Pediococcus pentosaceus</i>	ID
<i>Rothia mucilaginosa</i>	ID
<i>Staphylococcus aureus</i>	ID/AST
<i>Staphylococcus auricularis</i>	ID/AST
<i>Staphylococcus capitis</i>	ID/AST
<i>Staphylococcus caprae</i>	ID/AST
<i>Staphylococcus carnosus</i>	ID/AST
<i>Staphylococcus chromogenes</i>	ID/AST
<i>Staphylococcus cohnii</i> ssp. <i>cohnii</i>	ID/AST
<i>Staphylococcus cohnii</i> ssp. <i>urealyticum</i>	ID/AST
<i>Staphylococcus epidermidis</i>	ID/AST
<i>Staphylococcus equorum</i>	ID/AST
<i>Staphylococcus felis</i>	ID/AST

<i>Staphylococcus gallinarum</i>	ID/AST
<i>Staphylococcus haemolyticus</i>	ID/AST
<i>Staphylococcus hominis</i>	ID/AST
<i>Staphylococcus hyicus</i>	ID/AST
<i>Staphylococcus intermedius</i>	ID/AST
<i>Staphylococcus kloosii</i>	ID/AST
<i>Staphylococcus lentus</i>	ID/AST
<i>Staphylococcus lugdunensis</i>	ID/AST
<i>Staphylococcus pasteurii</i>	ID/AST
<i>Staphylococcus saprophyticus</i>	ID/AST
<i>Staphylococcus schleiferi</i> ssp. <i>coagulans</i>	ID/AST
<i>Staphylococcus schleiferi</i> ssp. <i>schleiferi</i>	ID/AST
<i>Staphylococcus sciuri</i>	ID/AST
<i>Staphylococcus simulans</i>	ID/AST
<i>Staphylococcus vitulinus</i>	ID/AST
<i>Staphylococcus warneri</i>	ID/AST
<i>Staphylococcus xylosus</i>	ID/AST
<i>Streptococcus agalactiae</i> (<i>Strep.</i> group B)	ID
<i>Streptococcus anginosus</i>	ID
<i>Streptococcus bovis</i> (<i>Strep.</i> group D)	ID
<i>Streptococcus bovis</i> I (<i>Strep.</i> group D)	ID
<i>Streptococcus bovis</i> II (<i>Strep.</i> group D)	ID
<i>Streptococcus constellatus</i>	ID
<i>Streptococcus cristatus</i>	ID
<i>Streptococcus equi</i>	ID
<i>Streptococcus gordonii</i>	ID
<i>Streptococcus</i> group C/G (large colony)	ID
<i>Streptococcus intermedius</i>	ID
<i>Streptococcus mitis</i>	ID
<i>Streptococcus mutans</i>	ID
<i>Streptococcus oralis</i>	ID
<i>Streptococcus parasanguinis</i>	ID
<i>Streptococcus pneumoniae</i>	ID
<i>Streptococcus porcinus</i>	ID
<i>Streptococcus pyogenes</i> (<i>Strep.</i> group A)	ID

<i>Streptococcus salivarius</i>	ID
<i>Streptococcus sanguinis</i>	ID
<i>Streptococcus sobrinus</i>	ID
<i>Streptococcus uberis</i>	ID
<i>Streptococcus vestibularis</i>	ID

¹ Not all species encountered during clinical performance evaluations.

Table B
List of Reagents and Principles Employed
in the Phoenix System

Substrate Name	Code	Principle
4MU-BD-CELLOBIOSIDE	M_BDCEL	Enzymatic hydrolysis of the amide or glycosidic bond results in the release of a fluorescent coumarin or 4-methylumbelliferone derivative.
L-ALANINE-AMC	A_LALT	
4MU-BD-GLUCOSIDE	M_BDGLU	
L-PROLINE-AMC	A_LPROB	
L-PYROGLUTAMIC ACID-AMC	A_LPYR	
L-PHENYLALANINE-AMC	A_LPHET	
L-TRYPTOPHAN-AMC	A_LTRY	
4MU-PHOSPHATE	M_PHOS	
METHIONINE-AMC	A_META	
4MU-AD-GLUCOSIDE	M_ADGLU	
ARGININE-ARGININE-AMC	A_ARARR	
GLYCINE-PROLINE-AMC	A_GLPRB	
4MU-BD-GLUCURONIDE	M_BDGLC	
L-LEUCINE-AMC	A_LLEUH	
4MU-N-ACETYL-BD-GLUCOS AMINIDE	M_NAG	
L-ARGININE-AMC	A_LARGH	
4MU-PHOSPHATE (with Trehalose)	M_PHOT	
L-HISTIDINE-AMC	A_LHIST	
L-ISOLEUCINE-AMC	A_LISO	
4MU-BD-GALACTOSIDE	M_BDGAL	

