It's a fast, fancy PCR- but does that make it better? How molecular diagnostics can impact infection prevention efforts

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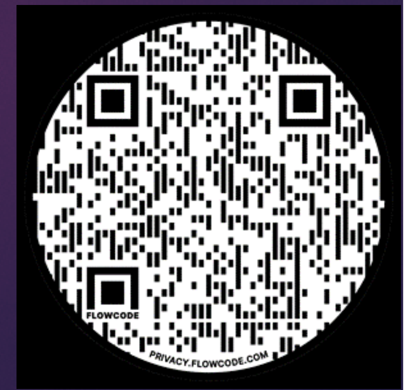
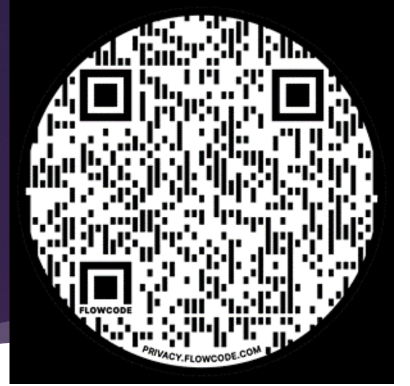
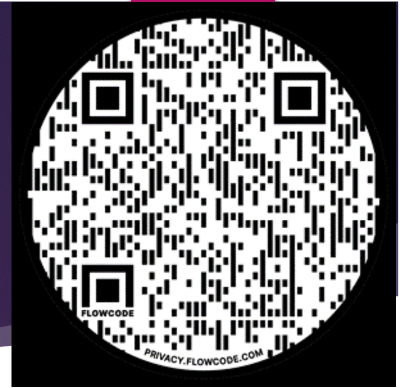


Disclosures



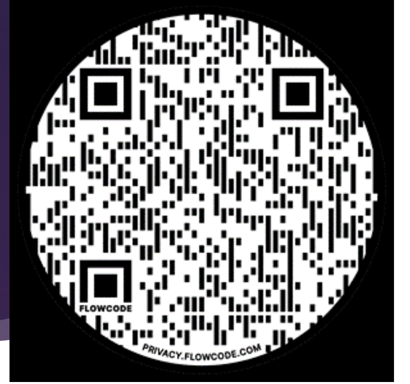
- Clare McCormick-Baw: No disclosures to report
- Francesca Lee: No disclosures to report

Objectives



- 1) Describe the differences between traditional and molecular diagnostic testing
- 2) Understand benefits and limitations of rapid molecular assays
- 3) Describe potential ramifications for patient care and public reporting based on test selection and utilization

What is molecular testing?



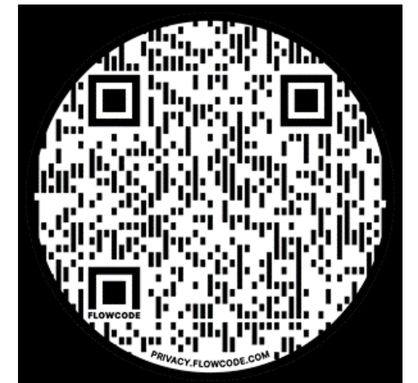
- Molecular dogma:
  - DNA → mRNA → Protein

- Diagnostic methods centered around molecular dogma:
  - Polymerase Chain Reaction (PCR) → DNA (bacterial chromosomal nucleic acid)
  - Enzyme Immunoassay (EIA) → Protein (toxin produced by the bacteria)

Phenotype vs. Genotype Diagnostic Testing

- Phenotype
  - Looking for the end product or reaction
  - Protein focused
    - Toxin production
    - Beta lactamase production

- Genotype
  - Looking for the nucleic acid that encodes the target
  - Gene focused



Clostridioides difficile – The IP Boogeyman

- Laboratory ID determines whether a patient is infected per federal standards.

