

Pre-Analytical in Clinical Bacteriology: Specimen Quality, Workflow and Error Prevention

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Learning and Terminal Objectives

At the End of This Session, Participants Will Be Able To:

- Define the pre-analytical phase and explain its critical role in clinical bacteriology
- Identify common pre-analytical errors and evaluate their impact on diagnostic accuracy and patient outcomes
- Differentiate biological influencing factors vs technical interferences and interpret their effects on laboratory results
- Assess specimen quality and determine suitability for microbiological testing
- Apply best practices for specimen collection, transport, and handling to preserve organism viability
- Recognize how pre-analytical factors influence Gram stain, culture, and antimicrobial susceptibility testing (AST)
- Utilize quality indicators (QIs) to monitor and improve laboratory performance
- Integrate pre-analytical practices into diagnostic stewardship and antimicrobial resistance (AMR) control

Outline

Introduction & Importance

Clinical–Laboratory Workflow

Pre-Analytical Errors

Specimen Quality

Influencing vs Interference vs Contamination

Transport & Storage

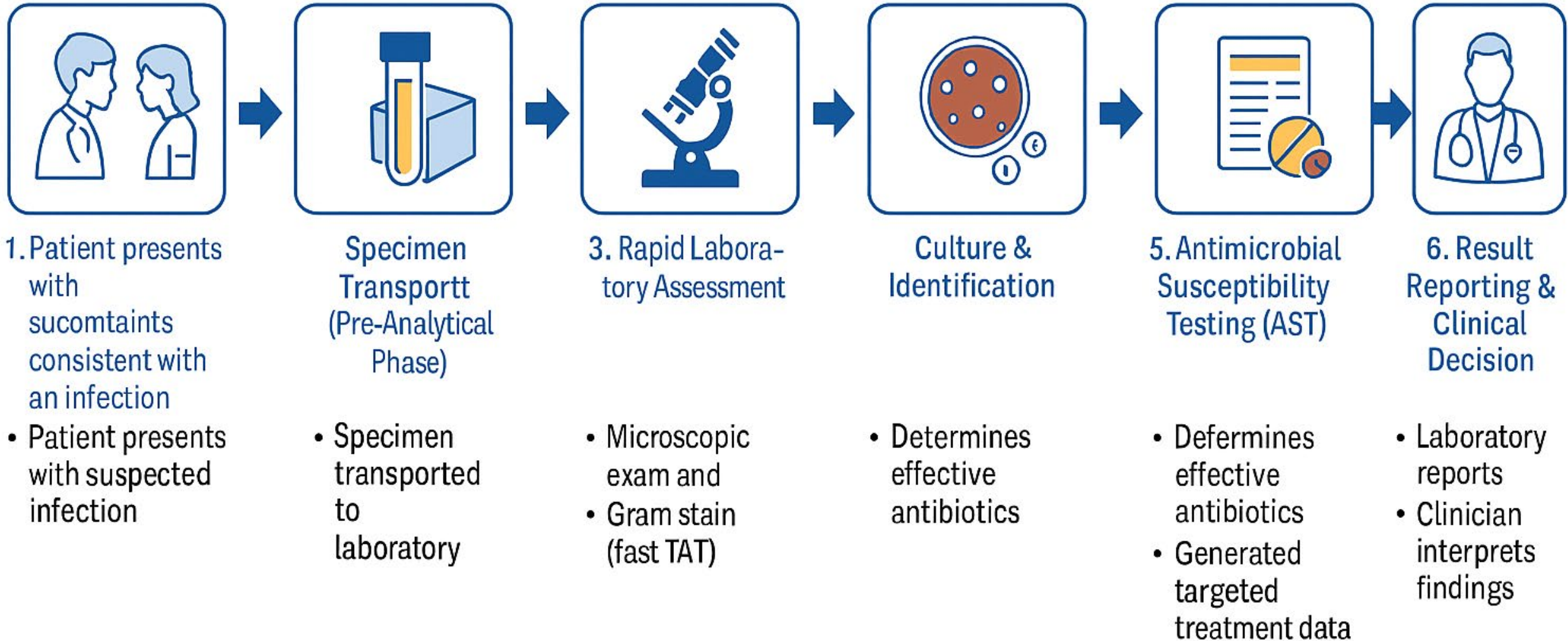
Workflow Impact (Gram stain → AST)

