



Antibacterial Resistance Leadership Group (ARLG)

Rapid Identification and Phenotypic Susceptibility Testing for Gram-Negative Bacteremia (RAPIDS-GN)

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List of Abbreviations

ANC	Absolute neutrophil count
ARLG	Antibacterial Resistance Leadership Group
AS	Antimicrobial stewardship
AST	Antimicrobial susceptibility testing
AXDX	Accelerate Pheno™System
CDC	Centers for Disease Control and Prevention
CRF	Case report form
DCRI	Duke Clinical Research Institute
DOOR/RADAR	Desirability of outcome response and response adjusted for days of antibiotic risk
EMR	Electronic medical record
FDA	Food and Drug Administration
FISH	Fluorescent in situ hybridization
FSTRF	Frontier Science Technology and Research Foundation
GNB	gram-negative bacilli
ICMJE	International Committee of Medical Journal Editors
ICU	Intensive care unit
ID	Infectious diseases
IRB	Institutional review board
IV	By vein
MALDI-TOF MS	Matrix-assisted laser desorption/ionization mass spectrometry
MDRO	Multi-drug resistant organism
NIAID	National Institutes of Allergy and Infectious Disease
NIH	National Institutes of Health
NHSN	National Healthcare Safety Network
PCR	Polymerase chain reaction
PO	By mouth
SD	Standard deviation
SDMC	Statistics and data management center
SOC	Standard of care
VAP	Ventilator-associated pneumonia

Protocol Synopsis

Protocol Title:	Rapid Identification and Phenotypic Susceptibility Testing for Gram-Negative Bacteremia (RAPIDS-GN)
Study Design:	Multi-center, prospective, randomized, controlled trial to evaluate the following strategies for patients with confirmed gram-negative bacillus bacteremia: <ol style="list-style-type: none"> 1) Standard culture and antimicrobial susceptibility testing (AST); or 2) Rapid identification and AST using the Accelerate PhenoTest™ BC Kit, performed on the Accelerate Pheno™ System (AXDX)
Primary Study Objectives:	To evaluate the impact of rapid identification and AST on time to first antibiotic modification in the first 72 hours after randomization
Secondary Objectives	To evaluate the impact of rapid identification and AST on antimicrobial utilization and clinical outcomes. <ul style="list-style-type: none"> • 30-day mortality • Length of stay in the hospital • Days in the ICU • Duration of broad-spectrum gram-negative coverage in the first 72 hours following randomization • Mean time to antibiotic escalation • Mean time to antibiotic de-escalation • Hospital-onset <i>Clostridium difficile</i> • Acquisition of new hospital-acquired infections (HAIs) and/or multidrug resistant organisms (MDROs)
Study Population	All patients with blood culture bottles growing gram-negative bacilli (GNB) detected on Gram staining will be eligible
Number of subjects	500 subjects
Number of sites	2 sites
Clinical Samples	Clinically collected blood cultures with growth of GNB detected by Gram stain
Start of Enrollment:	October 2017
End of Enrollment:	October 2018

1.0 Background Information and Scientific Rationale

1.1 Background Information

Novel molecular diagnostics to identify bacteria and determine antimicrobial susceptibility from clinical cultures are quickly entering the arsenal of tools used by clinical microbiology laboratories. However, the clinical benefit of these methods has not been well-quantified.

The goal of this study is to determine the impact of rapid bacterial identification and phenotypic antimicrobial susceptibility testing (AST) on antimicrobial usage and clinical outcomes.

The Accelerate PhenoTest™ BC Kit, performed on the Accelerate Pheno™ System (together termed AXDX) (Accelerate Diagnostics, Tucson, AZ) is an FDA-approved fully automated platform that performs rapid bacterial identification within 1.5 hours followed by determination of minimum inhibitory concentration (MIC)-based phenotypic susceptibility within approximately 7 hours directly from positive blood cultures.¹ This novel system uses fluorescence *in situ* hybridization (FISH) for rapid bacterial identification for a panel of organisms followed by time-lapse microscopy for determining phenotypic AST directly from positive blood culture bottles (see Table 1). In a study of 1850 blood cultures with gram-positive, gram-negative, or yeast isolates, AXDX was found to have overall 97.4% sensitivity and 99.3% specificity for organism identification compared to conventional culture methods (see Table 2). Among 728 gram-negative isolates, AXDX had 97.6% sensitivity and 99.6% specificity for pathogen identification, and had excellent essential (device result agrees exactly with or within one two-fold dilution of the reference result) and categorical (the device and reference result interpretive categories agree) agreement with the gold-standard methods for drug susceptibility testing, according to Food and Drug Administration (FDA) and/or Clinical and Laboratory Standards Institute (CLSI) breakpoints (see Table 3)¹.

