LABORATORY PROCEDURE

BD Phoenix™ NMIC/ID Panels
BD Phoenix™ NMIC Panels
BD Phoenix™ NID Panels

INTENDED USE

The BD Phoenix™ Automated Microbiology System is intended for the *in vitro* rapid identification (ID) and quantitative determination of antimicrobial susceptibility by minimal inhibitory concentration (MIC) of Gram Negative aerobic and facultative anaerobic bacteria belonging to the family *Enterobacteriaceae* and non-*Enterobacteriaceae*.

SUMMARY AND EXPLANATION OF THE TEST

Micromethods for the biochemical identification of microorganisms were reported as early as 1918[1]. Several publications reported on the use of the reagent-impregnated paper discs and micro-tube methods for differentiating enteric bacteria[1-9]. 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 specimens[12]. 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 solution[14].

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 and Phoenix™ PID Panels), AST only (Phoenix™ NMIC Panels and Phoenix™ PMIC Panels), or ID/AST combination (Phoenix™ NMIC/ID Panels and Phoenix™ PMIC/ID Panels). Unused wells are reserved for future se.

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 agent[15]. 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 Panel 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 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: 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 aerobic and/or facultatively anaerobic Gram Negative organisms are acceptable for testing. The test isolate <u>must</u> 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., 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. 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.

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 method[18]. 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 Panel 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
- Nonselective 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 PhoenixSpec 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 McFarland 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 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 reader 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 removed 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 2-8ºC 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 or AST 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 tube either before or after inoculating 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 the 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 a 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 entire well. 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 for testing. 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 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 (NMIC/ID and NID panels):

Escherichia coli ATCC™ 25922

Pseudomonas aeruginosa ATCC™ 27853

AST (NMIC/ID, NMIC panels):

Escherichia coli ATCC™ 25922

Pseudomonas aeruginosa ATCC™ 27853

Escherichia coli ATCC™ 35218

Klebsiella pneumoniae ATCC™ 700603

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 antimicrobic/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 antimicrobic/organism combinations.

Antimicrobial Agent	Organism(s)	Applied Range (µg/mL)
Amikacin	*Morganella morganii*	2-64
	Proteus penneri	2-64
	Proteus vulgaris	2-64
	Providencia species	2-64
Aztreonam	*Providencia stuartii*	2-64

Cefotaxime	*Providencia* species	2-64
Cefotetan	*Proteus mirabilis*	4-64
Gentamicin	*Escherichia coli*	1-16
Piperacillin	*Morganella morganii*	4-128
	Achromobacter species	4-128
Piperacillin/ Tazobactam	*Achromobacter* species *Serratia marcescens*	2/4 – 128/4 4/4 – 128/4
	Serratia species	4/4-128/4
Tetracycline	*Morganella morganii*	1-16
Ticarcillin	*Achromobacter* species	4-128
	Alcaligenes species	4-128
	Brevundimonas species	4-128
	Chryseobacterium species	4-128
	Delftia acidoverans	4-128
	Myroides species	4-128
	Ochrobactrum anthropi	4-128
	Providencia species	4-128
	Ralstonia species	4-128
	Salmonella species	4-128
	Serratia species	4-128
	Shewanella species	4-128
	Shingobacterium species	4-128
	Wautersia species	4-128
Ticarcillin/ Clavulanate	*Citrobacter freundii* *Morganella morganii*	4/2 – 128/2 4/2 – 128/2
Tobramycin	*Enterobacter aerogenes*	0.5-16
Trimethoprim	*Enterobacter aerogenes*	1-16
	Proteus mirabilis	1-16

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 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 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 Negative Identification

In two internal studies, the performance of the Phoenix Gram Negative identification was evaluated. The 0.5 inoculum density configuration and the 0.25 inoculum density configuration were tested with 721 strains (0.5) and 784 strains (0.25), respectively. Enteric and non-enteric results were evaluated against commercial and non-commercial methods.