Quality Indicators

AMR & Stewardship

Patient Journey in Clinical Bacteriology

From Symptoms to Targeted Treatment



Why Pre-Analytical Quality Matters in Clinical Bacteriology

- **Critical Pre-Analytical Phase:** Pre-analytical steps from ordering, patient preparation, sample collection, transportation, preparation for analysis, to storage are the most error-prone and impact test accuracy significantly.
- The pre-analytical phase is the most error-prone, accounting for up to 70% of laboratory errors. (PMID: 16729864)
- **Consequences of Poor Quality:** Errors during pre-analytical phase cause false negatives, misidentification, and unnecessary antimicrobial use. (PMID: 21517699; PMID: 19952034)
- **Patient Safety and Stewardship:** Pre-analytical quality affects patient safety, diagnosis timeliness, healthcare costs, and antimicrobial resistance.
- **Importance in Resource-Limited Settings:** Ensuring pre-analytical excellence is vital where test repetition or sample recollection is difficult or costly.



From Patient to Result: Where Errors Begin



Pre-Analytical Risk Points

Most errors happen before analysis, during test ordering, specimen collection, and transport stages.

Impact of Specimen Quality

Specimen type, aseptic technique, volume, and timely transport critically affect diagnostic accuracy.

Collaborative Workflow Improvement

Collaboration among clinicians, nurses, and lab staff reduces errors and improves specimen quality.

The clinical–laboratory workflow demonstrates that most diagnostic errors in bacteriology originate in the pre-analytical phase—during test ordering, specimen collection, and transport—meaning the accuracy of microbiology results ultimately depends on the quality of the specimen provided and collaboration across the care pathway. (PMID: 19952034; PMID: 23845951; PMID: 21517699)

Scope and Definition of Pre-Analytical Activities

- **Key Pre-Analytical Steps:** Includes patient ID, specimen selection, timing, container choice, and transport conditions critical for accurate results.
- **Shared Responsibility:** Pre-analytical responsibilities extend beyond the lab, involving clinicians and healthcare staff across the system.
- **Quality and Error Reduction:** Understanding pre-analytical scope enables targeted quality indicators and training to reduce errors efficiently.

Scope and Definition of Pre-Analytical Activities

- **Formal Definition and Standards:** Pre-analytical activities cover all steps from clinician's request to the start of analytical processing, as per ISO 15189.

ISO 15189 states that the pre-analytical phase includes everything that happens before the sample is actually tested—and requires strict quality control because it is a major source of laboratory error.

Key implication for microbiology

It confirms that:

- The laboratory is responsible not only for testing
- But also for ensuring quality across the entire pre-analytical process

- **Formal Definition and Standards:** Pre-analytical activities cover all steps from clinician's request to the start of analytical processing, as per ISO 15189.

What Makes a Good Microbiology Specimen

- **Specimen Quality Attributes:** Accurate **site representation** and organism viability are essential for high-quality microbiology specimens.
- **Microbiology vs Chemistry:** Microbiology specimens contain living organisms requiring careful handling unlike stable chemical analytes.
- **Common Collection Challenges:** Contamination and **improper volume** can invalidate results, exemplified by saliva in sputum masking pathogens.
- **Transport and Timing:** Timely transport under appropriate conditions preserves specimen quality and organism viability.

Volume Matters (Blood Cultures)

- **Key concept:** Blood volume is the most critical variable in detecting bloodstream infections (PMID: 31092594)

- **Impact of low volume:**

- Low volume → increased false negatives (missed bacteremia)
- Many BSIs have very low organism burden (≤ 1 CFU/mL), requiring sufficient volume to detect (PMID: 30901027)

- **Why volume matters:**

- Sensitivity increases directly with volume collected
- Approximate effect:
 - ~3% decrease in sensitivity for every 1 mL underfilled (PMID: 23269730)
 - ~13% increase in detection odds per additional mL (PMID: 31092594)

- **Takeaway:** Underfilling blood cultures is one of the most common and correctable causes of diagnostic failure



Case: “The Missing Pathogen”



Scenario

- A patient with suspected pneumonia
- Sputum sample sent to lab
- ✓ Findings:
 - GS: numerous epithelial cells
 - Culture: mixed normal flora
 - Report: “No significant pathogen”



Outcome:

- Patient worsens
- Later BAL sample shows *Klebsiella pneumoniae*



Questions for audience:

- What went wrong?
- Biological issue or technical issue?
- Should the sample have been accepted?

“Poor specimen quality (saliva contamination) can mask the true pathogen—this is a pre-analytical failure.”



Biological Influences and Technical Interferences

Biological Influencing Factors

Biological factors impact the patient's true condition, such as antibiotic use or infection stage, affecting test results.

