Clinical Study Protocol

Biomarker-signature Supported Antibiotic Treatment Decisions in ICU (BAST-ICU)

Version 1.6

Date: 03-Sep-2025

Full Title:	Biomarker-signature Supported Antibiotic Treatment Decisions in ICU (BAST-ICU)
Principal-Investigator:	Makeda Semret, MD FRCP(C)
Study Support/Funding:	Canadian Institutes of Health Research (CIHR)
Study Management Group:	Mi4 Clinical Research Platform (Mi4-CRP)
Central Research Laboratory:	Research Institute of McGill University Health Centre **Glen site** 1001 Decarie Blvd. Bloc E Montreal, QC, H4A 3J1, Canada
Date:	03-Sep-2025

GCP Statement

This clinical study will be conducted in accordance with applicable Health Canada regulations, International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH guidelines on current Good Clinical Practice (GCP), and the Declaration of Helsinki.

Confidentiality Statement

This clinical study protocol contains information which is of a confidential, trade-secret or proprietary nature. The protocol is for the use of Dr. Makeda Semret and her designated representatives participating in the investigational trial. It is not to be disclosed to any other person or party without the prior written approval of Dr. Makeda Semret.

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INVESTIGATOR AGREEMENT

Protocol Title:	Biomarker-signature Supported Antibiotic Treatment Decisions in ICU (BAST-ICU)
Date:	03-Sep--2025

This clinical study will be conducted in accordance with applicable Health Canada regulations, ICH guidelines on current GCP ISO 14155:2011 (E), Clinical investigation of medical devices for human subject - Good Clinical Practice, 2011-02-01 and ISO 14971:2007, and the Declaration of Helsinki.

I confirm that I have read and understand this protocol and I agree to conduct this clinical study in accordance with the design and specific provisions of the protocol, with the exception of a change intended to eliminate an immediate hazard to participants. Any deviation from the study protocol will be documented in the case report form.

I agree to promptly report to the applicable ethics boards any changes in the research activity and all unanticipated problems involving risks to human participants or others. Additionally, I will not make any changes in the research without prior ethics and sponsor approval, except where necessary to ensure the safety of study participants.

Name	Signature	Date (dd-mmm-yyyy)

STUDY CONTACT DETAILS

Name	Role	Responsibility	Email
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Don Vinh	Co-A	Laboratory testing for BV signature, biobanking of specimens and post-hoc analysis for potential additional biomarker signatures Member of the steering committee 2 hours/week	donald.vinh@mcgill.ca
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Alexandre Cherton	PWLE (non-academic)	Review of informed consent form and strategies for participant recruitment; input on additional outcomes of interest; knowledge translation strategies and policy translation 2 hours/month	alexandrecherton@hotmail.com
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Jean Bourbeau	Collaborator	Training of students (MSc and PDF) in clinical trials, core component of HQP development plan; assistance with studentship awards	
Guy Rouleau	Collaborator	ACT/AEC Trial promotion and awareness raising through the network communication program; help in identifying potential additional study sites for future	

ABBREVIATIONS AND DEFINITIONS

Acronym / Abbreviation	Definition
AEs	Adverse Events
AMR	Antimicrobial Resistance
AMU	Antimicrobial Use
AMS	Antimicrobial Stewardship
AWare	Antibiotic Watch and Reserve Classification
BAST- ICU	Biomarker-Supported Antibiotic Stewardship Trial in ICU
BV	MeMed BV® (Diagnostic Assay)
CIHR	Canadian Institute of Health Research
Co-I	Co-investigators
CTCAE	Common Terminology Criteria for Adverse Events
CRP	C-Reactive Protein
DOOR	Desirability of Outcome Ranking
ICU	Intensive Care Unit
IP-10	Interferon Gamma-Induced Protein 10
MGH	Montreal General Hospital
MUHC	McGill University Health Centre
NIH	National Institutes of Health
PCT	Procalcitonin
RADAR	Response Adjusted for Antibiotic Risk
RCT	Randomized Controlled Trial
REB	Research Ethics Board
REDCap	Research Electronic Data Capture
RVH	Royal Victoria Hospital
TRAIL	Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand
WHO	World Health Organization

SUMMARY

Reducing excessive or inappropriate antibiotics is critical for Canada's National Action Plan to contain Antimicrobial Resistance (AMR), which has been declared a global threat by the World Health Organization (WHO). A major limitation of that objective is that diagnostic uncertainty and fear of under-treating patients, particularly in critical care settings, drive a large proportion of antibiotic overuse and misuse in human healthcare. We propose to accelerate improved clinical decision-making for antibiotic therapy in the ICU setting, using a novel biomarker, MeMedBV which exploits a targeted host-protein signature to distinguish between infections that require antibiotics versus those that do not. The BV test is a promising assay to evaluate in an antibiotic *cessation* decision matrix, given its performance characteristics (high negative predictive value for bacterial infections), rapid results (1 hour), and the fact it does not seem to be affected by the presence of colonizing bacteria.

The central question of this study is whether a biomarker-signature supported antibiotic treatment decision matrix can have a beneficial impact on antibiotic use and patient outcomes in an ICU population.

We propose to pragmatically combine the evaluation of the diagnostic test with an antibiotic treatment decision matrix, in a manner that mimics real-life but is as close as possible to a "best-case" scenario. The study will be a Randomized clinical trial to determine the effect of implementing a BV-supported antibiotic treatment decision matrix (efficacy) on antibiotic use *and* on a combined clinical endpoint among study participants (safety). As secondary outcomes, individual endpoints of Antibiotic use (days of treatment, daily defined doses), antibiotic-free days in ICU, use of reserve/restricted antibiotics, adherence to recommendations; incidence of clinically significant (grade IV) adverse events, recurrent infections, infectious complications, length of stay in ICU, death, colonization with multi-drug-resistant organisms will be analyzed.

This clinical trial will establish the efficacy and safety of a new diagnostic assay on a real-world ICU patient population, an important step in the translational pathway to establish this platform for diagnostic testing in the critically ill. By improving antimicrobial stewardship interventions in the ICU, the study will help curtail the emergence of AMR and potentially improve the outcomes of critically ill patients.

PROTOCOL SYNOPSIS

Full Title	Biomarker signature-supported Antibiotic Treatment Decisions in ICU
Short Title	BAST-ICU
Protocol and Version No.	V1.5 02-Apr-2025
Study Duration	Enrollment period: October 1, 2024 – March 31, 2027
	Study period: August 1, 2024 – July 31, 2027
Sponsor-Investigator	RI-MUHC, Dr. Makeda Semret
Number of Centers	MUHC; Royal Victoria Hospital and Montreal General Hospital
Diagnostic Test Description	Diagnostic test: MeMed BV Medical Device Licence Number: 104240 Test result combined with clinical assessment to facilitate decision on antibiotic treatment.
Pilot Study Design	Standard procedures for the Validation of the BV Score in Differentiating Bacterial and Non-Bacterial Infections
Pilot Sample Size	N = 40
Pilot Study Population	Participants who are admitted to Intensive Care Unit (ICU) and: non-infected, confirmed bacterial infection, presumed or confirmed viral pneumonia.
Pilot Objective	To evaluate the feasibility of the study procedures and the performance of the BV Score before the full-scale study begins
Pilot Eligibility Criteria	18 years old, admitted to ICU
Study Design	Pivotal Study, Observer-Blind, Randomized Controlled Clinical Trial
Sample Size	N = 1200
Study Population	Inpatients admitted to ICU and started on antibiotic treatment within the preceding 72 hours (approximately 3 days).
Control	Results of BV test masked
Randomization	Randomly assigned 1:1 ratio to either the control group or the intervention group.
Primary Objective	To evaluate the combined endpoint of efficacy and safety at 28 days (approximately 4 weeks). This will include: 1. Efficacy: Assessed by the use of antibiotics. 2. Safety: Assessed by clinical outcomes. The assessment at 28 days will determine the overall effectiveness and safety of the intervention.
Secondary Objectives	To evaluate antibiotic-free days in ICU, use of reserve/restricted antibiotics, adherence to recommendations; incidence of clinically significant (grade IV) adverse events, recurrent infections, infectious complications, length of stay in ICU, death, colonization with multi-drug-resistant organisms.
Primary Outcome	To evaluate the combined endpoint of efficacy and safety at 28 days (approximately 4 weeks) using Desirability of Outcome

	Ranking (DOOR) and Response Adjusted for Antibiotic Risk (RADAR).
Secondary Outcomes	To evaluate the following parameters; antibiotic-free days in ICU, adverse events, adherence to treatment recommendations, death, and length of stay in ICU.
Exploratory Outcomes	To assess the incidence of colonization or infection with drug-resistant organisms.
Interventional arms	Treatment decision matrix (intervention) plus standard of care compared to standard of care alone (control).
Duration of Intervention	1 Day (BV testing)
Statistical Analysis	Wilcoxon-Mann-Whitney statistic with a 2-sided 95% CI to compare differences between groups, specific to each component and comparison of DOOR distribution between groups. Descriptive analyses for secondary outcomes, and modeling to examine impact of confounding factors. Chi-Square tests will be used for categorical measures and t-tests or Wilcoxon tests for continuous measures.