Table 1. FDA-approved gram-negative bacilli and antibiotic combinations available on AXDX

	Ampicillin-sulbactam	Piperacillin-tazobactam	Cefepime	Ceftazidime	Ceftriaxone	Ertapenem	Meropenem	Amikacin	Gentamicin	Tobramycin	Ciprofloxacin	Aztreonam
<i>E coli</i>	x	x	x	x	x	x	x	x	x	x	x	x
<i>Klebsiella spp.*</i>	x	x	x	x	x	x	x	x	x	x	x	x
<i>Enterobacter spp.*</i>		x	x	x	x	x	x	x	x	x	x	x
<i>Proteus spp.*</i>	x	x	x	x	x	x	x	x	x	x	x	x
<i>Citrobacter spp.*</i>		x	x	x	x	x	x	x	x	x	x	x
<i>S. marescens</i>		x	x	x	x	x	x	x	x	x	x	x
<i>P. aeruginosa</i>		x	x	x			x	x	x	x	x	
<i>A. baumannii</i>		x						x				

**Klebsiella spp.* includes *pneumoniae* and *oxytoca*; *Enterobacter spp.* includes *cloacae* and *aerogenes*; *Proteus spp.* includes *mirabilis* and *vulgaris*; *Citrobacter spp.* includes *freundii* and *koseri*

Table 2. Clinical trial results of AXDX performance for gram-negative bacilli identification (N = 728)

Organism	Sensitivity (%)	Specificity (%)
<i>A. baumannii</i>	98.6	99.7
<i>Citrobacter spp.</i>	96.8	99.3
<i>E. coli</i>	97.3	99.7
<i>Enterobacter spp.</i>	97.3	99.5
<i>Klebsiella spp.</i>	96.1	99.6
<i>P. aeruginosa</i>	100	99.4
<i>Proteus spp.</i>	97.7	99.6
<i>Serratia marcescens</i>	100	99.9
Gram-negative bacilli, total	97.6	99.6

Table 3. Clinical trial results of AXDX performance for gram-negative bacilli antibiotic susceptibility.

Antibiotic	EA (%)	CA (%)	# of fresh isolates	# of challenge isolates
Amikacin	93.8	93.8	202	200
Ampicillin-sulbactam	91.0	82.7	174	92
Aztreonam	96.4	97.6	198	137
Cefepime	96.2	95.5	215	209
Ceftazidime	92.4	92.1	203	163
Ceftriaxone	94.7	96.4	180	124
Ciprofloxacin	98.4	98.4	216	209
Ertapenem	98.8	98.5	200	139
Gentamicin	99.5	98.7	207	165
Meropenem	96.7	96.9	215	208
Piperacillin-tazobactam	91.0	90.8	207	205
Tobramycin	96.3	96.0	210	165

EA, essential agreement; CA, categorical agreement; challenge isolates with key resistance phenotypes and genotypes were seeded into blood culture broths.

1.2 Scientific Rationale

Bacteremia due to gram-negative bacilli (GNB) poses a dangerous threat due to increasing rates of resistance and high mortality with ineffective antibiotic therapy.²⁻⁶ Conversely, treatment with overly broad antibiotics increases risks of adverse drug events and drives further development of resistance.^{7,8} An important solution to address this conundrum is development of methods for rapid detection of drug resistant GNB, which would allow for rapid de-escalation or escalation of antibiotics to appropriate definitive therapy.⁹ Standard turn-around-time for clinical microbiology laboratories to isolate, identify, and perform antimicrobial susceptibility testing (AST) of bacterial isolates is 48-96 hours from the time a blood culture turns positive, while rapid testing methods can provide results within 6-24 hours. Strategies for rapid phenotypic AST of GNB are preferred over methods for genotypic resistance detection, which cannot reliably predict the numerous antibiotic resistance mechanisms in GNB.^{2,10,11}

Prior single-center, observational studies have demonstrated decreased time to appropriate antibiotics, lower mortality, shorter durations of hospital and ICU stay, and reduced costs when rapid identification and AST methods are coupled with antimicrobial stewardship.¹²⁻¹⁴ Limitations to these studies include the fact that they were retrospective, subject to temporal trends, single-center, and uncontrolled. A previous single-center, randomized, controlled trial demonstrated that rapid polymerase chain reaction (PCR)-based identification paired with antimicrobial stewardship intervention was associated with more rapid antibiotic de-escalation, shorter time to appropriate antibiotic therapy, and less use of broad-spectrum antibiotics.¹⁵ However, the diagnostic test evaluated in that study had limited impact on management of patients with GNB bacteremia

because the test detected only a single GNB resistance determinant (*bla_{KPC}*), and did not provide rapid phenotypic susceptibility information for a full panel of antibiotics.