The Phoenix Gram Negative identification performance is outlined below:

	McFarland	Agreement	No Agreement	No ID
Species Level	0.5	95.6%	3.6%	0.8%
	0.25	98.1%	1.4%	0.5%

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.

Confirmatory ESBL Test

To determine the accuracy of the Phoenix Confirmatory ESBL test, accuracy testing was performed at multiple sites using Clinical and Challenge isolates. The results from the ESBL test resident on the Phoenix panels were compared to the results obtained from the CLSI

reference confirmatory ESBL test.

For Challenge organisms this result is an expected result and for Clinical isolates this result was obtained from concurrent testing in the CLSI reference broth microdilution method. Additionally, a challenge set of 30 previously characterized organisms was tested at one site.

Positive Percent Agreement = 183/189 = 96.8%

Negative Percent Agreement = 780/812 = 96.1%

Overall Percent Agreement = 963/1001 = 96.2%

Gram Negative 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 (µg/mL)	EA N	EA %	CA N	CA %
5-Fluoroquinolone	Ciprofloxacin	CIP	0.25-4	2853	98.8	2853	95.1
5-Fluoroquinolone	Gatifloxacin	GAT	0.25-8	2213	98.8	2213	95.8
5-Fluoroquinolone	Levofloxacin	LVX	0.25-8	2934	98.5	2934	95.8
5-Fluoroquinolone	Moxifloxacin	MXF	0.12-8	2202	98.3	2202	97.6
5-Fluoroquinolone	Norfloxacin	NOR	0.25-16	2792	97.5	2792	94.3
5-Fluoroquinolone	Ofloxacin	OFX	0.25-8	2926	98.5	2926	94.6
Aminoglycoside	Amikacin	AN	0.5-64	2598	94.7	2598	96.7
Aminoglycoside	Gentamicin	GM	0.25-16	2751	96.2	2751	96.3
Aminoglycoside	Tobramycin	NN	0.12-16	2658	93.3	2658	95.3
B-Lac/B-Lac. Inh	Amoxicillin/ Clavulanate	AMC	0.5/0.25- 32/16	2249	96.7	2249	90.9
B-Lac/B-Lac. Inh	Ampicillin/ Sulbactam	SAM	0.5/0.25- 32/16	1305	97.2	1305	87.5
B-Lac/B-Lac. Inh	Ticarcillin/ Clavulanate	TIM	1/2-128/2	1527	92.5	1527	89.7
B-Lactam Pen	Ampicillin	AM	0.5-32	1712	97.0	1712	94.6
B-Lactam Pen	Piperacillin	PIP	0.5-128	1781	94.3	1781	93.8
B-Lac/B-Lac. Inh	Piperacillin/ Tazobactam	TZP	0.5/4- 128/4	1546	93.2	1546	94.9
B-Lactam Pen	Ticarcillin	TIC	1-128	2882	94.7	2882	92.7

Carbapenem	Imipenem	IPM	1-16	2680	97.2	2680	96.8
Carbapenem	Meropenem	MEM	0.25-16	2905	97.6	2905	98.3
Cephem	Cefazolin	CZ	0.5-32	1331	96.7	1331	94.4
Cephem	Cefepime	FEP	0.5-64	1789	95.2	1789	92.9
Cephem	Cefotaxime	CTX	0.5-64	2268	95.0	2268	92.7
Cephem	Cefotetan	CTT	2-64	1175	96.6	1175	96.7
Cephem	Cefoxitin	FOX	0.5-64	1397	96.9	1397	93.3
Cephem	Ceftazidime	CAZ	0.5-64	1796	96.5	1796	94.4
Cephem	Ceftriaxone	CRO	0.5-64	1872	95.8	1872	90.9
Cephem	Cefuroxime	CXM	1-64	1068	96.3	1068	93.3
Cephem	Cephalothin	CF	1-64	2025	96.4	2025	89.0
Folate Antagonist	Trimethoprim	TMP	0.5-16	1856	95.5	1856	98.7
Folate Antagonist	Trimethoprim-Sulfamethoxazole	SXT	0.5/9.5- 16/304	2212	96.0	2212	97.7
Monobactam	Aztreonam	ATM	0.5-64	1470	96.2	1470	96.2
Nitrofurantoin	Nitrofurantoin	FM	8-512	2130	95.8	2130	84.4
Quinolone	Nalidixic Acid	NA	2-32	2103	96.2	2103	98.6
Tetracycline	Tetracycline	TE	0.5-16	2837	95.5	2837	92.3