1. INTRODUCTION, BACKGROUND, AND STUDY RATIONALE

1.1 The need for a trial

Antimicrobial resistance (AMR) poses a major challenge for human health and is in large part fueled by overuse of antibiotics[1,2]. During the COVID-19 pandemic, global use of antibiotics was positively correlated with COVID-19 cases despite bacterial infection rates of less than 10%, resulting in further amplification of antimicrobial use (AMU)[3]. In Canada, overall AMU for human health decreased in community settings between 2017-2021, but consumption of drugs in the "watch" and "reserve" categories of AWaRe (the WHO tiered classification system for antibiotics[4]) has increased by 25%, with concomitant increases in rates of infections caused by priority drug-resistant organisms[5], including non-susceptibility to some of the newest antibiotics approved for use in Canada[6]. A large proportion of any hospital's use of broad-spectrum antibiotics occurs in the intensive care units (ICUs), where the baseline risk of drug-resistant infections is higher[7–9]. Among a worldwide sample of ICU patients in 2017, 54% had a suspected or proven infection but 70% were receiving antibiotics[10]. In Canadian hospitals, ICUs account for at least 49% of all AMU, with > 50% of patients in ICU receiving antibiotics on any given day[8]. Because many patients in ICU have an undifferentiated illness, empirical antibiotic treatment is much broader than would ultimately be required if the infectious etiology was known[11]; even once a pathogen has been identified, appropriate narrowing of the spectrum of antibiotics occurs in only 30-40% of cases[12]. Most recent guidelines clearly differentiate between septic shock and infection or sepsis without shock and introduce the possibility of delaying or withholding antibiotics if shock is absent[18]; yet, aggressive antibiotic prescribing remains the norm, with broadening of coverage when the clinician perceives lack of improvement (even if too early to expect improvement) or worsening (even in the absence of objective evidence of deterioration)[11–13].

Antibiotic prescription decisions in the critically ill are primarily driven by fear of under-treatment, with a general impression that antibiotics "can't hurt", and this is further compounded by diagnostic uncertainty[14,15]. The cumulative frequency of adverse events associated with antibiotic use and patient level risk (*C. difficile* infection, nephrotoxicity associated with certain drugs or combinations, selection for AMR within the patient or within the ICU, breakthrough infections with drug-resistant organisms, drug-drug interactions, bystander effect on the microbiome which may detrimentally affect response to treatment of the underlying disease[16] and other risks) are unknown. Such knowledge would more effectively counterbalance the fear of under treatment than metrics of antibiotic use or societal arguments on the risks of AMR.

Antimicrobial stewardship (AMS) is a systems wide approach to promote appropriate AMU[17]**.** Studies have shown the benefit of different AMS strategies on clinical outcomes, particularly prospective audit-feedback in which a targeted review of the indication and appropriateness of antibiotics on a case-by-case basis (patient-level) lead to specific antibiotic treatment recommendations[18–20]. However, the lack of uniformity in methodologies, context, and selection of outcomes limit the generalizability

of specific interventions and systematic comparisons between studies[21–25]. Recently, a pragmatic cluster-randomized study of an audit-feedback strategy among patients hospitalized with COVID-19 in Alberta demonstrated that a simple intervention, with pre-specified definitions of appropriateness and graded treatment recommendations, designed with minimal bias and prioritizing clinical outcomes rather than only antibiotic use, can lead to reduction in antibiotic exposure and serves as a useful template for further studies aiming to optimize AMU[26].

Optimization of AMU is dependent on accurate decision-making when appropriate diagnostic tests are obtained at the onset of infection. Although microbiologic tests (culture or nucleic acid-based amplification tests) are helpful to detect specific pathogens, they are not sufficient to lead to significant reductions in AMU or changes in clinical outcomes for hospitalized patients[27,28]. Further, positive bacterial results can result from colonization or contamination and are not always indicative of infection, in fact can lead to over-treatment[10,29,30,31-33]. Host response-based diagnostics tests could more accurately discriminate between non-infectious etiologies vs. microbial colonization vs. infectious disease, in an objective and quantifiable format[34]. Procalcitonin (PCT) is the best studied of such markers, with several trials showing that test results folded into antibiotic decision support guidelines curb the use of antibiotics in some infectious syndromes with no apparent harm[35–38] but other studies have demonstrated that a strategy of PCT combined with graded treatment recommendations provided no added value beyond usual care, despite the careful use of quality-improvement principles in the deployment of the intervention[42.]

MeMed BV® (referred to as BV) is a novel diagnostic assay that exploits a targeted, multiplexed host protein signature to distinguish between bacterial and non-bacterial infection, or no infection (e.g colonization) [47]. The selection of the proteins was based on extensive bioinformatic screening followed by computational models to identify the combination with the best discriminatory power. The score performs robustly across different patient subgroups (age, gender, comorbidities, time from onset of symptoms, pathogen species), and is superior to individual or combinations of known biomarkers in differentiating between bacterial vs non-bacterial infections[48–50]. Its diagnostic accuracy has now been validated in >20,000 patients presenting with respiratory infections or fever without a source in emergency and inpatient wards in different countries, with BV score >65 predicting bacterial infection with a sensitivity of 98%, and BV<35 having a negative predictive value of 98.8% [51–53]. The test is now Health Canada-approved, but whether it can be used to support antibiotic treatment decisions in ICU populations is not yet known as critically ill patients were excluded from previous studies.

1.2 Study Rationale

The central question of this study is whether a biomarker-signature -supported antibiotic treatment decision matrix can have a beneficial impact on antibiotic use and patient outcomes in an ICU population.

We propose to combine the evaluation of this test pragmatically with an antibiotic treatment decision matrix in a manner that mimics real-life but is as close as possible to a “best-case” scenario - modeling our study on a successful NIH-funded multicenter trial of PCT-guided use of antibiotics which avoided many of the biases that previously led to overestimating the benefit of PCT[42]. This study will inform researchers, practitioners, and health-care administrators whether and how this recently commercialized diagnostic assay should be incorporated in routine practice, ensuring diagnostic stewardship considerations are integrated within AMS considerations.

1.3 Potential impact

We anticipate this study will demonstrate that an evidence-based implementation strategy of a BV-supported antibiotic treatment decision matrix can lead to a significant reduction of antibiotic use without compromising patient safety in an ICU population. If antibiotic exposure is not reduced in the intervention group (non-superior), we would not recommend implementation of the BV test widely. We would evaluate if the finding can be attributed to an unexpectedly lower antibiotic use in the control arm (compared to usual practice in ICU) and would recommend hospitals choose either the BV test or clinical assessment of prescription quality to reduce antibiotic use in ICU. If our findings demonstrate reduction in antibiotic use but evidence of harm in the intervention group, we will not recommend widespread implementation of the BV test but would carefully analyze our adverse outcome results to determine if select events are driving the overall finding. We would further perform subgroup analyses to assess if these outcomes occurred predominantly in specific groups.

2. MEDICAL DEVICE

2.1 Description

The LIASON® MeMed BV® is an innovative immunodiagnostic solution to differentiate bacterial from viral infection. The assay measures and integrates the serum levels of circulating host-response protein signatures: TRAIL (tumor necrosis factor-related apoptosis inducing-ligand), IP-10 (interferon gamma-induced protein 10) and CRP (C-reactive protein). The result is a qualitative score – in less than an hour – discriminating between the bacterial and viral etiology of an infection.

3. STUDY OBJECTIVES AND DESIGN

3.1 Overall Study Design

BAST-ICU is a Pivotal study, observer-blind, randomized controlled clinical trial designed to evaluate the effect of implementing a BV-supported antibiotic treatment decision matrix on antibiotic use and clinical outcomes, comparing the treatment decision matrix (intervention) plus standard of care to standard of care alone (control) in an ICU population.

This RCT will be used to determine the effect of implementing a BV-supported antibiotic treatment decision matrix on antibiotic use (efficacy) *and* on a combined clinical endpoint among study participants (safety).