  - Not so fast…the clinical presentation and circumstances are not always clear cut for who is truly suffering from a C. *difficile* infection (CDI)

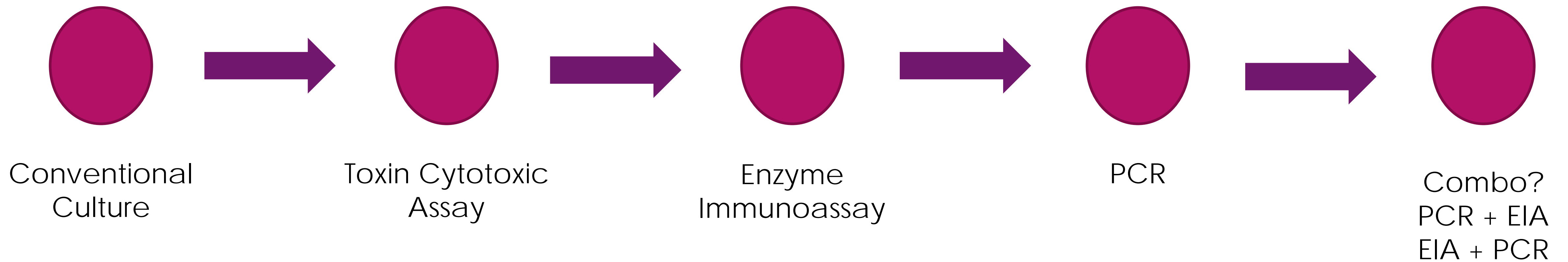
CDI Conundrum

Patients colonized with toxigenic C. diff with diarrhea

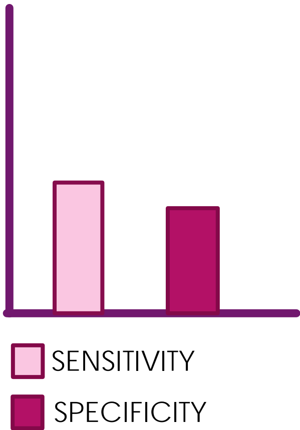
CDI

Patients not colonized with C. diff with diarrhea

History of *C. diff* Diagnostic Testing



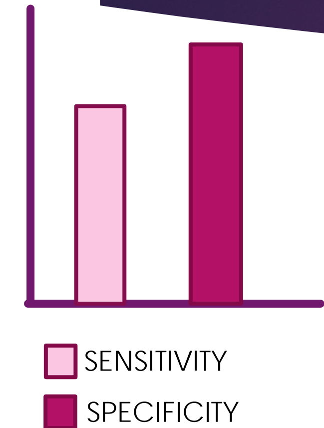
Conventional Culture



- Grow the organism
  - Requires anaerobic environment
  - Requires specialized media
  - **Days** to incubate and read
  - No toxin determination immediately available (another test required)
  - Pre-analytical variables impact culture results and interpretation

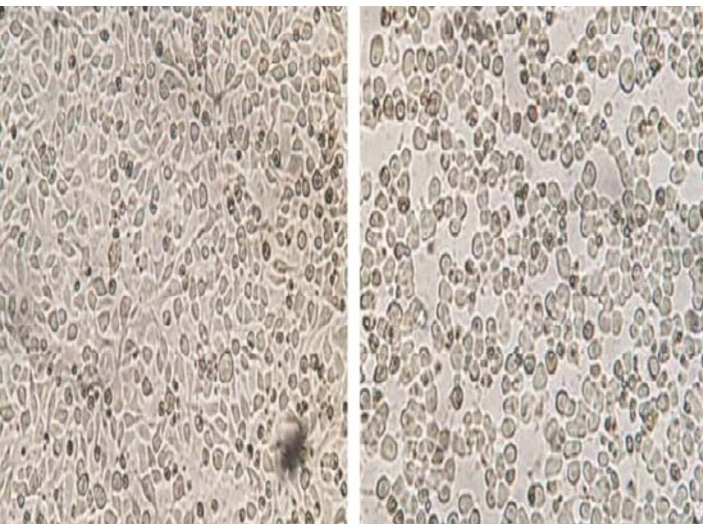
- Not a very sensitive assay to determine the cause of diarrhea in patients
- Was available before any other method
- Answers the MOST BASIC question…does this patient have ANY *C. difficile*?