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TECHNICAL INFORMATION

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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 Negative (0.5 McFarland)

Gram Negative Taxa[1]	ID, AST, ID/AST
Achromobacter piechaudii	AST
Achromobacter species	ID/AST
Achromobacter xylosoxidans ssp*. denitrificans*	AST
Achromobacter xylosoxidans ssp*. xylosoxidans*	AST
Acinetobacter baumannii	ID/AST
Acinetobacter baumannii/calcoaceticus complex	ID/AST
Acinetobacter calcoaceticus	AST
Acinetobacter haemolyticus	ID/AST
Acinetobacter johnsonii	AST
Acinetobacter junii	AST
Acinetobacter lwoffii	ID/AST
Acinetobacter radioresistens	AST
Acinetobacter species	ID/AST
Actinobacillus lignieresii	ID
Actinobacillus suis	ID
Actinobacillus ureae	ID
Aeromonas allosaccharophila	AST
Aeromonas caviae	ID/AST
Aeromonas eucrenophila	AST
Aeromonas hydrophila	ID/AST
Aeromonas jandaei	AST
Aeromonas media	AST
Aeromonas salmonicida	AST
Aeromonas salmonicida ssp. *achromogenes*	AST

Aeromonas salmonicida ssp. *masoucida*	ID/AST
Aeromonas salmonicida ssp. *pectinolytica*	AST
Aeromonas salmonicida ssp. *salmonicida*	ID/AST
Aeromonas salmonicida ssp. *smithia*	ID/AST
Aeromonas schubertii	ID/AST
Aeromonas sobria	ID/AST
Aeromonas trota	AST
Aeromonas veronii	ID/AST
Alcaligenes faecalis	ID/AST
Bergeyella zoohelcum	ID
Bordetella bronchiseptica	ID
Brevundimonas diminuta	ID/AST
Brevundimonas vesicularis	ID/AST
Burkholderia cepacia	ID/AST
Burkholderia gladioli	ID
Cardiobacterium hominis	ID
CDC group EF-4a	ID
CDC group EF-4b	ID
CDC group EO-2	ID
CDC group Vb-3	ID
Cedecea davisae	ID/AST
Cedecea lapagei	ID/AST
Cedecea neteri	ID/AST
Cedecea species 3	AST
Cedecea species 5	AST
Chromobacterium violaceum	ID
Chryseobacterium gleum	ID/AST
Chryseobacterium indologenes	ID/AST
Chryseobacterium meningosepticum	ID/AST
Chryseobacterium scophthalmum	AST
Citrobacter amalonaticus	ID/AST
Citrobacter braakii	ID/AST
Citrobacter farmeri	ID/AST
Citrobacter freundii	ID/AST
Citrobacter gillenii	AST