3.2 Objectives

3.2.1 Primary Objective(s)

To evaluate the combined endpoint of efficacy and safety at 28 days (approximately 4 weeks). This will include:

- Efficacy: Assessed by the use of antibiotics.
- Safety: Assessed by clinical outcomes.

3.2.2 Secondary Objective(s)

The assessment at 28 days will determine the overall effectiveness and safety of the intervention. To evaluate the following parameters:

- Antibiotic-free Days in ICU: The number of days patients remain in the ICU without antibiotic treatment.
- Use of Reserve/Restricted Antibiotics: The frequency and duration of the use of reserve or restricted antibiotics.
- Adherence to Recommendations: The degree of adherence to the recommended treatment guidelines and protocols.
- Incidence of Clinically Significant (Grade IV) Adverse Events: The occurrence of severe adverse events classified as Grade IV.
- Recurrent Infections: The rate of infection recurrence during the study period.
- Infectious Complications: The incidence of complications arising from infections.
- Length of Stay in ICU: The duration of the patient's stay in the intensive care unit.
- Death: The mortality rate during the study period.
- Colonization with Multi-Drug-Resistant Organisms: The incidence of colonization by multi-drug-resistant organisms.

3.2.3 Exploratory Objectives(s)

- To assess the incidence of colonization or infection with drug-resistant organisms.
- To assess the degree to which age, sex, gender, ethnicity may affect antibiotic prescription, appropriateness, and adherence to treatment recommendation (physician prescription behavior)

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

Study Procedures will occur in the ICU from both MGH and RVH sites, with the RVH site serving as the coordinating center.

4.1 Number of Participants

Our target recruitment goal is 600 participants per group (1200 total participants) at participating MUHC sites.

During the pilot phase of the study, it was documented that the study population in ICU are often incapable of providing informed consent for themselves due to their health condition or due to the fact they are intubated. As of 17-Jun-2025, 40% of otherwise eligible patients could not be approached by the research team as they suddenly lost their capacity to consent for themselves. Without the possibility to approach the Legally Authorized Representative who makes health decisions on behalf of the patient, there is a significant risk the BAST-ICU trial may not meet its enrollment target therefore study objectives.

As this study represents minimal risks to participants, that medical decision-making remains in the treating team's hands, and the objectives of the study would not be met if only patients with capacity to consent are approached, the inclusion criteria are updated to include:

- Participant who have suddenly lost capacity to consent due to their health condition (intensive care) and for whom a Legally Authorized Representative can be approached and provide consent for the patient. The research team will seek the participant's consent (confirmation of consent or withdrawal) as soon as the participant regains capacity to consent.

4.2 Inclusion Criteria

All participants must meet ALL of the following inclusion criteria:

i. Adults (≥18 years) at the time of consent;
ii. Admitted to the Intensive Care Unit (ICU);
iii. Started on antibiotics for any suspected or confirmed infection in the preceding 72 hours (about 3 days);
iv. Treating doctor(s) willing to consider BV test result in antibiotic treatment decision making.
v. Being able to provide informed consent. In case of sudden lost of capacity to consent due to the health condition (requires Intensive care), a Legally Authorized Representative will be approached by the study team.

4.3 Exclusion Criteria

Potential participants who meet ANY of the following criteria will be excluded:

i. Severe immunocompromise/immunosuppression
   a. Congenital immunodeficiency
   b. HIV with CD4 < 200
   c. Active chemotherapy and profound neutropenia (ANC < 100) expected to last > 7 days;

d. solid organ or stem cell transplant within preceding 6 months AND active GVHD
e. Receiving high dose steroids (Pred > 20mg/day for > or = 2 weeks)

ii. Advanced metastatic cancer irrespective of treatment
iii. Palliative intent, death imminent and inevitable within 4 weeks
iv. Antibiotic to be discontinued within 24h (ex. Prophylaxis)
v. Active infection diagnosed and treated with antibiotics within preceding 2 weeks
vi. Previously included during the same hospitalization

4.4 Strategies for Recruitment

Participants will be recruited from two ICU clinic sites (MUHC: MGH and RVH). The ICU treating team will identify potential participants and will inform them about the research project. If they are interested, treating teams will ask the patient for authorization to be approached by the research team. If the potential participants are interested, study coordinator will present the study, and the consent process can begin.

Consent process:
A delegated research staff will read the ICF in its entirety with the participant and explain the study protocol. Time and opportunity to discuss with the treating team and relatives will be given, and all questions will be answered. After reflection, if the participant agrees to participate in the study, the ICF will be signed by the participant and the person obtaining the consent. A copy of the signed ICF will be given to the participant for their own records. The ICF will also be scanned and placed in their hospital medical chart.

E-consent:
1) The consent process for e-consent is the same as for wet-ink signature. REDCap, a secure FDA part 11 compliant platform, is used to collect signatures from the participant and the person obtaining consent and for participant's research file storage during the study. If the participant opts for electronic signature, they will receive a link by email which will lead them to the REB approved ICF in pdf format uploaded to REDCAP (there is no need to submit a e-consent form as the same consent is used).

4.5 Enrollment Procedures

Upon confirmation of eligibility and consent (Appendix I), each participant will be randomly allocated a randomization number and a unique Participant Identification (ID) number which will serve as their identifier throughout the study. Once a randomization number and Participant ID number have been assigned, they cannot be re-assigned to any other participant. This measure ensures that each participant's data remains distinct and accurately attributed. A participant cannot receive more than one ID number, ensuring that each participant's contributions to the study are accounted for singularly and accurately.

All participants will be given a participant identification card identifying them as participants in a research study. The card will contain study site contact information

(including direct telephone numbers) to be used in the event of an emergency. The investigator or qualified designee will provide the participant with a participant identification card immediately after the participant provides documented informed consent.

4.6 Co-enrollment Guidelines

Patients can be enrolled in other clinical trials if there is no conflict with the inclusion or exclusion criteria of the BAST-ICU study, and the other protocol is not in conflict with the means of data collection and publication of this protocol.

4.7 Pregnancy and/or breastfeeding

Pregnant or breastfeeding women can be included in this study if they meet the eligibility criteria. Since the study intervention is providing advice on antibiotic therapy that has already been prescribed by the treating team, there is no contraindication to exclude pregnant or breastfeeding women. As per standard of care, the pregnancy and breastfeeding status of potential participant will be reviewed and documented by the treating team.

5. PILOT Testing of Enrollment Processes and BV Score Validation

Before the implementation of the main study, a pilot phase will be conducted to refine the study enrollment procedures and validate the BV Score. Participants will be screened and enrolled but will not be randomized for the intervention, and their test results will not be used for decision-making.

5.1 Number of Pilot Participants

A total of 40 pilot participants will be included. Target population:

- Non-infected post-op patients (eg. post-cardiac surgery with no clinical, radiological or microbiological evidence of infection) (n= 10)
- Non-infected and non post–op patients (eg. CHF, trauma) (n=10)
- Patients with confirmed bacterial infection (eg. confirmed ventilator-associated pneumonia, bacteremia) (n = 10)
- Patients with presumed or confirmed viral pneumonia (without a confirmed bacterial superinfection) (n = 10)

5.2 Pilot Participant Eligibility Criteria

Study team members will screen eligible participants that are 18 years old belonging to any of the target populations.

5.3 Pilot Procedures

For each of the participants, a blood sample for BV testing will be collected on the day of enrolment and brought to the research laboratory for testing.

5.4 Pilot Data Collection and Analysis

Study team members (PI/Co-I and Pharmacist) will assess clinical and laboratory parameters and establish a consensus-based determination of bacterial/no-bacterial infection independent of BV results. Subsequently, BV results will be plotted on a receiver operating characteristic (ROC) curve to verify the cut-off values established by the manufacturer (BV <35: low likelihood of bacterial infection; 35 ≤65: equivocal; >65: high likelihood of bacterial infection) in this population.

6. WITHDRAWAL OF PARTICIPANTS

6.1 Withdrawal criteria

A participant may be withdrawn from the study if they meet the following criteria:
- It is deemed in the participant's best interest based on the Investigator's clinical judgment.
- The sponsor decides to terminate the study.
- A new health condition arises that necessitates withdrawal.
- The participant or their LAR chooses to withdraw.

In such cases, data collected up to the point of withdrawal will be retained and analyzed, unless the participant explicitly requests that their data be destroyed and excluded from the analysis. If a participant requests withdrawal after data collection and analysis have been completed, the request will be considered on a case-by-case basis.