Cytotoxic Assay



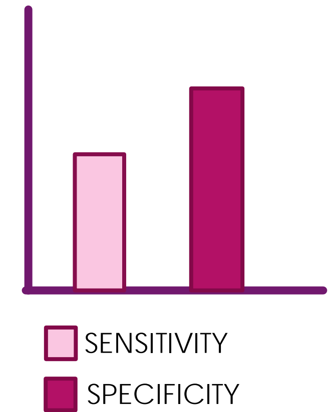
- Toxin Cytopathic Effect Assay
  - Stool from a suspected patient is filtered to remove the solids
  - Alternatively, an isolate is incubated in broth and the fluid used in this assay
  - The filtrate is overlayed on a monolayer of tissue culture cells and incubated (picture on the left)
  - The tissue culture cells are evaluated for cytologic changes (picture on the right)

- More sensitive and much more specific than conventional culture
- Still takes DAYS to perform and interpret
- Specialized laboratories (reference lab)
- Answers the question: Does the patient have *C. diff* toxin in their stool?
  - Phenotypic testing



Mirzaei et al. Diagnosis of Clostridium difficile infection by toxigenic culture and PCR assay. Iranian Journal of Microbiology. 2018:10(5):287-93

Enzyme Immunoassay (EIA)



- The *C. diff* pregnancy test!
- Uses raw stool sample
- Can detect the organism itself with one protein and toxin B
- Testing time is ~20 minutes, start to finish

- Most EIAs require approximately 1 nanogram of toxin to be detectable (cytotoxic assay is more sensitive)
- Answers the question: Does the patient have toxigenic *C. diff* producing detectable toxin?
  - Phenotypic testing

Polymerase Chain Reaction

- Multiple assays available (most commonly encountered are listed):
  - Cepheid Xpert *C. diff*
    - Toxin B detection (*tcdB*)
  - Cepheid Xpert *C. difficile* Epi
    - Toxin B detection
    - Epidemic strain 027/NAP1/BI detection

- Raw stool sample
- Internal control to ensure proper processing of the sample
- Testing time 40 min and 45 min, respectively
- FDA cleared for SOFT or UNFORMED stool only → need to reject formed stool
- Answers the question: Does this patient carry a toxigenic strain of *C. difficile*?
  - Genotype testing

www.Cepheid.com

C. diff PCR

Pros

- Most sensitive test available
- Most specific test available
- Little hands-on time
- Objective read-out (detected/not-detected)
- Testing time is 40 or 45 min depending on assay

Cons

- **NOT a test of cure**
  - No molecular test should EVER be used
- Only detects the gene for the toxin
  - Not whether it is functional
  - Not whether it is present in high enough quantities to cause disease

Combo Testing?
PCR→ EIA or EIA→ PCR

- Fusion of both phenotypic and genotypic diagnostic strategies!

- Used in tandem, but order is determined by each laboratory:
  - High prevalence of toxin producing *C. diff* → consider screening with EIA first
    - Looking for threshold amount of toxin production then PCR confirms