Accelerate's AXDX is the first FDA-approved platform to provide both identification and *rapid phenotypic antibiotic susceptibility* testing of organisms directly from positive blood cultures. We will evaluate the clinical impact of AXDX testing for GNB bacteremia in a multicenter, prospective, randomized, controlled clinical trial. This study will evaluate the impact of AXDX testing in the setting of baseline antimicrobial stewardship activities, because current regulations mandate that all acute care settings have antimicrobial stewardship programs,^{16,17} and several prior studies demonstrate benefit of combining rapid testing with antimicrobial stewardship. Results from this study will inform strategies to improve diagnostic testing for GNB bacteremia.

2.0 Hypotheses

We hypothesize that subjects with GNB bacteremia who receive rapid testing of blood cultures using AXDX will have faster time to antibiotic modification within the first 72 hours following randomization compared to subjects who receive standard bacterial culture and AST.

3.0 Objectives

3.1 Primary Objectives

The primary objective is to evaluate the impact of rapid identification and AST on time to first antibiotic modification in the first 72 hours after randomization.

3.2 Secondary Objectives

Secondary objectives will be to evaluate the impact of rapid identification and AST on antibiotic utilization and clinical outcomes.

4.0 Study Design

This study will be a multicenter, prospective, randomized, controlled trial evaluating antimicrobial utilization and clinical outcomes among patients with GNB bacteremia. Patients will be randomized to one of the following arms:

1. Standard culture and AST
2. Rapid identification and AST using AXDX

Baseline antimicrobial stewardship will occur in both arms. The blood sample for arm 2 will also undergo standard culture and AST in addition to the rapid testing.

4.1 Inclusion Criteria

Patients who meet the following inclusion criteria, and none of the exclusion criteria, will be included in the study:

- Positive blood culture with Gram stain showing GNB identified during local laboratory business hours.

4.2 Exclusion Criteria

Patients who meet any of the following criteria will be excluded from the study:

- Identification of GNB outside of local laboratory business hours (e.g. whenever laboratories are staffed to perform both rapid testing and routine testing)
- Positive blood culture for GNB at the same institution within prior 7 days (if known at the time of randomization).
- Deceased at the time of randomization.
 - GNB plus gram-positive organism, gram-negative cocci, and/or yeast detected on Gram stain
 - Previous enrollment in this study
- No Minnesota research authorization (*Rochester site only*)

If the patient does not meet any of the above exclusion criteria, he/she will be randomized, and laboratory testing will be performed according to group assignment.

5.0 Study Procedures

5.1 Screening and Enrollment

Eligible patients will be identified based on positive blood culture bottles with Gram stain showing GNB. The Microbiology Laboratory Technologists will screen for exclusion criteria and then will enroll patients who do not meet any exclusion criteria.

This study will not require informed consent because this FDA approved test is being used for its approved indication, in addition to standard of care. Furthermore, the rapid test is not being evaluated for its diagnostic accuracy (which has been determined by studies used to gain FDA approval), but rather to determine whether its use in the clinical setting can improve quality of patient care.

5.2 Randomization

Patients meeting enrollment criteria will be assigned, in a 1:1 ratio using permuted blocks, to one of the two arms outlined in Section 4.0. The randomization scheme will be stratified by site. Randomization will occur at the time the Gram stain detecting GNB is identified by the lab. Randomization will be performed by the Laboratory Technologists, using an electronic system.

5.3 Interventions

5.3.1 Notifications

Once study group is assigned, the Laboratory Technologists will immediately notify the Study Coordinator via electronic or telephone page. The Study Coordinator will then notify the Antimicrobial Stewardship (AS) team that a subject has been randomized to either arm. Each subject's medical record number, group assignment and subject ID will be recorded in an electronic log.

For subjects in both arms, the Laboratory Technologists will notify ordering clinicians by telephone when the Gram stain results are available, as is done in routine clinical practice.

Sites are responsible for notifying appropriate agencies of reportable infectious diseases per institutional policy.

5.3.2 Standard Subculture and AST

For all patients, the local standard of care for identification and AST of GNB from positive blood cultures will be performed, including standard subculture and AST as well as matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS). If the primary or AS teams desire to have additional drug susceptibility information that is not routinely reported, they may request this information from the Microbiology Laboratory.