7. RANDOMIZATION AND BLINDING PROCEDURES

7.1 Randomization

Eligible participants will be randomized in a 1:1 ratio to either the intervention or control groups. Randomization will be stratified by study center to account for site-specific participant characteristics and physician practices. This stratification ensures that any potential confounding factors associated with the different sites are balanced across the treatment groups.

To further ensure the randomization process is robust, block randomization will be utilized within each site. The blocks will have varying sizes, determined by an internet-based randomization tool, to prevent predictability and maintain the integrity of the allocation process. The specific block sizes will remain undisclosed to the research team and participants to ensure concealment and prevent potential bias.

7.2 Blinding during Enrollment and Clinical Assessments

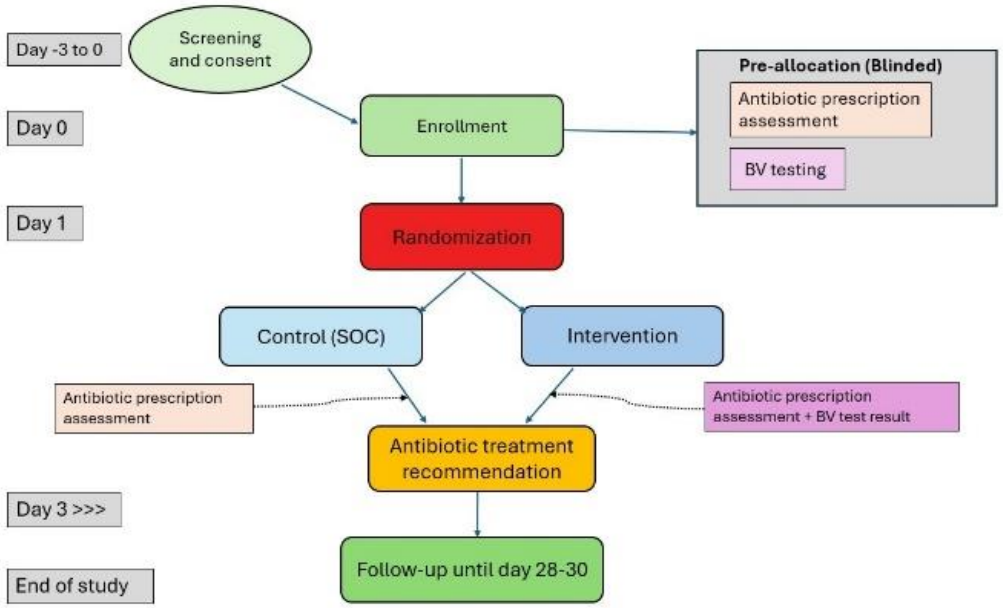
All team members remain blinded during the Enrollment and Clinical Assessment Stages. The study team, (PI/CO-Is that are ID physicians, pharmacist, and laboratory technologist performing BV testing), will remain blinded while conducting the clinical assessment and laboratory testing.

7.3 Unblinding at the Intervention Stage

The results of the MeMed BV test, a diagnostic tool used in the study, will only be disclosed for participants in the experimental group. This means that only those in the experimental arm and their treating teams may have access to this specific information, which may influence their treatment. The study and treating team will be informed of the treatment group assignment when the intervention begins, specifically when the clinical assessment is completed.

8. STUDY PROCEDURES

8.1 Study Flow Chart



8.2 Schedule of Events

Procedures	Screening	Day 0	Day 1[1]	Day 3	Day 7	Day 14	Day 28
Visit Window	-3 days			± 1 day	± 1 day	± 1 day	+ 2 days
Enrollment							
Informed Consent	X						
Eligibility Assessment	X						
Randomization			X				
Interventions							
Prescription assessment		X					
Research Blood Collection for BV Testing		X					
BV test reporting[2]			X				
Treatment recommendation			X				
Assessments							
Demographics	X						
Medical History	X						
Diagnostics/microbiological results	X	X	X	X	X	X	X
Adherence to treatment recommendation				X	X	X	X
Signs/symptoms of infection	X	X	X	X	X	X	X
Antibiotic use	X		X	X	X	X	X
Adverse events, safety outcomes			X	X	X	X	X
Outcome / disposition							X

[1]Day 1 is defined as the day of randomization until 11:59 pm of the same day.

[2]BV test result for intervention group only.

8.3 Screening Period (-3 days to 0 days)

Prior to enrollment, potential participants will be evaluated to determine that they fulfill the entry requirements as set forth in Sections 3.2 and 3.3. Participants are expected to enroll as soon as possible after eligibility is confirmed.

The Investigator will perform a preliminary assessment of the participant's eligibility. Prior to screening, the Investigator or designee will explain the study to the participant/LAR in lay terms. If the participant/LAR consents to participation in the study, during a 1 hour visit the following will be performed:

- Complete the informed consent process with participant/LAR.
- Provide participant/LAR with a copy of signed and dated consent form.
- Assign Participant ID from REDCap.
- Perform the assessment of inclusion/exclusion criteria.
- Obtain the participant's medical history, pre-existing or active conditions.
- Collect the following data on participant:
    - Demographics: Age, sex, gender, ethnicity, ;
    - Admission data: date of admission to the hospital and ICU
    - Medical History
    - Antibiotic prescription; name of drug, dose, start and end date
    - Clinical status; vital signs, temperature (T max day -2 to Day 1), ventilation status; need for vasopressor support (type and dose)
    - Data on infection; site, microbiological results, signs and symptoms of infection
    - Basic laboratories; hematological, biochemical (routine)
    - Imaging; pertinent X-ray and/or CT scans
    - Baseline status of MDRO colonization: swab results for MRSA, VRE, CPE

8.4 Baseline Visit (Day 0)

Study team (Pharmacist and PI/Co-I) perform a clinical assessment of the participant to establish a baseline assessment of the antibiotic prescription (indication and appropriateness of antibiotic) and enter the assessment into the eCRF.

- Collect the following data on participant:
    - Antibiotics prescribed (start and end dates, name, dose)
    - Microbiological results
    - Colonization status for MDRO
    - Signs and symptoms of infection
    - Adverse events related to antibiotics if any
    - Laboratory values for creatinine, liver, hematology
- Research Blood Collection; Collect up to 5 mL (1 teaspoon) of blood for research laboratory BV testing and analysis

8.5 Randomization and Intervention (Day 1)

Study team will enter study ID in randomization system and obtain a study group assignment. Based on the assignment, BV test result is communicated to the blinded team.

The study team (Pharmacist and PI/Co-I) finalize treatment recommendation for both groups (BV value only available for the intervention group) by placing the recommendation in the medical chart.

- Collect the following data on participant:
    - Antibiotics prescribed (start and end dates, name, dose)
    - Microbiological results
    - Colonization status for MDRO
    - Signs and symptoms of infection
    - Adverse events related to antibiotics if any
    - Laboratory values for creatinine, liver, hematology

8.6 Follow-Up (Days 3, 7, & 14 ± 1 day)

- Collect the following data on participant:
    - Review adherence of antibiotic treatment recommendation.
    - Antibiotics prescribed (start and end dates, name, dose)
    - Microbiological results
    - Colonization status for MDRO
    - Signs and symptoms of infection
    - Adverse events related to antibiotics if any
    - Laboratory values for creatinine, liver, hematology

8.7 End of Study Visit EOSV (Day 28 + 2 days)

If participant is discharged prior to Day 28, provide a BAST-ICU discharge packet (28-day antibiotic use diary and report any symptoms). Every effort should be made to complete the Day 28 visit by conducting a remote call.

- Collect the following data on participant:
    - Review adherence of antibiotic treatment recommendation.
    - Antibiotics prescribed (start and end dates, name, dose)
    - Microbiological results
    - Colonization status for MDRO
    - Signs and symptoms of infection
    - Adverse events related to antibiotics if any
    - Laboratory values for creatinine, liver, hematology
    - Vital status: date of death, date of discharge from ICU, date of discharge from hospital
    - Disposition upon discharge from ICU

- Final clinical outcomes and DOOR ranking

8.8 Study Procedures common to both groups: Assessment of antibiotic prescription

Before recruitment of the first patient, the investigators at each site (MGH and RVH) ensure that all legal and regulatory requirements are met. No patients are to be screened or included until the trial starts by the project coordinator.

For all participants, the study team (Pharmacist and PI/Co-I) will review the medical chart and complete a standard prescription quality assessment form. All participants will have baseline data consisting of:

- Sociodemographic information
- Comorbidities, current medications
- Vital signs
- Test results and therapeutics relevant to current illness
- Antibiotics prescribed and received

Based on this assessment, they will arrive at a rating of the antibiotic prescription that includes 3 domains: Appropriateness of diagnostics, necessity of antibiotics, and choice of antibiotics.