Technical Interference Factors

Technical errors like contamination or improper handling introduce false signals that obscure accurate findings.

Importance of Distinction

Differentiating biological influences from technical interferences helps identify true patient status and correct errors.

Distinguishing between biological influences and technical interferences is essential to determine whether a test result reflects the patient's true condition or a correctable pre-analytical error.

Pre-Analytical Phase in Diagnostic Stewardship

- Most stewardship interventions should target:
 - Sample selection: Ordering the right specimen type based on clinical syndrome and diagnostic yield
 - Collection quality: Ensuring proper technique, timing, handling, and transport to avoid contamination and false results
- Why this phase matters:
 - The pre-analytical phase is a major driver of diagnostic error and inefficiency
 - Suboptimal collection or specimen choice can lead to:
 - Inaccurate or delayed diagnoses
 - Unnecessary repeat testing
 - Increased healthcare costs (PMID: 39431829)

Pre-Analytical Phase in Diagnostic Stewardship

- Operational reality:
 - Most pre-analytical steps occur outside the laboratory, performed by frontline clinical teams
 - Requires strong collaboration, education, and communication between lab, ID, nursing, and providers (PMID: 39431829)
- Key opportunity:
 - Highest impact Dx stewardship interventions often occur before the sample reaches the lab



Case: “The Vancomycin Spiral”



Scenario

A 68-year-old patient presents to the emergency department with:

- Low-grade fever (38.1°C) Mild fatigue
- No clear source of infection

Labs:

- WBC mildly elevated
- Hemodynamically stable
- 👉 Blood cultures are drawn, and the patient is discharged on oral antibiotics



What Happens Next...

48 hours later:

- Blood culture flags positive
- Gram stain: Gram-positive cocci in clusters
- 👉 The patient is urgently called back and admitted
- 👉 Started on IV vancomycin for suspected bacteremia



Case: “The Vancomycin Spiral”



Clinical Course

- Hospitalized for 3 days
- Develops rising creatinine (AKI)
- Infectious Diseases consult questions diagnosis

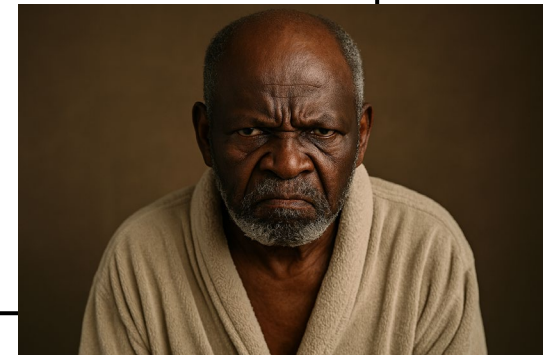
Repeat cultures:

- Negative
- Final interpretation:
👉 Contaminated blood culture (Staphylococcus epidermidis)

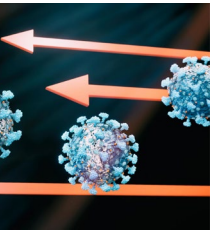


Outcome

- Unnecessary hospitalization
- Exposure to broad-spectrum antibiotics
- Drug-related toxicity (AKI)
- Associated additional healthcare cost



How Contamination Occurs and How to Prevent It



Common Contamination Pathways

Contamination often occurs via skin flora or oral flora entering specimens during venipuncture or sample collection.

Clinical Consequences

False-positive cultures cause unnecessary antibiotics, longer hospital stays, and increase antimicrobial resistance.

Preventive Strategies

Strict aseptic technique, proper skin antisepsis, staff training, and monitoring contamination rates reduce errors.

Contamination occurs when normal flora enter specimens during collection, leading to false results and inappropriate treatment, but can be significantly reduced through strict aseptic technique, proper training, and continuous quality monitoring.

Blood Culture Contamination – Current Benchmarks

- CLSI/CDC target: $\leq 3\%$ contamination (ideal $\leq 1\%$)
- Many institutions exceed this $\rightarrow$ major quality gap
- Variation across sites due to technique and systems

True Cost of Contamination

- Adds \$2,900–\$5,800 per case (PMID: 40911304)

- Leads to:
 - Longer hospital stay
 - Unnecessary antibiotics
 - Repeat testing

Diagnostic Stewardship – What It Means

- **Core principle:** “Right test, right patient, right time”

- **Key components:**
 - Ordering: Ensuring appropriate test selection based on clinical indication and pre-test probability
 - Collection: Optimizing specimen quality (timing, site, technique) to minimize contamination and false results
 - Interpretation: Integrating results with clinical context to guide appropriate management

- **Goal:**
 - Improve diagnostic accuracy, reduce unnecessary testing and antibiotic use, and enhance patient outcomes while lowering costs

Transport, Storage, and Media: Preserving Organism Viability Before Analysis

- **Impact of Transport Conditions:** Transport time and temperature critically affect organism survival and recovery, preventing death or contamination overgrowth.
- **Role of Culture Media:** Appropriate culture media and transport systems maintain viability and suppress contaminants during specimen transport.
- **Link to Diagnostic Accuracy:** Proper transport and storage improve Gram stain accuracy, culture yield, and antimicrobial susceptibility testing outcomes.