If standard culture methods detect a gram-positive organism, gram-negative cocci, and/or yeast *instead* of a gram-negative organism (as was originally detected on Gram stain), the culture result will be reported in the medical record, as per standard of care, and the subject will be considered unevaluable as he or she will not have GNB bacteremia.

5.3.3 Rapid identification and AST

For patients randomized to the rapid testing arm, testing will be performed during local laboratory business hours, using the AXDX system. Several milliliters of material from the positive bottle will be used to perform the testing, while the remainder will be used for standard of care testing. If no targets are identified using the rapid testing panel (see Table 1, Section 1.1), then identification and AST will be done through standard methods only. Combinations of bacteria/antibiotics that are reported by AXDX but not routinely reported by each laboratory will be suppressed for study patients. GNB/antibiotic combinations that are not reported by AXDX, but are routinely reported by the lab using standard of care testing, will be added to the final AST report for study patients.

If there is disagreement between the rapid AST and the standard AST results, any “intermediate” or “resistant” result will be reported, regardless of testing method. The primary team caring for the patient will be notified by phone of discrepant results, and additional susceptibility testing will be performed as needed and determined by the site Laboratory Director. Discrepant results will be reviewed by the Microbiology Laboratory Director as detailed in section 6.0. If the primary or AS teams desire to have additional drug susceptibility information that is not reported by AXDX or standard of care testing, they may request this information from the Microbiology Laboratory.

If the AXDX assay detects a gram-positive organism, gram-negative cocci, and/or yeast (that was not originally noted on Gram stain) *in addition* to GNB, the laboratory technologist will review the Gram stain. If the gram-positive organism or yeast is seen upon review, the following FDA-approved gram-positive or yeast organism identifications along with AST will be reported in the medical record: *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Enterococcus faecalis*, *Candida albicans*, *Candida glabrata*, coagulase-negative staphylococci, *Enterococcus* species, *Streptococcus* species. If the gram-positive bacteria or yeast is not seen upon review of the Gram stain, the AXDX result will not be reported, as this test is not FDA approved for clinical use if organisms are not visualized by Gram stain. If the AXDX assay detects a gram-positive organism, gram-negative cocci, and/or yeast *instead* of GNB (as was originally detected on Gram stain), the AXDX result will not be reported. In these cases, if standard culture methods detect other organisms *instead* of GNB, the standard culture result will be reported in the medical record, and the subject will be considered unevaluable as he or she will not have GNB bacteremia.

5.3.4 Antimicrobial Stewardship

All patients in both arms of the study will undergo prospective audit and feedback by the institutional AS programs. The AS physician or pharmacist will review the subject’s microbiology results and medical records. The AS provider will contact the primary service (or the consulting infectious diseases (ID) physician, if applicable) if modifications

to therapy are indicated, at any time following notification of positive Gram stain result, organism identification, and/or AST results. If the ID service is consulting on the subject, the AS team will contact the ID service rather than the primary service. If the rapid test result is ready when the AS team is unavailable (i.e. in the evening or weekend), the AS team will review the record and make recommendations at the beginning of the following working day. Local institutional treatment guidelines will be followed. Recommendations will be communicated to the treating teams via telephone or pager and documented per institutional standards. Recommendation types and acceptance rates will be recorded in the study database. Categories of recommendations include: intravenous to oral conversion, de-escalation of gram negative coverage, de-escalation of gram positive coverage, escalation of gram negative coverage, escalation of gram positive coverage, dose optimization, discontinuation of therapy, therapeutic drug monitoring, reduce/define duration of therapy, ID consultation, and other. More than one stewardship recommendation category may be chosen.

In addition to prospective audit and feedback, other routine institutional AS activities will continue, such as implementation of institutional treatment guidelines, prior authorization of restricted antibiotics, review of all patients on selected antibiotics with feedback to clinicians, and daily review of specific culture results.

5.4 Blinding

Primary service/provider: The primary service, including the prescribing provider, will be unaware of group assignment at the time of randomization, so initial antibiotic choice will not be affected by group assignment. Once rapid results become available and/or AS interventions are made, treating providers may become aware of group assignment.

The AS team and laboratory technologists will not be blinded.

5.5 Assessments

For this study, all measurements and assessments will be taken from the electronic medical record (EMR) and are done as routine standard of care practice.

5.5.1 Baseline Assessment

Once a patient is randomized, information about the patient's demographics, comorbid conditions, severity of illness (e.g. components of the Pitt Bacteremia Score¹⁸⁻²⁰), and microbiology and antimicrobial susceptibility results will be recorded. Whether a patient is on comfort care will be recorded, as determined by review of the primary physician team notes and any relevant consultations (e.g. Palliative Care).