8.9 Antibiotic treatment decision procedure specific to the control group

For the control group, the BV test result will remain masked to investigators and treating team members.

The antimicrobial stewardship study team (Pharmacist and PI/Co-I) will issue antibiotic treatment suggestions following the standard decision matrix outlined below. The recommendation will be shared with the treating team.

Necessity of antibiotics	**Recommendation on antibiotic treatment**
Clearly not indicated	STOP (strong recommendation)
Somewhat indicated	Stop (moderate recommendation) or continue --> review choice*
Indicated (Justified)	Continue antibiotic --> review choice *
Cannot be rated	Consider expert (ID) consultation

For all cases where necessity of antibiotics was rated as “somewhat indicated” or “indicated”, the choice of antibiotics will be rated. The final recommendation (to stop, continue or change antibiotic) is shared with the treating team.

Choice of antibiotic	Recommendation on antibiotic treatment
Far too broad	Change drug (strong recommendation)
Somewhat too broad	Consider changing drug (moderate recommendation)
Appropriate choice	Continue same drug
Somewhat too narrow	Change drug
Cannot be rated	Consider expert consultation (ID)

8.10 Antibiotic treatment decision procedure specific to the experimental group

The result of the BV test will be unmasked for the investigators and combined with the prescription assessment form using the decision matrix below. The final recommendation (to stop, continue or change antibiotic treatment) will be shared with the treating team.

Necessity of antibiotics	BV score < 35	BV ≥ 35 ≤ 65	BV > 65
Clearly not indicated	STOP*	STOP*	Expert consultation
Somewhat indicated	STOP*	STOP**	Continue ***
Justified	Expert consultation	Continue ***	Continue ***
Cannot be rated	STOP*	STOP**	Continue ***

*Strong recommendation; **Moderate recommendation; ***review choice of antibiotic

In all cases of “continue antibiotic treatment***”, rate the choice of antibiotic. The final recommendation (to stop, continue or change the antibiotic) will be shared with the treating team.

Choice of antibiotic:	Recommendation
Far too broad	Change drug
Somewhat too broad	Change drug
Appropriate choice	Continue same drug
Somewhat too narrow	Change drug
Cannot be rated	Expert consultation

8.11 Table 1 Data Collection

At enrollment
Demographics: Age, sex, gender, ethnicity
Admission data: Date of admission to hospital, date of admission to ICU
Reason for admission to hospital, reason for admission to ICU
Comorbidities (diabetes, cardiovascular, liver, renal, ...)

Antibiotic prescription: name of drug, dose, start and end date Suspected/confirmed Diagnosis (according to treating team)
Clinical status: Vital signs, temperature (T max day -2 to Day 1), ventilation status; Need for vasopressor support (type and dose)
Data on infection: Site, Microbiological results Signs and symptoms of infection
Basic laboratories: Hematological, biochemical (routine)
Imaging: Pertinent Xray, CT scans
Baseline status of MDRO colonization: swab results for MRSA, VRE, CPE
During study period
Antibiotics prescribed (start and end dates, name, dose)
Antibiotic treatment recommendation, date of recommendation, adherence to recommendation
Microbiological results
Colonization status for MDRO
New signs and symptoms of infection
Adverse events related to antibiotics
Laboratory values for creat, liver, hematology
End of study
Vital status: date of death, date of discharge from ICU, date of discharge from hospital Disposition upon discharge from ICU Final clinical outcomes and DOOR ranking

9. CLINICAL AND LABORATORY EVALUATIONS

9.1 Clinical Evaluations

After informed consent has been obtained, a research member with appropriate training will record the listed information on the eCRF. The initial evaluation will document reasons for ICU admission, current presentation and symptoms, working diagnosis, the reason for antibiotic therapy, and results of laboratory and diagnostic investigations. Patients will be monitored for 28 days or until death, with an End of Study Visit (EOSV) conducted at the conclusion.

Data collected during follow-up visits will include antibiotic prescriptions, symptoms of infections, laboratory and radiological results from the treating team, and adverse events. Antibiotic data will be extracted from the APSS (Lumed) application, while clinical diagnosis and evolution data will be sourced from patient charts and direct observation. Adverse events will be reported as part of the primary safety outcome.

All data will be collected on an electronic case report form (CRF). Original study forms (source documents) will be kept on file at the study site. All data will be kept for at least 7 years after the study's close.

9.2 Schedule of Clinical Evaluations and Data Collection

	Clinical data	Laboratory data	Microbiological data	Radiological data	Antibiotic use data
Baseline	Signs and symptoms of infection; syndrome	MeMedBV; CRP and PCT if collected by treating team	Micro collected since enrollment by treating team	Pertinent radiological findings if available	Start dates for all antibiotics prescribed since enrollment
Day 3	New signs of infection	Standard of care (if available: CBC, SMA-7, LFTs, CRP, PCT)	Results of all Micro cultures collected since enrollment)	findings pertinent for source of infection	Start and end dates for all antibiotics; follow-up adherence to treatment recommendation
Day 7	New signs of infection Or adverse events from antibiotics	Standard of care (if available: CBC, SMA-7, LFTs, CRP, PCT)	Results of all Micro cultures collected since enrollment)		Start and end dates for all antibiotics
Day 14	New signs of infection or adverse events from antibiotics	Standard of care (if available: CBC, SMA-7, LFTs, CRP, PCT)	Results of all Micro cultures collected since enrollment)	Imaging findings pertinent for new infection or source of initial infection	Start and end dates for all antibiotics
Day 28 +/- 2	Outcome Status	Standard of care (if available: CBC, SMA-7, LFTs, CRP, PCT)	Results of all Micro cultures collected since enrollment)	Start dates for all antibiotics prescribed since enrollment	Start and end dates for all antibiotics

Obtain blood sample; freshly collected sample should be processed (clotting, centrifugation and serum separation) **within two hours for testing (see lab manual)**.

9.3 Specimen Collection and Laboratory Evaluations

Personnel who collect specimens from participants must use personal protective equipment (PPE) in accordance with their institution guidelines. Collection kits will be provided to participating sites along with a laboratory manual describing the collection of quality samples as well as instructions for specimen processing, safe storage requirements and packaging to the research lab for analysis.

Aside from the initial BV testing, the study team will not request any additional blood work but will collect data on laboratory, microbiological and pertinent radiological findings requested by the treating team.

The initial blood tests requested for BV testing will be collected from all participants on enrollment day. Testing will be performed according to the manufacturer's instructions in the research space of the MI4 CRP (see lab manual). The personnel performing the measurements will be blinded to clinical information and results of other investigations. Within 24 hours of testing, BV results will be provided to the study team only for the intervention group; results of the control group will remain hidden. All lab materials used for this testing will be supplied by research funds.

9.3.1 Research Blood Collection Serum Specimen

Blood will be collected in 1 x 6mL yellow top SST vacutainer on Enrollment. Study personnel will collect equal amounts of serum into pre-labelled 2 mL cryovials. Research blood samples should be processed within two hours for testing. Detailed instructions for the collection, storage and shipment of the serum samples are provided in the Laboratory Manual.

10. RISK MANAGEMENT

10.1 Risk Management

We have implemented several procedures to minimize potential risks to participants. These include:

- Specific study inclusion and exclusion criteria to minimize risks for specific high-risk populations
- Participants will have one additional blood sample (about 3-5mL) to be combined with usual bloodwork, on the day of enrollment. The risk of blood sampling includes bleeding, bruising and pain at the drawing site. These risks will be reduced by trying, as much as possible, to collect blood when other standard of care blood tests are being collected.
- The study ensures that standard of care is provided to all participants, with the clinician making final treatment decisions. While the study team's recommendations on antibiotic therapy may inform the treating team, they are not obligatory. The study team mitigates risks associated with potentially insensitive or non-specific biomarker assessments by prioritizing clinical judgment and suggesting expert consultations in cases of significant discrepancies.
- Confidentiality risks are minimized by coding participant information and securely storing it in compliance with MUHC REB policies.

11. EVALUATION, RECORDING, AND REPORTING OF ADVERSE EVENTS

In this RCT, the primary safety outcome will encompass all significant potential adverse events related to the cessation or withholding of antibiotics. These adverse outcomes will be integrated into the primary safety outcome measure and will not be reported separately as Adverse Events (AEs) or Serious Adverse Events (SAEs) elsewhere in the study.