Citrobacter koseri	ID/AST
Citrobacter murliniae	AST
Citrobacter rodentium	AST
Citrobacter sedlakii	ID/AST
Citrobacter werkmanii	ID/AST
Citrobacter youngae	ID/AST
Comamonas terrigena	ID
Comamonas testosteroni	ID
Delftia acidovorans	ID/AST
Edwardsiella hoshinae	ID/AST
Edwardsiella ictaluri	ID/AST
Edwardsiella tarda	ID/AST
Eikenella corrodens	ID
Empedobacter brevis	ID
Enterobacter aerogenes	ID/AST
Enterobacter amnigenus	AST
Enterobacter amnigenus biogroup 1	ID/AST
Enterobacter amnigenus biogroup 2	ID/AST
Enterobacter asburiae	ID/AST
Enterobacter cancerogenus	ID/AST
Enterobacter cloacae	ID/AST
Enterobacter cowanii	AST
Enterobacter dissolvens	AST
Enterobacter gergoviae	ID/AST
Enterobacter hormaechei	ID/AST
Enterobacter intermedius	ID/AST
Enterobacter kobei	AST
Enterobacter nimipressuralis	AST
Enterobacter sakazakii	ID/AST
Escherichia blattae	AST
Escherichia coli	ID/AST
Escherichia fergusonii	ID/AST
Escherichia hermannii	ID/AST
Escherichia vulneris	ID/AST
Ewingella americana	ID

Hafnia alvei	ID/AST
Kingella denitrificans	ID
Kingella kingae	ID
Klebsiella granulomatis	AST
Klebsiella oxytoca	ID/AST
Klebsiella pneumoniae ssp. *ozaenae*	ID/AST
Klebsiella pneumoniae ssp. *pneumoniae*	ID/AST
Klebsiella pneumoniae ssp. *rhinoscleromatis*	ID/AST
Kluyvera ascorbata	ID/AST
Kluyvera cryocrescens	ID/AST
Kluyvera georgiana	AST
Leclercia adecarboxylata	ID/AST
Leminorella grimontii	ID
Leminorella richardii	ID
Mannheimia haemolytica	ID
Methylobacterium extorquens	ID
Moellerella wisconsensis	ID/AST
Moraxella (Branhamella) catarrhalis	ID
Moraxella species	ID
Morganella morganii	ID/AST
Myroides odoratus/odoratimimus	ID/AST
Ochrobactrum anthropi	ID/AST
Oligella ureolytica	ID
Oligella urethralis	ID
Pantoea agglomerans	ID/AST
Pantoea ananatis	AST
Pantoea dispersa	AST
Pantoea stewartii ssp. *indologenes*	AST
Pantoea stewartii ssp. *stewartii*	AST
Pasteurella aerogenes	ID
Pasteurella multocida	ID
Pasteurella pneumotropica	ID
Photobacterium damselae	ID
Plesiomonas shigelloides	ID
Pragia fontium	ID

Proteus hauseri	AST
Proteus mirabilis	ID/AST
Proteus myxofaciens	AST
Proteus penneri	ID/AST
Proteus vulgaris	ID/AST
Providencia alcalifaciens	ID/AST
Providencia heimbachae	AST
Providencia rettgeri	ID/AST
Providencia rustigianii	ID/AST
Providencia stuartii	ID/AST
Pseudomonas aeruginosa	ID/AST
Pseudomonas alcaligenes	AST
Pseudomonas fluorescens	ID/AST
Pseudomonas luteola	ID/AST
Pseudomonas mendocina	ID/AST
Pseudomonas monteilii	AST
Pseudomonas oryzihabitans	ID/AST
Pseudomonas pertucinogena	AST
Pseudomonas pseudoalcaligenes	ID/AST
Pseudomonas putida	ID/AST
Pseudomonas species	ID/AST
Pseudomonas stutzeri	ID/AST
Pseudomonas veronii	AST
Rahnella aquatilis	ID
Ralstonia pickettii	ID/AST
Ralstonia solanacearum	AST
Ralstonia species	AST
Raoultella ornithinolytica	ID/AST
Raoultella planticola	AST
Raoultella terrigena	AST
Rhizobium radiobacter	ID
Salmonella aberdeen	AST
Salmonella abortus-equi	AST
Salmonella adelaide	AST
Salmonella aderike	AST