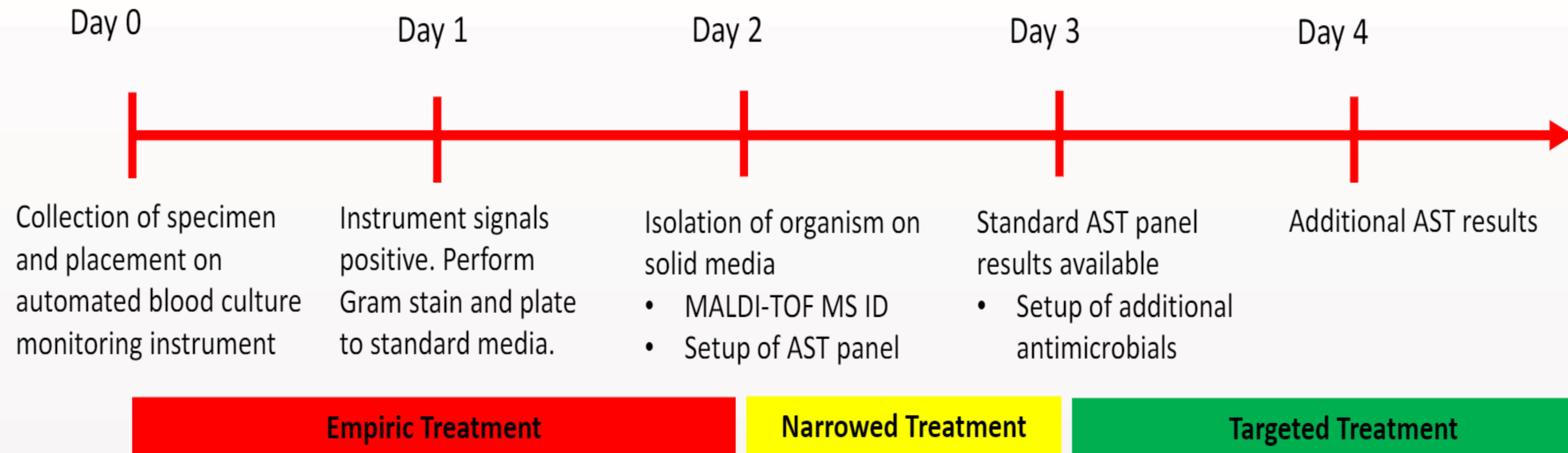
  - Lower prevalence of toxin producing *C. diff* or high prevalence of non-toxigenic *C. diff* strain carriage → consider PCR first
    - Looking for the groups that COULD make toxin, then determine if enough toxin is present to correlate with infection

Why IP is so important…

- **Your leadership is CRUCIAL to providing education and guidance about the appropriate situations for testing**
- Laboratory testing CANNOT distinguish who is truly suffering from infection versus who is colonized

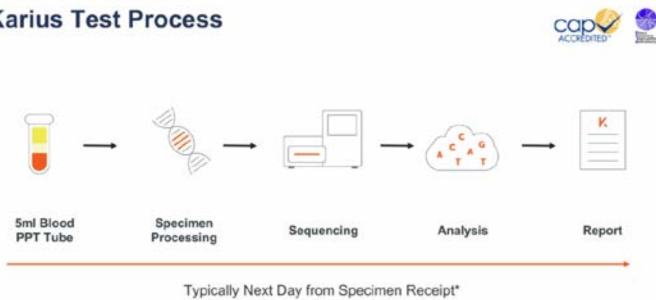
Blood cultures, CLABSIs, and MDROs…oh my!

Current Paradigm for BSI Diagnostics

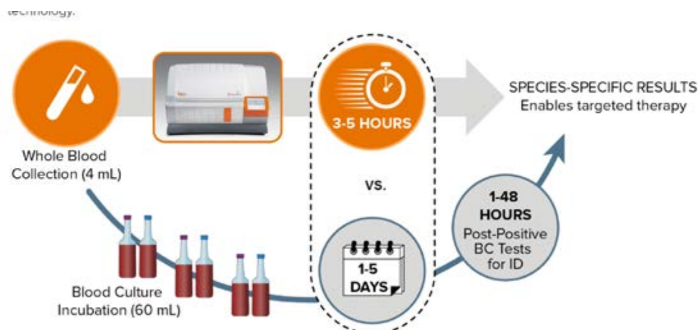


Newer Technologies

Direct from Blood – no incubation
(culture independent)



1250 pathogens
No AMR



5 Candida
6 Bacteria
No AMR

Multiplex detection after incubation
(culture dependent)