Despite our comprehensive approach, unforeseen adverse events may still occur. Therefore, ongoing monitoring of AEs is essential to ensure participant safety and maintain data integrity.

11.1 Definitions

11.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a participant or clinical investigation participant, administered a study medication/intervention (e.g., recommendation for antibiotic cessation), which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) study medication/intervention, whether related to the medicinal (investigational) study medication/intervention.

11.1.2 Serious Adverse Events (SAEs)

An SAE is any untoward medical occurrence that:

- Results in death;
- Is life-threatening (i.e., event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- Requires inpatient hospitalization or prolongation of existing hospitalization (i.e., all medical events leading to hospitalizations will be recorded and reported as Serious Adverse Events, except for: hospitalization planned before inclusion into the study or out-patient treatment with no hospitalization). Note that an elective hospital admission will not be considered as a serious adverse event;
- Results in persistent or significant disability/incapacity (i.e., persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions);
- Is a congenital anomaly/birth defect

11.2 Event Reporting

We will capture all major potential adverse events related to antibiotic cessation/withholding in the combined primary safety outcome. The adverse outcomes

comprising the primary safety outcome will be included in outcome reporting and not reported a second time as Adverse Events (AE) or Serious Adverse Events (SAE).

However, as with any procedure or intervention, there may be adverse events that are currently unknown. Monitoring of AE is critical to the participants safety and data integrity. An AE will be defined as any untoward medical occurrence (unfavorable and unintended sign, symptoms or disease) in a participant temporally associated with the study intervention (eg. recommendation for cessation of antibiotics) but not necessarily related to participation in the study. A SAE will be defined as an untoward medical occurrence that results in death, is life-threatening or requires prolongation of ICU stay, results in disability or incapacitation.

AE and SAE will be recorded when reported by the treating team or study team nurse or when documented in the medical record. If a reportable SAE, the site PI will be review the pertinent records (including medical notes, laboratory and radiographic data). The information, along with the site PI's impression of the diagnosis, will be recorded in the eCRF. The site PI will assess causality between the event and the intervention using best clinical judgement.

11.3 Criteria for Adverse Events related to antibiotics

Adverse event[1]	Definition
Non-*C. difficile* associated diarrhea	> 3 loose stools per day associated with Antibiotic use, in absence of laxative or pre-existing enteritis (C. diff excluded from this category)
***C. Difficile* infection**	Clinical signs and symptoms consistent with CDI and PCR test positive (and no laxatives)
Nausea/vomiting	Nausea and vomiting associated with antibiotic administration, in the absence of alternative explanation
Hematologic	Anemia: hemoglobin <10g/dL Leucopenia: WBC < 4500 cells/uL Thrombocytopenia: platelets <150x10^3/uL with levels below patient's baseline and in absence of bleeding or myelosuppressive therapy
Hepatobiliary	Cholestasis (bili > 51umol/L) or transaminitis (ALT, AST > 3x baseline) in the absence of existing hepatobiliary disease or recent biliary instrumentation
Renal	Increase in serum creatinine > 1.5x patient's baseline in the absence of precipitating factors such as intravenous contrast or other nephrotoxic agents, *and* not related to sepsis
Neurologic	Altered mental status, peripheral neuropathy, seizures in the absence of pre-existing neurological conditions or substance-related toxic events *and* unrelated to infectious syndrome
Dermatologic	Rash, including hives, non-hives rashes, red man syndrome – temporally associated with antibiotics; resolution with antibiotic cessation

Cardiac	QTc>440 ms in males or >460ms in females in absence of pre-existing arrhythmias, based on >2 ECGs
Anaphylaxis	Acute onset respiratory compromise, hypotension, or end organ dysfunction within minutes after antibiotic administration, without alternative explanations
Myositis	Increase in creatine kinase level >5 x patient's baseline, in absence of statin use or pre-existing myopathy

[1]Adapted from Tamma et al, JAMA 2017

11.3.1 AE Descriptions and Reporting

AEs and SAEs will be documented when reported or noted in the participant's medical records by the treating team.

11.3.2 Intensity

The severity of each AE, including laboratory and testing abnormalities and results, will be graded according to Common Terminology Criteria for Adverse Events (CTCAE).

11.4 Reporting and Evaluation of SAEs

11.4.1 SAEs

The Site PI will review relevant records provided by the research team such as medical notes, laboratory results, and radiographic data. This information, along with the Site PI's assessment and diagnosis, will be recorded in the electronic Case Report Form (eCRF).

12. STATISTICAL CONSIDERATIONS

12.1 Rationale

Instead of evaluating safety and efficacy separately, we will apply an approach that is increasingly used in trials comparing therapeutic interventions for selected infectious diseases to provide a global evaluation of the benefits and risks of the intervention: Desirability of Outcome Ranking (DOOR), which can further be combined with antibiotic use outcomes, Response Adjusted for Duration of Antibiotic Risk (RADAR) [55–59]. In this approach, trial participants are ranked with respect to the desirability of their overall outcome, and during study analysis, the distribution of DOORs are compared between study arms. These are further analyzed with antibiotic use outcomes under the principle that less antibiotic use is better, but not at the expense of clinical outcomes. All participants will have a DOOR assigned based on a review of the individual patient during the course of the study, where the ranking will be constructed from a defined number of mutually exclusive hierarchical levels ranging from least desirable (death) to most desirable (e.g., cure, no adverse events). Using such a combined endpoint will

enable us to determine if BV-supported antibiotic treatment recommendation will yield better outcomes than usual care alone.

12.2 Definitions for clinical outcomes and ranking of outcome categories (DOOR)

Points are assigned for overall outcomes, with pre-specified definitions for treatment failure, complications and adverse events related to antibiotic use. All clinical outcomes will be reviewed by a 3-member clinical review panel, and all AE will be reviewed by a different 3-member panel (the Data Safety and Monitoring Committee). The panels will consist of experienced ID, Pharmacy and ICU experts (to be named) blinded to intervention and patient identity.

Clinical outcome	Definition
Treatment failure	Signs and symptoms of initial event (infectious illness) within 72h of end of antibiotic treatment
Infection persistence	Continued presence of signs and symptoms and of original causative pathogen at end of antibiotic treatment
Infection recurrence	New infection due to at least one of the original causative agents found at baseline (initial event)
Infectious complication	Signs and symptoms requiring surgical or other procedure in organ involved in initial event (eg. empyema post pneumonia) occurring >72h after end of antibiotic treatment

We will determine how many DOOR events (AE, infection recurrence etc.) occurred for each participant, then assign a mutually exclusive rank.

RANK	Criteria
1	Survival, clinical benefit of treatment, with NONE of treatment failure, persistence, recurrence, complication AND NONE of Grade IV adverse event (AE)
2	Survival, clinical benefit of treatment, with NONE of treatment failure, persistence, recurrence, complication, AND at least one of AE
3	Survival, at least one of treatment failure, persistence, recurrence, complication, AND NONE of AE
4	Survival, at least one of treatment failure, persistence, recurrence, complication AND at least one AE
5	Death

12.3 RADAR Score

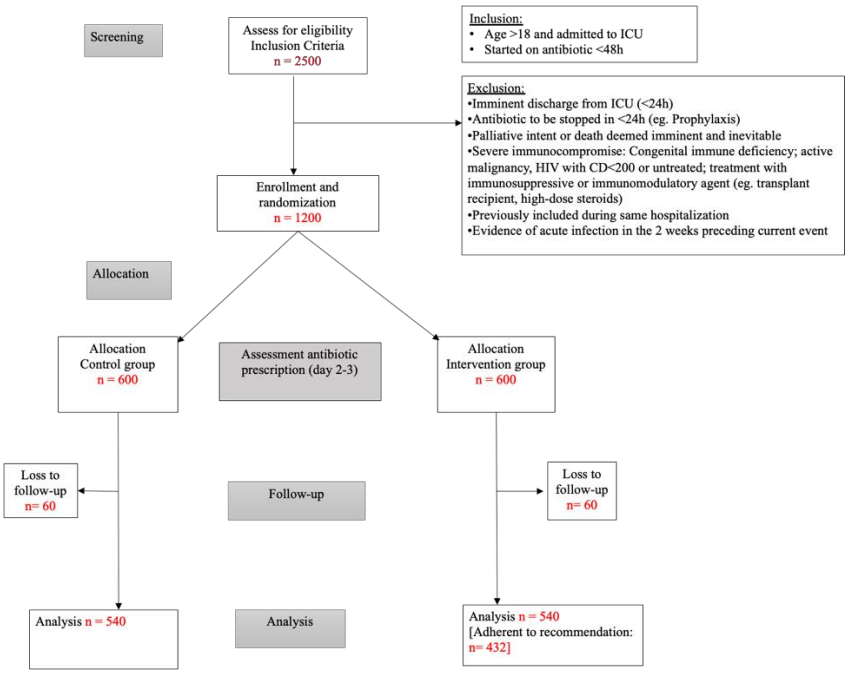
The antibiotic use will be defined as number of days with antibiotic treatment (DOT, aggregate sum of days for which any amount of a specific antibiotic is given, eg. if 2 different antibiotics for 5 days, DOT = 10 / days in ICU.