Substrate Name	Code	Principle
COLISTIN	C_CLST	Resistance to the antimicrobial agents results in a reduction of the resazurin based indicator.
POLYMYXIN B	C_PXB	
D-GLUCONIC ACID	C_DGUA	Utilization of a carbon source results in a reduction of the resazurin based indicator.
3-METHYL GLUTARIC ACID	C_3MGA	
D-FRUCTOSE	C_DFRU	
IMINODIACETIC ACID	C_IMN	
ALPHA-KETOGLUTARIC ACID	C_KGA	
D-MANNITOL	C_DMNT	
3-METHYLADIPIC ACID	C_MAA	
THYMIDINE	C_THY	
FLOURESCENT POSTIVE CONTROL	FLR_CTL	Control to standardize fluorescent substrate results.
FLUORESCENT POSITIVE CONTROL	FLR_CTL	
ALANINE-ALANINE-PNA	N_ALALH	Enzymatic hydrolysis of the colorless amide substrate releases yellow p-nitroaniline.
L-PROLINE-PNA	N_LPROT	
VALINE-ALANINE-PNA	N_VAALA	
PNP-AD-GLUCOSIDE	P_PAGLU	Enzymatic hydrolysis of the colorless aryl substituted glycoside release yellow p-nitrophenol.
PNP-PHOSPHATE	P_PHOL	
BETA-GENTIOBIOSE	R_BGEN	Utilization of carbohydrate results in lower pH and change in indicator (phenol red).
D-SUCROSE	R_DSUC	
MALTOTRIOSE	R_MTT	
N-ACETYL-GLUCOSAMINE	R_NGU	
D-TREHALOSE	R_DTRE	
D-TAGATOSE	R_DTAG	
MALTOSE	R_MAL	
DEXTROSE	R_DEX	
UREA	S_URE	Hydrolysis of urea and the resulting ammonia change results in pH rise and change in fluorescent indicator.
ESCULIN	T_ESC	Hydrolysis of esculin results in a black precipitate in the presence of ferric ion.
NITROCEFEN	L_NCF	Enzymatic hydrolysis of the β -lactam ring results in a color change.

Table C
Recommended Media and Approved Use

Recommended Media	Approved Use	
	ID	AST
Trypticase Soy Agar with 5% Sheep Blood	Yes	Yes
Bromthymol Blue (BTB) Lactose Agar	Yes ²	Yes
BBL™ CHROMagar™ Orientation	Yes	Yes ¹
Chocolate Agar	Yes	Yes
Columbia Agar with 5% Horse Blood	Yes	Yes
Columbia Agar with 5% Sheep Blood	Yes	Yes
Columbia CNA Agar with 5% Sheep Blood	Yes	No
Cystine-Lactose-Electrolyte-Deficient (CLED) Agar	Yes ³	Yes
Phenylethyl Alcohol Agar	Yes	No
Trypticase™ Soy Agar without Blood	Yes	No
Trypticase™ Soy Agar with Lecithin and Tween™ 80	Yes	No

¹The use of CHROMagar Orientation may produce false susceptibility results when testing erythromycin with Gram positive organisms. Antimicrobial susceptibility test results should be confirmed using Trypticase Soy Agar with 5% Sheep Blood.

²The use of Bromthymol Blue lactose Agar for Gram Positive organism identification should be restricted to Staphylococci for both the 0.5 and 0.25 GP systems.

³The use of Cystine-Lactose-Electrolyte Deficient Agar for Gram positive organism identification should be restricted to Staphylococci for the 0.25 GP system.