Assay* (Manufacturer)	Pathogens	AMR
MALDI-TOF MS ID Sepsityper™, Lysis centrifugation	Broad Gram-positive & Gram-negatives	None
Verigene* (Luminex)	12 Gram pos/9 Gram neg	*mecA*, *vanA*, *B* bla_{CTX}, bla_{KPC}, $bla_{OXA\text{-}48\text{-}like}$, bla_{NDM}, bla_{VIM}
FilmArray (bioMerieux)	8 Gram pos/11 Gram neg; 5 *Candida*	*mecA*, *vanA*, *vanB*, bla_{KPC}
ePlex BCID* (GenMark)	20 Gram pos/21 Gram neg; 16 fungi	*mecA*, *mecC*, *vanA*, *B* bla_{CTX}, bla_{KPC}, $bla_{OXA\text{-}23,\text{-}48}$, bla_{NDM}, bla_{VIM}

Courtesy of Dr. Trish Simner

How do these technologies impact IC?

Culture-independent

- Molecular organism identification
  - Number of targets dependent on assay
- No susceptibility results
- No Gram stain

Culture alone

- Gram stain
- Organism identification
- Susceptibility results
  - Phenotypic resistance information

Culture plus BCID

- Gram stain
- Molecular organism identification
  - Dependent on assay targets
- Molecular AMR information
  - Dependent on assay targets
- Organism identification
- Susceptibility results
  - Phenotypic resistance information

Case Study

50 year old male admitted with fevers, sepsis. He has a right internal jugular permacath for hemodialysis.

Workup for sepsis is initiated.

Empiric vancomycin, piperacillin-tazobactam started

- Culture independent

- T2MR-
  - Candida: no targets detected
  - Bacteria:
    - *E. faecium*
    - *S. aureus*
    - *K. pneumoniae*
    - *P. aeruginosa*
    - ***E. coli***

- No change in management

Case Study

50 year old male admitted with fevers, sepsis. He has a right internal jugular permacath for hemodialysis.

Workup for sepsis is initiated.

Empiric vancomycin, piperacillin-tazobactam started

Cultures alone

- Day 1
  - Blood cultures positive at 18 hours
  - Gram stain: GPC and GNR
- No change in management
- Day 2:
  - MALDI-TOF identification
    - *Staphylococcus lugdunensis*
    - *Escherichia coli*
- No change in management
- Day 3:
  - AST results:
    - *S. lugdunensis:* oxacillin susceptible
    - *E. coli: ESBL* producer
  - Vancomycin and Piperacillin-tazobactam stopped
  - **Meropenem started**
  - **Contact isolation initiated**

Case Study

50 year old male admitted with fevers, sepsis. He has a right internal jugular permacath for hemodialysis.

Workup for sepsis is initiated.

Empiric vancomycin, piperacillin-tazobactam started

Cultures plus BCID

- Day 1
  - Blood cultures positive at 18 hours
  - Gram stain: GPC and GNR
  - BCID
    - *Staphylococcus lugdunensis*
    - *Escherichia coli*
      - *CTX-M*
- Piperacillin-tazobactam changed to **Meropenem**
- Vancomycin continued
- **Contact isolation initiated**
- Day 2:
  - MALDI-TOF identification
    - *Staphylococcus lugdunensis*
    - *Escherichia coli*
- No change in management
- Day 3:
  - AST results:
    - *S. lugdunensis:* oxacillin susceptible
    - *E. coli: ESBL* producer
  - Vancomycin stopped

Impact of rapid diagnostics

- Faster time to de-escalation
  - S. aureus and NO MecA/MecC = MSSA => Nafcillin/Cefazolin

- Faster time to appropriate antibiotics when resistance genes detected
  - VanA/VanB = VRE => Daptomycin
  - MecA/MecC = MRSA => Vancomycin
  - CTX-M = ESBL => Carbapenem
  - KPC/NDM/IMP/OXA/VIM = Carbapenemase => stewardship-driven therapy

- Faster time to appropriate contact isolation

How do these tests affect CLABSI reporting?