12.4 Sample Size and Justification

DOOR-RADAR requires fewer patients than conventional non-inferiority analysis but since a major objective of this study is to enable a detailed analysis of adverse events related to antibiotic use, we have calculated a sample size that would enable separate analysis of clinical outcomes and antibiotic use (Sample size calculations detailed in Appendix I).

Testing hypothesis 1 (H1): We desire at least 80% power to test our primary hypothesis that BV-supported antibiotic treatment decision can lead to reducing antibiotic exposure using a one-sided significance of 0.05. Demonstrating H1 would require a sample size of 352 participants per group for a mean difference in antibiotic days of 1.5 (SD 8 days). Based on studies evaluating AMS interventions in the ICU, we estimate a 1.5 days difference between the 2 groups to be significant[14,18,36]. Further, it would be equivalent to a 20% decrease in total antibiotic days, a reasonable assumption considering antibiotics are “clearly not indicated” or “only somewhat (dubious) indicated” in about 30% of cases based on prior retrospective audits of prescription quality.
Testing hypothesis 2 (H2): BV-supported antibiotic treatment algorithm does not lead to worse clinical outcomes. Studies using combined endpoints of adverse outcomes have estimated between 10-25% rates of combined adverse outcomes (death, septic shock, mechanical ventilation, renal failure)[38,42,43]. A meta-analysis of PCT-guided antibiotic treatment in patients with infection and sepsis in ICU reported all-cause mortality rates of 23% at 30-days[35]. Studies evaluating adverse events specifically related to antibiotic use in hospitalized patients suggest rates of 12% within 30 days. We expect a combined adverse outcome rate of 18-20% and will set a non-inferiority margin at 4%. A sample size of 529 patients per group (1060 in total) will therefore provide 80% power to demonstrate H2 (a robust safety evaluation), and yet be exceptionally well powered for efficacy (with preserved power under a wide range of assumptions).

12.5 Expected Study Recruitment Flow Chart



Based on data for MUHC (2019-2020):
Admissions per year: n = 2800 (n = 8400 for 3 years)
Discharge <48h: 1050/y, n = 3150
Admissions > 48h: 1750/year; n = 5250 for 3 years
Inclusion: 60% of all admissions are started on antibiotics, n = 3150
Potential exclusions: 13% of admitted patients are severely immunocompromised, so n= 410
Enrollment: expect 50% of approached patient to consent; n = (3150-410)*50% = 1370
Maximal sample size required: 1060

12.6 Estimations of Adverse Outcomes in ICU

- Combined severe (Grade IV) adverse events secondary to antibiotics (excluding renal): 5-10%
- Overall Acute kidney injury in ICU (all cause): 26-67%
- Drug induced liver injury: rare (16-19/100,000)
- All cause ICU Mortality: 3-28% (MUHC ICU mortality: 13%; median length of stay in (IQR): 3.2 days (1.5, 7.6))

12.7 Primary Analysis

The primary analysis will be an intent-to-treat (ITT) and will include 2 interim analyses at 1/3 and 2/3 enrolment. The final analysis will use Lan-DeMets stopping rules[62]. We will also perform per-protocol analyses where the treatment recommendations were followed.

12.8 Frequency of Analyses

Our approach for interim monitoring is to test H1 first (null H1: no difference between intervention and control in terms of antibiotic exposure). Should the null H1 be rejected at the first interim, then the second hypothesis (H2: no significant difference in combined endpoint of adverse events between the 2 groups) will be assessed and decision to continue will be based on H2. If H1 is not rejected at the interim, then H2 will not be assessed until the final analysis. We estimate that H1 testing may cross the pre-specified boundary at one of the interim analyses, but H2 testing may only meet non-inferiority once full sample size is enrolled.

12.9 Final Analysis

We will compare the difference in antibiotic use between groups by a one-sided test comparing the mean duration of antibiotic use.We will also compare the DOOR distribution between groups, and the DOOR probability of having a more desirable outcome in intervention group versus control. A DOOR probability of 50% will indicate no statistical difference between groups. We will additionally calculate the probabilities specific to each component (AE, infectious complications, etc), using methods described in prior studies[57]. We will further perform descriptive analyses for secondary outcomes, and modeling to examine whether confounding factors (Age, qSOFA or Charlson comorbidity scores) impacted estimates of intervention effect and safety. Randomization quality will also be evaluated for potential differences between arms for baseline variables. Chi-Square tests will be used for categorical measures and t-tests or Wilcoxon tests for continuous measures.

We anticipate that the percentage of missing data will be negligible as the outcomes of interest are part of routine data collection in the ICU setting and the observation period of interest is while the patient is in the hospital. In the event some data is lost (eg. Medical chart is misplaced or lost, electronic medical data inadvertently deleted), we will not replace those lost to follow-up and the data from those participants will be included in the Intention-to-Treat analysis. We will also conduct a sensitivity analysis of those lost to follow-up to ensure there is no systematic bias.

12.10 Sub-Group Analyses:

Studies have shown significant gender differences in antibiotic use with women being 27% more likely to receive antibiotics in their lifetime than men[63]. This has at least in part been attributed to health-seeking behavior, and gender disparities tend to diminish with older age and comorbidities[64]. More recently, studies conducted in the US have shown significant disparities in rates of inappropriate antibiotic prescriptions related to race and ethnicity, with blacks and Hispanics more at risk of receiving inappropriate prescriptions than whites[65]. We will specifically conduct subgroup analyses to determine if sex, gender, ethnicity impact not only antibiotic prescription quality and clinical outcomes, but also the treating team's adherence to BV-guided treatment recommendations. Our study sites service a large proportion of the First Nations, Inuit and Metis populations of Quebec, with medivac transfers for procedures and hospitalizations.

13. DATA MANAGEMENT

All participant data will be entered by study staff into the secure web-based REDCap data entry system and uploaded to the study database server via secure hospital server. Each study team member will have specific access and rights defined by their study role. The system will fulfil standards of good epidemiological practice, and security

and data protection standards. Data will be entered accurately and comprehensively by the responsible site staff.

Any identifying data will be maintained separately from clinical, laboratory and follow-up data for added security and confidentiality.

13.1 Data Monitoring and Auditing

Data monitoring will be performed by data monitoring study team member based at the central (Glen) site, for completeness and plausibility, logic checks for accuracy, and will send queries to the study nurses in cases of ambiguity or errors. Data documentation will be performed continuously. Data verification for primary outcomes will be a priority.

The study coordinator may perform on-site monitoring of documentation by comparing data entry with source documents (eg. participant records) for completeness and chronology, logic checks for accuracy and trend analysis to identify problem areas. The site PIs will cooperate in resolving any queries or findings made during the monitoring process.

The Data safety and monitoring board (DSMB) will provide independent monitoring of interim results and make recommendations for continuation, modification or termination of the study. The DMSB will meet at two planned interim analyses for safety, at completion, and at any other needed interval.

13.2 Data Safety and Monitoring Committee (DSMC)

This committee will be composed of at least 3 members, with at least one expert for each of infectious diseases, ICU, and pharmacy. The committee will be independent from the steering committee, and will initially define all the event triggers that would prompt unscheduled review, as well as review of interim and cumulative data for evidence of efficacy and safety. They will review data quality and completeness, factors that might compromise data confidentiality or affect outcomes. They will conclude each review with recommendation to the steering committee whether the study should continue without change, be modified, or terminated.

13.3 Record Keeping

13.3.1 Source Documents

Source documents are where data are first recorded, and from which participants' eCRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarized into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. eCRF entries will be considered source data if the eCRF is the site of the original recording (e.g. there is no other written or electronic record of data).