Salmonella agona	AST
Salmonella alachua	AST
Salmonella anatum	AST
Salmonella arizonae	AST
Salmonella avana	AST
Salmonella bahrenfeld	AST
Salmonella blockley	AST
Salmonella bongori	AST
Salmonella braenderup	AST
Salmonella bredeney	AST
Salmonella bunn	AST
Salmonella california	AST
Salmonella carrau	AST
Salmonella cerro	AST
Salmonella champaign	AST
Salmonella chittagong	AST
Salmonella cholerasuis	AST
Salmonella choleraesuis ssp. *arizonae*	ID/AST
Salmonella choleraesuis ssp. *choleraesuis*	ID/AST
Salmonella choleraesuis ssp. *diarizonae*	AST
Salmonella choleraesuis ssp. *houtenae*	AST
Salmonella choleraesuis ssp. *indica*	AST
Salmonella choleraesuis ssp. *salamae*	AST
Salmonella cubana	AST
Salmonella dakar	AST
Salmonella daressalaam	*AST*
Salmonella derby	AST
Salmonella dessau	AST
Salmonella DT	AST
Salmonella dublin	AST
Salmonella duesseldorf	AST
Salmonella enteritidis	AST
Salmonella fresno	AST
Salmonella gallinarum	ID/AST
Salmonella give	AST

Salmonella haardt	AST
Salmonella hadar	AST
Salmonella hamburg	AST
Salmonella hartford	AST
Salmonella heidelberg	AST
Salmonella illinois	AST
Salmonella infantis	AST
Salmonella inverness	AST
Salmonella java	AST
Salmonella javiana	AST
Salmonella kentucky	AST
Salmonella kirkee	AST
Salmonella kunduchi	AST
Salmonella kvittingfoss	AST
Salmonella lansing	AST
Salmonella litchfield	AST
Salmonella liverpool	AST
Salmonella london	AST
Salmonella luciana	AST
Salmonella manhattan	AST
Salmonella mbandaka	AST
Salmonella meleagridis	AST
Salmonella memphis	AST
Salmonella michigan	AST
Salmonella minneapolis	AST
Salmonella minnesota	AST
Salmonella montevideo	AST
Salmonella muenchen	AST
Salmonella muenster	AST
Salmonella newington	AST
Salmonella newport	AST
Salmonella nottingham	AST
Salmonella ohio	AST
Salmonella onderstepoort	AST
Salmonella oranienburg	AST

Salmonella panama	AST
Salmonella paratyphi A	ID/AST
Salmonella paratyphi B	AST
Salmonella poona	AST
Salmonella pullorum	ID/AST
Salmonella quinhon	AST
Salmonella rubislaw	AST
Salmonella saintpaul	AST
Salmonella schwarzengrund	AST
Salmonella seftenberg	AST
Salmonella species	ID/AST
Salmonella tallahassee	AST
Salmonella thompson	AST
Salmonella typhi	ID/AST
Salmonella typhimurium	AST
Salmonella virginia	AST
Salmonella westerstede	AST
Salmonella worthington	AST
Serratia entomophilia	AST
Serratia ficaria	ID/AST
Serratia fonticola	ID/AST
Serratia grimesii	AST
Serratia liquifaciens	ID/AST
Serratia marcescens	ID/AST
Serratia odorifera	AST
Serratia odorifera 1	ID/AST
Serratia odorifera 2	ID/AST
Serratia plymuthica	ID/AST
Serratia proteamaculans ssp. *proteamaculans*	AST
Serratia proteamaculans ssp. *quinovora*	AST
Serratia rubidaea	ID/AST
Shewanella algae	AST
Shewanella putrefaciens	ID/AST
Shigella boydii	ID/AST
Shigella dysenteriae	ID/AST