5.5.2 Daily Evaluations

Information about additional microbiology results and antibiotic treatment will be gathered from the EMR during the three days following randomization. Suspected source and site of infection will be recorded by the AS providers.

5.5.3 Antibiotic spectrum and antibiotic intensity score

The antibiotic intensity score will be determined as follows: Each antibiotic is assigned an antibiotic spectrum rank from 1-5 (see Table 4). The antibiotic rank will be multiplied by the duration each antibiotic is received (in hours) within the first 72 hours following randomization. For patients receiving multiple antibiotics, the total antibiotic intensity

score will be the summation of the rank x duration product for each antibiotic. If a single dose of antibiotic is administered, duration will be considered 1 hour. For all antibiotics given for more than one dose, the duration will be the time of administration of the last dose minus the time of administration of the first dose (or time of randomization, if subject was receiving antibiotic at randomization) plus one hour. Patients who die before 72 hours will be assigned the highest intensity score among patients in the study who were alive at 72 hours.

Table 4. Antibiotic spectrum rank, categorized by clinical use and spectrum

Intensity score	Rank	Antibiotics
1	Narrowest	Amoxicillin, ampicillin, first-generation cephalosporins, clindamycin, dalbavancin, daptomycin, dicloxacillin, linezolid, macrolides, metronidazole, nafcillin, oritavancin, oxacillin, penicillin, quinupristin/dalfopristin, rifampin, tedizolid, vancomycin
2	Medium narrow	Amoxicillin/clavulanic acid, ampicillin/sulbactam, 2 nd -generation cephalosporins, 3 rd generation cephalosporins (except ceftazidime), PO fluoroquinolones (ciprofloxacin, moxifloxacin, levofloxacin), tetracyclines, trimethoprim-sulfamethoxazole,
3	Medium	Aminoglycosides (amikacin, gentamicin, tobramycin), ceftaroline, ceftazidime, IV fluoroquinolones (ciprofloxacin, moxifloxacin, levofloxacin), fosfomycin
4	Medium broad	Anti-Pseudomonal penicillin/penicillinase combinations, aztreonam, cefepime, ertapenem
5	Broadest	Anti-Pseudomonal carbapenems, ceftazidime/avibactam, ceftolozane/tazobactam, polymyxins, tigecycline

5.5.4 Outcomes

Final outcomes for each enrolled patient will be gathered retrospectively from the EMR. Patients will be followed for the duration of their hospitalization, or up to 30 days if they remain hospitalized.

Inpatient hospitalization cost data will be obtained from Vizient/University Health Consortium (UHC).

6.0 Monitoring

The site Microbiology Laboratory Director is responsible for monitoring and reviewing discordant results between the rapid and standard test methods for subjects in arm 2. AST discrepancies will be categorized as follows:

- **Very major errors:** susceptible by rapid test method and resistant by standard culture/AST.
- **Major errors:** resistant by rapid test method and susceptible by standard culture/AST
- **Minor errors:** intermediate by one method and susceptible or resistant by the other method

Sites will record all errors.

Discrepancies between the rapid and standard results will be handled as noted in section 5.3.3.

The proportion of discrepancies between testing methods for individual bacteria-drug combinations classified as very major errors, major errors, or minor errors will be calculated. Acceptable performance will be categorical agreement $\geq 90\%$, very major errors $\leq 3\%$ and major errors $\leq 3\%$.²¹.

7.0 Statistical Analysis Plan

7.1 Definitions

- 7.1.1 Escalation: Changing to a broader spectrum antibiotic (higher number category in Tables 5 and 6), addition of one or more antibiotics, or conversion of oral (PO) to intravenous (IV) route.
- 7.1.2 De-escalation: Changing to a narrower spectrum antibiotic (lower number category in Tables 5 and 6) cessation of one or more antibiotics, or changing from an IV to PO route of appropriate drug (i.e. PO ciprofloxacin or levofloxacin).