The Investigator must maintain adequate and accurate source documents upon which CRFs for each participant are based. They are to be separate and distinct from CRFs except for cases in which the Sponsor has pre-determined that direct data entry into specified pages of the participant's CRF is appropriate. These records should include detailed notes on:

- Oral and written communication with participant regarding the study treatment (risks/benefits)
- Participation in trial and signed and dated informed consent forms
- Inclusion and exclusion criteria details
- Visit dates
- Adverse events
- Results of relevant examinations
- Laboratory printouts
- Participant's exposure to any concomitant therapy (start/stop dates, dosing details)
- Reason for premature discontinuation (if applicable)
- Enrollment number
- Methods of contraception and fertility status (if applicable)
- Compliance/non-compliance protocol deviation information

13.3.2 Record Retention

The Investigator will maintain all study records according to the ICH-GCP and applicable regulatory requirement(s). Records will be retained for 7 years, in accordance with applicable regulatory requirement(s). If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility and the Sponsor notified. The Investigator should ensure that no destruction of medical records occurs without the written approval of the Sponsor.

14. GENERAL TRIAL CONDUCT CONSIDERATIONS

14.1 Trial Oversight

A trial steering committee will be composed of at least 5 of the study co-applicants including biostastician and the NPA. The committee will meet once before the study begins, and at least twice per year during the study. This committee will strive to ensure scientific integrity, progress towards timelines, adherence to protocol and analysis plan. They will consider all recommendations from the Independent Data Safety and Monitoring Committee (DSMC), advise on major decisions such as continuing or terminating the trial and timely dissemination of trial results.

14.2 Trial Coordination

The study will be coordinated by the Clinical Research Platform of the McGill Interdisciplinary Initiative in Infection and Immunity (CRP-MI4) and the ICU infections

platform both based at the McGill University Health Centre, which will provide support in the form of highly trained personnel, equipment, lab infrastructure and resources. Initial training for laboratory testing will be provided by the company Diasorin, who will have no other role in this trial. Adverse events that comprise the primary safety outcome will be included in the outcome reporting, and not reported a second time as AEs related to the intervention. The site PIs will be responsible to review pertinent records, assess causality between adverse events and intervention using their best clinical judgement, and notify the trial steering committee of any AE that is serious, unexpected and possibly related to the study intervention within 48 hours of recognition.

14.3 Participating MUHC Sites

The Montreal General Hospital (MGH) provides emergency services (including advanced trauma care), medical and surgical services (general, orthopedic, thoracic, plastics, neurosurgery) and 24-bed capacity ICU. The Royal Victoria Hospital (RVH provides emergency, medical, hematology-oncology, transplant (solid and stem-cell), and surgical services (general, urology, hepato-biliary, and cardiac), and a 32-bed capacity ICU. We expect to recruit the required sample size based on the volume of admissions to both ICUs. Both sites benefit from an established AMS program. Total days of antimicrobial treatment (DOT) in the ICUs of the MUHC was 1067/1000 patient-days (every ICU patient receives on average 1.1 antimicrobials/day spent in ICU). The study sites therefore have sufficient volume and diversity of eligible participants, infrastructure, and expertise to effectively implement the study procedures.

14.4 Protocol Deviations

Protocol deviations and incidents of non-compliance which do not involve risk to participants must be reported as “NC”. Examples of these include:

- Deviating from the protocol without risk to the participant
- Obtaining consent using an outdated form with no other substantive difference other than date

The study site coordinator will be responsible for notifying the DSMB regarding event reporting within 48h of their knowledge of AE that are categorized as serious, unexpected and related.

Other reportable events will be recorded on the eCRF, entered into the database and reported in the DSMB in routine reports.

14.5 Intervention Adherence

To improve intervention adherence, the pilot phase, start-up and education efforts will be focused on key logistic steps required to accomplish the intervention. We will conduct in-service training to raise awareness about the procedures leading to the BV-guided antibiotic treatment recommendations among ICU physicians, with the main message being to “pay attention, but the final decision is entirely yours”. We will further regularly monitor time to reporting BV results/issuing recommendation: time to draw

blood, lab receipt, assay time, assay completion to reporting; time to clinical assessment; time to receipt of treatment recommendation (delegated study team member will document information receipt). If clinicians choose to continue antibiotics even if the recommendation was to stop antibiotics, a study team member (Pharmacist or PI/Co-I) will gently query the clinician and record reasons for non-adherence (no other questions will be asked of the clinician). All cases of non-adherence to treatment recommendations will be reviewed by the investigators.

15. STUDY ETHICAL CONSIDERATIONS

15.1 Informed Consent

All participants or, when appropriate or the participant's legally authorized representative (LAR) will be given detailed information about the study. Informed Consent Form (ICF) describing in detail the study intervention(s), study procedures, anticipated benefits, and potential risks will be given to each participant. Participants/LAR must voluntarily provide their informed consent to participate in the study by reading and completing an ICF prior to any research procedures being perform. The informed consent will be signed and dated by the participant/LAR and the person who conducted the informed consent discussion. Each participant should have sufficient opportunity to discuss the study and consider the information in the consent process prior to agreeing to participate. Participants may withdraw consent at any time during the trial. The original signed informed consent form will be retained in the participant's study files and a copy will be provided to the participant.

15.2 Institutional Review Board, Ethics Committee, or Research Ethics Board

The McGill University Health Centre Research Ethics Board (REB) will review all appropriate study documentation to safeguard the rights, safety, and well-being of the participants. The study will be conducted only at sites where ethics approval has been obtained. A copy of the protocol (including protocol amendments), all versions of informed consent forms, and other information to be completed by participants and any proposed advertising/recruitment materials must be reviewed and approved by the REB prior to implementation of the trial. The PI will be responsible for obtaining REB approval of the annual Continuing Review throughout the duration of the study and will notify the REB of serious adverse events as applicable within seven days of the recorded event. The PI will seek prior ethics approval for any protocol deviations except when the change is intended to eliminate an immediate hazard to participants. In this case, the protocol deviation will be promptly reported.

15.3 Confidentiality

The trial will conform to the International Conference for Harmonization and Good Clinical Practice (ICH-GCP) regulations and guidelines, the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans 2 (2022), and the current revision of the Declaration of Helsinki. All the information collected during the trial will remain strictly confidential to the extent required and provided by law. For risk mitigation

of disclosing personal information from study participants, we will use double coding of participants' ID and remove identifying information like name, address, and date of birth. Participants will be identified only by means of a participant ID specific to each participant. All records will be kept in a secure locked location, accessible only by special computer login and password that are provided only to authorize study personnel. Participant confidentiality and privacy will be strictly held in trust by the participating investigators, their staff, and the sponsor. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data generated, will be released to any unauthorized third party without prior written approval of the PI.

Sensitive information including any data containing protected health information (PHI) will be stored on a separate secured database under the responsibility of the site PI. The study participant’s contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for 7 years.

16. DISCLOSURE AND PUBLICATION POLICY

Study results will be published in a PubMed referenced journal, regardless of findings (positive or negative trial), without the use of professional writers. Collaborators with satisfactory contributions to the study will be invited to participate on the writing of eventual manuscripts. Authorship policy will follow ICMJE criteria. Paid research personnel (eg. study nurses) and other contributors (eg. members of treating teams who provided medical care of study participants) who do not meet criteria for authorship, will be acknowledged but not listed as authors.

The protocol outline will be posted on ClinicalTrials.Gov, and we will plan to publish a trial methodology and the statistical plan. We will provide access to data as soon as the study is completed, and the main manuscripts are published. Our final dataset will include de-identified demographic and clinical information, outcome data and BV values. Protected health information or patient identifiers will not be part of any dataset. We will employ a Data Use and Publications Request form to review all requests; only after these have been approved by the steering committee will we generate datasets to share with other researchers in response to specific needs.

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18. Appendix I: Sample Size Calculations

For all calculations below – Type I error = 0.05, Power = 0.8, One-sided test

Sample size required for Hypothesis 1 that BV-supported antibiotic treatment recommendation leads to reducing antibiotic exposure.

Expected difference in mean # of days on antibiotics	Standard deviation of Days of antibiotics in ICU	Sample size required per group
1.5 days	10 days	550
1.5 days	8 days	352
1.5 days	6 days	198
1.5 days	4 days	89

Sample size required for Hypothesis 2 that BV-guided antibiotic treatment recommendation does not lead to worse clinical outcomes.

Risk of adverse events in BV group	Risk of adverse events in control group	Non-inferiority margin	Sample size required Per group
32%	30%	4%	6612
30%	30%	4%	1624
28%	30%	4%	708
22%	20%	4%	5128
20%	20%	4%	1238
18%	20%	4%	529
17%	15%	4%	4154
15%	15%	4%	986
13%	15%	4%	414