Shigella flexneri	ID/AST
Shigella sonnei	ID/AST
Shigella species	ID/AST
Sphingobacterium multivorum	ID/AST
Sphingobacterium spiritivorum	ID/AST
Sphingobacterium thalpophilum	ID/AST
Sphingomonas paucimobilis	ID
Stenotrophomonas maltophilia	ID/AST
Suttonella indologenes	ID
Tatumella ptyseos	ID
Vibrio alginolyticus	ID
Vibrio cholerae	ID
Vibrio fluvialis	ID
Vibrio hollisae	ID
Vibrio metschnikovii	ID
Vibrio mimicus	ID
Vibrio parahaemolyticus	ID
Vibrio vulnificus	ID
Wautersia gilardii	AST
Wautersia paucula	ID/AST
Weeksella virosa	ID
Yersinia aldovae	AST
Yersinia bercovieri	AST
Yersinia enterocolitica	ID/AST
Yersinia frederiksenii	ID/AST
Yersinia intermedia	ID/AST
Yersinia kristensenii	ID/AST
Yersinia mollaretii	AST
Yersinia pseudotuberculosis	ID/AST
Yersinia rohdei	AST
Yersinia ruckeri	ID/AST
Yokenella regensburgei	ID

[1] Not all species encountered during clinical performance evaluations.

Gram Negative (0.25 McFarland)

Gram Negative Taxa[1]	ID, AST, ID/AST
Achromobacter species	ID/AST
Acinetobacter baumannii/calcoaceticus complex	ID/AST
Acinetobacter haemolyticus	ID/AST
Acinetobacter lwoffii	ID/AST
Actinobacillus lignieresii	ID
Actinobacillus suis	ID
Actinobacillus ureae	ID
Aeromonas caviae	ID/AST
Aeromonas hydrophila	ID/AST
Aeromonas salmonicida ssp. *masoucida*	ID/AST
Aeromonas salmonicida ssp. *salmonicida*	ID/AST
Aeromonas salmonicida ssp. *smithia*	ID/AST
Aeromonas schubertii	ID/AST
Aeromonas sobria	ID/AST
Aeromonas veronii	ID/AST
Alcaligenes faecalis	ID/AST
Bergeyella zoohelcum	ID
Bordetella bronchiseptica	ID
Brevundimonas diminuta	ID/AST
Brevundimonas vesicularis	ID/AST
Burkholderia cepacia	ID/AST
Burkholderia gladioli	ID
Cardiobacterium hominis	ID
CDC group EF-4a	ID
CDC group EF-4b	ID
CDC group EO-2	ID
CDC group Vb-3	ID
Cedecea davisae	ID/AST
Cedecea lapagei	ID/AST
Cedecea neteri	ID/AST
Chromobacterium violaceum	ID

Chryseobacterium gleum	ID/AST
Chryseobacterium indologenes	ID/AST
Chryseobacterium meningosepticum	ID/AST
Citrobacter amalonaticus	ID/AST
Citrobacter braakii	ID/AST
Citrobacter farmeri	ID/AST
Citrobacter freundii	ID/AST
Citrobacter koseri	ID/AST
Citrobacter sedlakii	ID/AST
Citrobacter werkmanii	ID/AST
Citrobacter youngae	ID/AST
Comamonas terrigena	ID
Comamonas testosteroni	ID
Delftia acidovorans	ID/AST
Edwardsiella hoshinae	ID/AST
Edwardsiella ictaluri	ID/AST
Edwardsiella tarda	ID/AST
Eikenella corrodens	ID
Empedobacter brevis	ID
Enterobacter aerogenes	ID/AST
Enterobacter amnigenus biogroup 1	ID/AST
Enterobacter amnigenus biogroup 2	ID/AST
Enterobacter asburiae	ID/AST
Enterobacter cancerogenus	ID/AST
Enterobacter cloacae	ID/AST
Enterobacter gergoviae	ID/AST
Enterobacter hormaechei	ID/AST
Enterobacter intermedius	ID/AST
Enterobacter sakazakii	ID/AST
Escherichia coli	ID/AST
Escherichia fergusonii	ID/AST
Escherichia hermannii	ID/AST
Escherichia vulneris	ID/AST
Ewingella americana	ID
Hafnia alvei	ID/AST