Table 5. Gram negative agents

Category	Spectrum	Antibiotics
1	Narrowest	Ampicillin, cefazolin
2	Narrow	Amoxicillin/clavulanic acid, ampicillin/sulbactam, 2 nd -generation cephalosporins, 3 rd generation cephalosporins (except ceftazidime), PO fluoroquinolones (ciprofloxacin, moxifloxacin, levofloxacin), metronidazole, tetracyclines, trimethoprim-sulfamethoxazole,
3	Medium	Aminoglycosides (amikacin, gentamicin, tobramycin), ceftaroline, ceftazidime, IV fluoroquinolones (ciprofloxacin, moxifloxacin, levofloxacin), fosfomycin
4	Medium broad	Anti-Pseudomonal penicillin/penicillinase combinations, aztreonam, cefepime, ertapenem
5	Broadest	Anti-Pseudomonal carbapenems, ceftazidime/avibactam, ceftolozane/tazobactam, polymyxins, tigecycline

Table 6. Gram positive agents

Category	Spectrum	Antibiotics
1	Narrow	Ampicillin, cefazolin, clindamycin, erythromycin, nafcillin, oxacillin, trimethoprim-sulfamethoxazole, tetracycline, azithromycin
2	Medium	Dalbavancin, IV vancomycin
3	Broad	Ceftaroline, daptomycin, IV/PO linezolid, oritavancin, quinupristin/dalfopristin, IV/PO tedizolid

7.2 Outcome Measures

7.2.1 Primary Outcome Measures

- Mean time until first modification of antibiotic therapy (in hours) within 72 hours post randomization

7.2.2 Secondary Outcome Measures

- In-hospital mortality within 30 days of randomization
- Length of stay in the hospital after randomization, up to 30 days, for those patients alive at 30 days. Length of stay will be date of discharge minus date of randomization.
- < 72 hours or ≥72 hours in the ICU after randomization.
- Mean time to antibiotic escalation in those who have antibiotic escalation within 72 hours from randomization. This will be further broken down by whether the escalation was in gram positive coverage, gram negative coverage, or both.
- Mean time to antibiotic de-escalation in those who have antibiotic de-escalation within 72 hours from randomization. This will be further broken down by whether the de-escalation was in gram positive coverage, gram negative coverage, or both.
- Hospital-onset *Clostridium difficile* within 30 days, as defined by the National Healthcare Safety Network (NHSN). This will be normalized to patient-days.²²
- Acquisition of new hospital-acquired infections (HAIs) and/or multidrug resistant organisms (MDROs) within 30 days during index hospitalization identified on routine clinical or surveillance samples. This will be normalized to patient-days. Cultures that will be tracked include the following, from any specimen source, unless otherwise indicated:
 - Methicillin-resistant *Staphylococcus aureus*
 - Vancomycin-resistant *Enterococcus*
 - 3rd generation cephalosporin non-susceptible *Enterobacteriaceae*
 - Carbapenem-resistant *Enterobacteriaceae*, as defined by the CDC: resistant to imipenem, meropenem, doripenem, or ertapenem OR documentation that the isolate possesses a carbapenemase²³
 - Multidrug-resistant *Pseudomonas aeruginosa* (resistant to aminoglycosides, cephalosporins, fluoroquinolones, and carbapenems)²⁴
 - Carbapenem-resistant *Acinetobacter*
 - *Candida* species (isolated from blood cultures only)

7.2.3 Exploratory Analyses

- Standardized costs, obtained from the UHC/Vizient database
- The primary endpoint will be analyzed comparing patients who are:
 - in the ICU at baseline versus not in the ICU at baseline
 - neutropenic at baseline versus not neutropenic at baseline
- The primary endpoint will be analyzed excluding antibiotic modifications made within the first hour after randomization, as these changes may be in response to Gram stain result rather than AXDX result
- 30-day readmission to the index hospital
- The proportion of discrepancies (as detailed in 6.0) between rapid testing and standard of care testing among subjects who receive rapid testing (arm 2)
- Antibiotic intensity score (as detailed in 5.5.3) in the first 72 hours post randomization

- Desirability of outcome response and response adjusted for antibiotic risk within 72 hours (DOOR-RADAR) score (see Table 7 below)

Table 7. Desirability of outcome response (DOOR) ranking table.

DOOR Rank	30-day mortality	In ICU at 72h post-randomization	HO-CDI*	New resistant or hospital-acquired organism**
1	Alive	No	No	No
2	Alive	No	Yes	No
3	Alive	No	No	Yes
4	Alive	No	Yes	Yes
5	Alive	Yes	No	No
6	Alive	Yes	Yes	No
7	Alive	Yes	No	Yes
8	Alive	Yes	Yes	Yes
9	Dead	--	--	--

*HO-CDI, Hospital onset *C. difficile* infection

**Newly acquired within 30 days post-randomization, from clinically collected or surveillance cultures

7.3 Sample Size

The study is designed to detect a difference in primary outcome, mean time to first antibiotic modification in the first 72 hours after randomization. We plan to enroll 500 unique participants (250 in each arm) in order to have at least 200 evaluable participants in each arm. Participants who died prior to randomization, were on comfort care at time of randomization, had GNB bacteremia at same or different institution within 7 days prior to randomization, or who were erroneously randomized due to laboratory issues such as spurious Gram stain result, will be considered unevaluable. This will provide 80% power with a 2-sided alpha=0.05 test, to detect a difference in the time to first modification of antibiotic therapy between the two arms of at least 9 hours, with a standard deviation (SD) of 32 hours. With 500 participants, if the SD is larger than 32 hours, there will still be sufficient power to detect a difference between the arms of 10 or more hours (see Table 8).