Proper transport, storage, and use of appropriate media are critical to preserving organism viability, as delays or suboptimal conditions can compromise culture recovery and downstream diagnostic accuracy.

WHO Transport Guidelines (2023–2024)

- **Core principle:** Safe transport of infectious materials requires compliance with standardized international regulations across the entire transport chain.

- **Key elements:**

- Packaging: Triple packaging (leak-proof primary, sealed secondary, rigid outer)
- Labeling: Proper hazard labels, UN classification, and documentation
- Temperature control: Maintain required conditions (refrigerated, frozen, dry ice) to preserve specimen integrity.

- **Operational requirement:**

- Mandatory training for all personnel involved in packaging, transport, and receiving
- Ensures safe handling, regulatory compliance, and risk mitigation

- **Takeaway:** Transport is a critical quality step affecting both safety and diagnostic accuracy

Pre-Analytical Impact on Laboratory Workflow From Gram Stain to AST: Downstream Consequences

Impact of Specimen Quality

Specimen quality directly affects the accuracy of Gram stain, organism identification, and antimicrobial susceptibility testing results.

Laboratory Workflow

Progression
Errors early in the workflow propagate through Gram stain, culture, identification, and AST, affecting final outcomes.

Recognizing and Reporting Red Flags

Identifying poor specimen quality or unusual organism patterns is essential to ensure accurate results and quality control.

Promoting Laboratory Quality Culture

Proactive communication and shared responsibility foster a culture of quality within the laboratory environment.

Poor specimen quality in the pre-analytical phase propagates through Gram stain, culture, identification, and AST, making early error recognition and communication essential for accurate laboratory results.

Pre-Analytical Factors Affecting AST

- **Specimen quality drives AST reliability:**

- Accurate AST depends on viable organisms from high-quality specimens
- Poor-quality or delayed specimens compromise downstream susceptibility results

- **Impact on clinical care:**

- Suboptimal specimens → delayed or inaccurate AST results
- Can contribute to inappropriate or prolonged empirical therapy

- **Link to rapid AST performance:**

- Rapid AST can deliver results in 1–8 hours, but only if workflows—from collection to lab receipt—are optimized
- Pre-analytical delays (collection, transport, processing) reduce the clinical impact (“actionability”) of rapid AST

- **Takeaway:**

- Even the fastest AST cannot overcome poor specimen quality or delayed processing

Emerging Technology vs Pre-Analytical Reality

- **Rapid AST transforms timelines:**

- Delivers MIC-based susceptibility results within hours (~6–13 h) vs 24–48 h conventional

- **BUT performance still depends on fundamentals:**

- High-quality specimen (positive blood culture) required
- Adequate viable organism load for phenotypic growth detection
- Timely workflow (collection → transport → processing)

- **Reality gap:**

- Delays or poor specimen quality limit actionability of rapid AST results
- Technology cannot overcome pre-analytical deficiencies

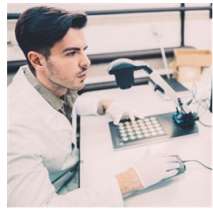
- **Takeaway:**

- Innovation + workflow excellence = clinical impact

Quality Indicators and Continuous Improvement: Measuring and Improving Pre-Analytical Performance -13



- **Key Quality Indicators:** Important metrics include specimen rejection rates, blood culture contamination, mislabeling, and turnaround time to monitor performance.



- **Routine Monitoring Benefit:** Regular review of quality indicators helps identify trends and evaluate corrective actions for continuous improvement.



- **Implementing Quality Monitoring:** Practical suggestions enable quality monitoring in resource-limited settings through data-driven training and process redesign.

Using quality indicators to routinely measure and analyze pre-analytical performance enables laboratories to identify errors, drive targeted improvements, and enhance patient care outcomes.