Klebsiella oxytoca	ID/AST
Klebsiella pneumoniae ssp. *ozaenae*	ID/AST
Klebsiella pneumoniae ssp. *pneumoniae*	ID/AST
Klebsiella pneumoniae ssp. *rhinoscleromatis*	ID/AST
Kluyvera ascorbata	ID/AST
Kluyvera cryocrescens	ID/AST
Leclercia adecarboxylata	ID/AST
Leminorella grimontii	ID
Leminorella richardii	ID
Mannheimia haemolytica	ID
Moellerella wisconsensis	ID/AST
Morganella morganii	ID/AST
Myroides odoratus/odoratimimus	ID/AST
Ochrobactrum anthropi	ID/AST
Oligella ureolytica	ID
Oligella urethralis	ID
Pantoea agglomerans	ID/AST
Pasteurella aerogenes	ID
Pasteurella multocida	ID
Pasteurella pneumotropica	ID
Photobacterium damselae	ID
Plesiomonas shigelloides	ID
Pragia fontium	ID
Proteus mirabilis	ID/AST
Proteus penneri	ID/AST
Proteus vulgaris	ID/AST
Providencia alcalifaciens	ID/AST
Providencia rettgeri	ID/AST
Providencia rustigianii	ID/AST
Providencia stuartii	ID/AST
Pseudomonas aeruginosa	ID/AST
Pseudomonas fluorescens	ID/AST
Pseudomonas luteola	ID/AST
Pseudomonas mendocina	ID/AST
Pseudomonas oryzihabitans	ID/AST

Pseudomonas putida	ID/AST
Pueudomonas stutzeri	ID/AST
Rahnella aquatilis	ID
Ralstonia pickettii	ID/AST
Raoultella ornithinolytica	ID/AST
Rhizobium radiobacter	ID
Salmonella choleraesuis ssp. *arizonae*	ID/AST
Salmonella choleraesuis ssp. *choleraesuis*	ID/AST
Salmonella gallinarum	ID/AST
Salmonella paratyphi A	ID/AST
Salmonella pullorum	ID/AST
Salmonella species	ID/AST
Salmonella typhi	ID/AST
Serratia ficaria	ID/AST
Serratia fonticola	ID/AST
Serratia liquifaciens	ID/AST
Serratia marcescens	ID/AST
Serratia odorifera 1	ID/AST
Serratia odorifera 2	ID/AST
Serratia plymuthica	ID/AST
Serratia rubidaea	ID/AST
Shewanella putrefaciens	ID/AST
Shigella boydii	ID/AST
Shigella dysenteriae	ID/AST
Shigella flexneri	ID/AST
Shigella sonnei	ID/AST
Sphingobacterium multivorum	ID/AST
Sphingobacterium spiritivorum	ID/AST
Sphingobacterium thalpophilum	ID/AST
Sphingomonas paucimobilis	ID
Stenotrophomonas maltophilia	ID/AST
Suttonella indologenes	ID
Tatumella ptyseos	ID
Vibrio alginolyticus	ID
Vibrio cholerae	ID