We assume that differences in the primary outcome will be due to the rapid testing results. We anticipate that most subjects will be receiving empiric broad-spectrum antibiotic therapy at randomization, and a difference between the arms of 9 hours or more in time to antibiotic escalation or de-escalation would be reasonable based on the turn-around-time of the rapid test, and clinically relevant with regards to risks of ineffective therapy or prolonged exposure to broad-spectrum agents. Estimates for the SD for time to first modification of antibiotic therapy are based on data from subjects with GNB bacteremia from the BCID trial¹⁵. We assume that the arm including stewardship in the BCID trial (BCID test plus AS), is a reasonable surrogate for the RAPIDS standard testing arm. This is because the BCID test provided minimal susceptibility results for GNB, and did not impact time to antibiotic modifications for subjects with GNB bacteremia¹⁵. Based on the BCID trial data, in the RAPIDS standard testing arm, we expect 63% will have antibiotic de-escalation with mean time of 31 hours (SD 27), 23% will have antibiotic escalation with mean time of 18 hours (SD 21), and 14% will have no antibiotic change. Incorporating the participants with no change, we expect the overall mean time to change in the standard testing arm will be 33.75 hours (SD 28.8). We anticipate that the rapid testing arm will have similar frequencies of antibiotic de-escalation, escalation, and no change. Based on the turn-around-time of the rapid test, we estimate that mean time to antibiotic de-escalation and escalation will be approximately 12 hours (SD 24). Incorporating subjects with no antibiotic change, we expect the overall mean time to antibiotic modification in the rapid testing arm will be

20.4 hours (SD 30.5). The sample size was selected to allow the study to be well powered if this is an optimistic estimate of the standard deviation.

Table 8: Power to detect a difference in means over a range of SD for a sample size of 250 per arm/500 total (200 per arm inflated 20% for death at randomization, comfort care at randomization, GNB bacteremia at same or different institution within 7 days, laboratory errors) for a 2-sided alpha=0.05 test

Difference in means (hours)	SD = 27 h	SD = 30 h	SD = 32 h	SD = 33 h	SD = 34 h
8	84%	76%	70%	68%	65%
9	91%	85%	80%	78%	75%
10	96%	91%	88%	86%	84%
12	99%	98%	96%	95%	94%

7.4 Analysis

7.4.1 Analysis of primary outcome

We will calculate the mean difference in time to first antibiotic modification within 72 hours post randomization in both treatment arms, together with a 95% confidence interval. Patients who do not have antibiotic modifications will be assigned a time of 72 hours. The primary test will be a t-test (relying on a normal approximation, allowing for unequal variances in the treatment groups) for whether the mean of the primary outcome is different between the arms. We will also carry out a Wilcoxon rank-sum test, to compare the distribution of the time to first antibiotic modification between the arms without the assumption of normality. We will estimate the probability of having an escalation or de-escalation of antibiotics between 0 and 72 hours after randomization using the empirical cumulative distribution function with pointwise 95% confidence intervals.

The primary analysis will be a modified intention to treat analysis (ITT). Participants who died prior to randomization, were on comfort care at randomization as assessed by the study coordinator, had GNB bacteremia at same or different institution within 7 days prior to randomization, or whose blood culture had erroneous Gram stain results in the laboratory will be considered unevaluable. These are all issues which may not be known to the laboratory technologist at the time of randomization and would otherwise be exclusion criteria. Participants randomized to the rapid testing arm will be analyzed as randomized, including cases where the rapid test does not return a result.

7.4.2 Analysis of secondary and exploratory outcomes

We will compare all secondary and exploratory outcomes between the rapid testing and standard testing arms. Where interest lies in mean differences, we will follow the same approach as for the primary outcome. The DOORs will be compared between arms to estimate the probability (with 95% CI) that a randomly selected participant will have a better DOOR outcome if assigned to rapid testing. We will determine the proportion of very major errors and very major or major errors among patients who receive rapid testing.