Standardization is the Future

- **Current gap: high variability across institutions:**

- Multiple contamination definitions (CAP, CLSI, NHSN) → different reported rates (PMID: 40643261)
- Inconsistent tracking of key quality indicators (e.g., single sets, positivity)
- Variable reporting and data sharing practices across teams

- **Why standardization matters:**

- Enables accurate benchmarking and comparison
- Reduces contamination, unnecessary antibiotics, and downstream harm
- Improves diagnostic stewardship and patient outcomes

- **Future direction:**

- Standardized definitions + quality indicators + reporting frameworks (e.g., CDC quality measures)

- **Takeaway:**

- Standardization is essential to unlock consistent quality and clinical impact at scale

Root Cause Analysis of Pre-Analytical Errors

Categories

- **Human:**

- Training gaps, protocol non-adherence, variable competency

- **System:**

- Workflow inefficiencies, lack of standardization, delays, poor communication

- **Environmental:**

- Time pressure, high-acuity settings, supply issues, transport conditions

- **Takeaway:**

- Errors are multifactorial → require system-level solutions (not just retraining)

Pre-Analytical Errors We Can Prevent

- 50–70% of lab errors occur in the pre-analytical phase (majority preventable) (PMID: 16729864)

- **Highest ROI Interventions:**

- Training: Standardize collection technique, reinforce competency, ongoing education
- SOP Standardization: Clear protocols for ordering, collection, transport, and handling

- **Additional High-Impact Levers**

- Checklists & decision support at point of care
- Audit & feedback (e.g., contamination rates, volume compliance)
- Collection kits/standardized supplies to reduce variability

- **Takeaway:**

- Most pre-analytical errors are preventable with simple, scalable interventions

Role of Feedback Loops

- Reporting contamination rates to clinicians improves collection practices and accountability
- Data transparency drives behavior change and sustained quality improvement

- **How feedback works:**

- Regular audit & feedback (e.g., contamination, volume, single sets)
- Benchmarking by unit, provider, or department
- Real-time or periodic performance dashboards

- **Multidisciplinary approach:**

- Requires collaboration across:
 - Laboratory, Nursing / Phlebotomy, Clinicians, Infection Prevention / Stewardship teams

- **Takeaway:**

- Feedback loops turn data into action—closing the gap between guidelines and practice

Pre-Analytical Quality and AMR - Linking Specimen Quality to Antimicrobial Stewardship - 14



- **Impact of Specimen Quality :** High-quality specimens reduce inaccurate results, preventing inappropriate antibiotic use and supporting effective treatment decisions.



- **Role of Antimicrobial Stewardship:** Improved specimen quality directly supports antimicrobial stewardship by guiding appropriate antibiotic use and reducing resistance.



- **Preamanalytical Excellence Importance:** Achieving excellence in pre-analytical processes is essential for modern clinical bacteriology and impacts patient outcomes and public health.

High-quality specimens are essential for accurate microbiology results, and improving pre-analytical processes directly supports antimicrobial stewardship and helps combat antimicrobial resistance.

The Future – Point-of-Care & Pre-Analytical Risk

- Rapid growth of decentralized / point-of-care (POC) testing
- Testing increasingly performed outside traditional lab control

- **Increased Pre-Analytical Risk**

- **Poor collection:** Variability in technique across diverse users
- **Poor handling:** Inconsistent transport, storage, and preparation
- **Reduced oversight:** Less direct lab supervision and quality control
- **Fragmented workflows:** Gaps between testing, reporting, and follow-up

- **Implication:**

- Greater need for standardization, training, and digital decision support at the point of care

- **Takeaway:**

- As diagnostics move closer to the patient, pre-analytical risk becomes the new bottleneck

Final Thoughts: Pre-Analytical Excellence Defines Diagnostic Success



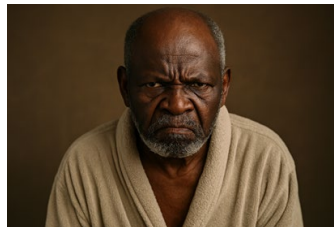
Most errors occur
before testing
even begins

“In microbiology, the
result doesn’t start in
the lab—it starts at the
bedside”

Specimen
quality is the
foundation of
every result

“The most advanced
diagnostic is only as
good as the specimen
it receives”

Small mistakes
have massive
clinical
consequences



Technology cannot
fix poor
fundamentals –

“You cannot out-
innovate poor
specimen quality”

This is a system
problem—not a
laboratory
problem

“Pre-analytical quality
is not a step in the
process—it is the
process”

👉 “You cannot detect what you do not collect—and that is the reality of diagnostic medicine.”