Vibrio fluvialis	ID
Vibrio hollisae	ID
Vibrio metschnikovii	ID
Vibrio mimicus	ID
Vibrio parahaemolyticus	ID
Vibrio vulnificus	ID
Wautersia paucula	ID/AST
Weeksella virosa	ID
Yersinia enterocolitica	ID/AST
Yersinia frederiksenii	ID/AST
Yersinia intermedia	ID/AST
Yersinia kristensenii	ID/AST
Yersinia pseudotuberculosis	ID/AST
Yersinia ruckeri	ID/AST
Yokenella regensburgei	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
L-PHENYLALANINE-AMC	A_LPHET	Enzymatic hydrolysis of the amide or glycosidic bond results in the release of a fluorescent coumarin or 4-methylumbelliferone derivative.
4MU-N-ACETYL-BD-GLUCOSAMINIDE	A_NAG	
L-GLUTAMIC ACID-AMC	A_LGTA	
L-TRYPTOPHAN-AMC	A_LTRY	
L-PYROGLUTAMIC ACID-AMC	A_LPYR	
L-PROLINE-AMC	A_LPROB	
L-ARGININE-AMC	A_LARGH	
ARGININE-ARGININE-AMC	A_ARARR	
GLYCINE-AMC	A_GLYB	
L-LEUCINE-AMC	A_LLEUH	
LYSINE-ALANINE-AMC	A_LYALD	
GLUTARYL-GLYCINE-ARGININE-AMC	A_GUGAH	
GLYCINE-PROLINE-AMC	A_GLPRB	
COLISTIN	C_CLST	Resistance to the antimicrobial agents results in a reduction of resazurin based indicator.
POLYMYXIN B	C_PXB	
D-MANNITOL	C_DMNT	Utilization of a carbon source results in a reduction of the resazurin-based indicator.
CITRATE	C_CIT	
ACETATE	C_ACT	
ADONITOL	C_ADO	
MALONATE	C_MLO	
ALPHA-KETOGLUTARIC ACID	C-KGA	
TIGLIC ACID	C_TIG	
FLUORESCENT POSITIVE CONTROL	FLR_CTL	Control to standardize fluorescent substrate results.
FLUORESENT POSTIVE CONTROL	FLR_CTL	
L-PROLINE-NA	N_LPROT	Enzymatic hydrolysis of the colorless amide substrate releases yellow p-nitroaniline.
GAMMA-L-GLUTAMYL-NA	N_LGGH	
BIS (PNP) PHOSPHATE	P_BPHO	Enzymatic hydrolysis of the colorless aryl substituted glycoside releases yellow p-nitrophenol.
PNP-BD-GLUCOSIDE	P_BDGLU	

Substrate Name	Code	Principle
BETA-ALLOSE	R_BALL	Utilization of carbohydrate results in lower pH and change in indicator (phenol red).
N-ACETYL-GALACTOSAMINE	R_NGA	
N-ACETYL-GLUCOSAMINE	R_NGU	
SORBITOL	R_DSBT	
SUCROSE	R_DSUC	
GALACTURONIC ACID	R_GRA	
MALTULOSE	R_MTU	
L-RHAMNOSE	R_LRHA	
BETA-GENTIOBIOSE	R_BGEN	
DEXTROSE	R_DEX	
D-GALACTOSE	R_DGAL	
D-FRUCTOSE	R_DFRU	
D-GLUCONIC ACID	R_DGUA	
D-MELIBIOSE	R_DMLB	
L-ARABINOSE	R_LARA	
METHYL-B-GLUCOSIDE	R_MBGU	
ORNITHINE	S_ORN	Utilization of ornithine results in pH rise and change in fluorescent indicator.
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.

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	Yes
BBL™ CHROMagar™ Orientation	Yes	Yes
Chocolate Agar	Yes	Yes
Columbia Agar with 5% Horse Blood	Yes	Yes
Columbia Agar with 5% Sheep Blood	Yes	Yes
Cystine-Lactose-Electrolyte-Deficient (CLED) Agar	Yes	Yes
Dey/Egley (D/E) Neutralizing Agar	Yes	No
Eosin Methylene Blue	Yes	Yes
Hektoen Enteric Agar	Yes	No
MacConkey Agar	Yes	Yes
Trypticase™ Soy Agar without Blood	Yes	No
Trypticase™ Soy Agar with Lecithin and Tween™ 80	Yes	No
Xylose Lysine Desoxycholate Agar	Yes	No