8.0 Ethics and Regulatory

8.1 Ethical Standards

The investigator will ensure that the study will be conducted in accordance with all applicable national, regional, and local regulations. The study does not involve direct interaction with human subjects, and all outcome data will be obtained as part of routine clinical care.

The purpose of this study is not to test the rapid assay's performance characteristics but rather to evaluate whether the use of this assay along with bacteremia-focused AS program will lead to improvement in the quality of care for patients. Because this study is considered a quality improvement initiative, we will request an exemption from IRB review at all sites, along with a waiver of informed consent.

8.2 Data Confidentiality

The study protocol, documentation, data, and all other information generated by this study will be maintained in a secure manner and will be kept confidential as required by law.

Database access will be limited to study personnel who are issued a unique user identification and password. Data will be entered at each site by study personnel. No information concerning the study or the data will be released to any third party without prior written approval of the sponsor. Study records may be reviewed in order to meet federal or state regulations. Reviewers may include the IRBs, the DCRI or the NIH.

Quality Control and Quality Assurance

The DCRI will provide direct access to the dataset for the purposes of monitoring, auditing and statistical analysis by ARLG staff and their affiliates and inspection by local and regulatory authorities. The local site principal investigator will ensure that study personnel are appropriately trained and applicable documentations are maintained.

The DCRI will implement a quality plan described in the Project Management Plan to ensure protocol training, data quality and data security are being undertaken.

Source Documents and Access to Source Data

Source documents will be stored at the sites. Redacted source documents may be requested by DCRI as needed to ensure data quality. Study monitoring is planned to be performed remotely, but may occur at the local site.

8.3 Data Handling and Record Keeping

Data Capture Methods

This study will use the REDCap data collection tool, which is HIPAA compliant. The site staff who will be entering data will receive training on the system, after which each person will be issued a unique user identification and password.

For security reasons, and in compliance with regulatory guidelines, it is imperative that only the persons who own the user identification and passwords access the system using their own unique access codes. Access codes are nontransferable. Site personnel who

have not undergone training may not use the system and will not be issued user identification and password until appropriate training is completed.

Study Records Retention

Applicable records and documents pertaining to the conduct of this study will be kept for a minimum of 6 years after final reporting or publication. Sites will remove medical record numbers from the electronic subject logs when notified by principal investigator (PI), after database lock.

8.4 Study Discontinuation

This study may be terminated at any time by the PI in consultation with the ARLG and/or NIH.

9.0 Publication Policy

Following completion of the study, the investigator may publish the results of this research in a scientific journal under the oversight of the Publication Committee of the ARLG.

The ARLG Publication Committee comprises representatives of the network cores, thought-leaders, statistics and data management center (SDMC), and is responsible for generation and coordination of the publications that report scientific findings of the network. All public presentations (abstracts, manuscripts, slides and text of oral or other presentations, and text of any transmission through any electronic media) by participating investigators, participating institutions, SDMC, and ARLG that use ARLG data and are intended to represent the ARLG or are supported by the ARLG will be reviewed by the Publication Committee per the Publication Committee charter and must include the following statement "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number UM1AI104681. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health."

The Publication Committee guarantees that the study results are presented by experts in the field that have working knowledge of the study design, implementation, data synthesis/analysis, and interpretation. The committee goals are to ensure that any confidential or proprietary information is protected, and that all appropriate statistical analyses have been included.

The ARLG Publication Committee will adhere to the trials registration policy adopted by the International Committee of Medical Journal Editors (ICMJE) member journals. This policy requires that all applicable clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase I trials), would be exempt from this policy.

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12

months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH-funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to: <http://publicaccess.nih.gov/>

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APPENDIX I – Schedule of Assessments

	Screening	Enrollment/baseline	24 hours	48 hours	72 hours	30 days	Close of study
Time of blood culture	x						
Evaluation of exclusion criteria	x						
Randomization		x					
Demographics		x					
Medical history		x					
Components of Pitt Bacteremia Score ¹⁸		x					
Comfort care		x					
Antibiotic administration		x	x	x	x		
Admission information		x					
Vital signs		x					
Source of bacteremia					X ^[2]		
Results of microbial identification					x		
Results of AST					x		
Evaluation of whether stewardship recommendation made and, if so, whether accepted ^[1]			x	x	x		
Date of discharge						x	
<i>Clostridium difficile</i> testing results						x	
Acquisition of new target HAI or MDRO						x	
Readmission						x	
Death status through 30 days							x
Cost data through 30 days							x

Footnotes:

1. Stewardship recommendations may occur at any time between randomization and 72h post randomization.
2. As assessed by the stewardship team within 72 hours of randomization