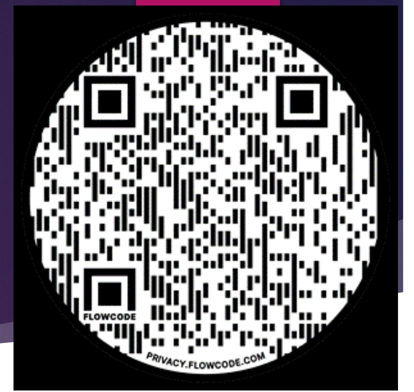
- Patient of any age has a recognized bacterial or fungal pathogen, not included on the NHSN common commensal list:
- 1. Identified from one or more blood specimens obtained by a culture OR
- 2. Identified to the genus or species level by non-culture based microbiologic testing (NCT)* methods (for example, T2 Magnetic Resonance [T2MR] or Karius Test).
  - Note: If blood is collected for culture within 2 days before, or 1 day after the NCT, disregard the result of the NCT and use only the result of the CULTURE to make an LCBI surveillance determination. If no blood is collected for culture within this time period, use the result of the NCT for LCBI surveillance determination. AND Organism(s) identified in blood is not related to an infection at another site (See Appendix B: Secondary BSI Guide).
  - *For the purposes of meeting LCBI-1, NCT is defined as a methodology that identifies an organism directly from a blood specimen without inoculation of the blood specimen to any culture media. **For instance, NCT does not include identification by PCR of an organism grown in a blood culture bottle or any other culture media.**

BCID doesn't count- only culture (?)

MDRO Definitions

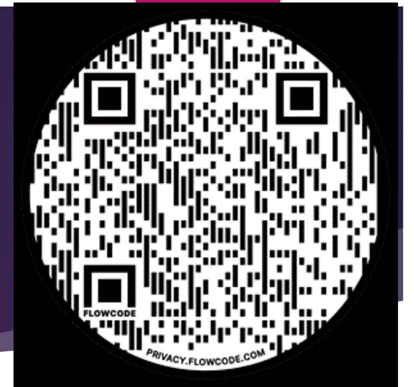
- Several reportable MDRO definitions include non-phenotypic components
  - MRSA- *S. aureus* with MecA/MecC genes
  - VRE- *E. faecalis/faecium/spp* with Van A/VanB genes
  - CRE- *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Klebsiella aerogenes* or *Enterobacte*r spp with KPC, NDM, VIM, IMP, OXA-48 genes
- Testing can be performed directly on the specimen (BCID) or after growth of the organism
  - MRSA
    - PCR from nasal swab
    - PCR for mecA gene on *Staphyloccus aureus*
  - CRE
    - PCR from rectal swab
    - PCR for multiple genes on *Klebsiella pneumoniae*

Questions will arise…



- Examples
  - CTX-M gene = ESBL
    - Labs only "define" ESBL for *E. coli*, *Proteus*, *Klebsiella*- what should you do if CTX-M is detected in a different bacteria?
      - Should isolation be implemented?
  - What if the BCID detects an organism, but this is NOT found on the culture plates?
    - Previously treated? (dead bug?) Lab error? Contamination?
  - What if a resistance gene is detected, BUT the phenotypic results are not consistent?
    - Labs need to have plans in place to investigate discrepancies
    - IP should have plans to address management of these patients

Objectives Review



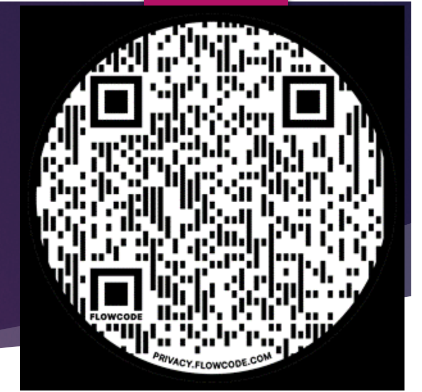
Describe the differences between traditional and molecular diagnostic testing

Understand benefits and limitations of rapid molecular assays

Describe potential ramifications for patient care and public reporting based on test selection and utilization

For *C. difficile* and bacteremia

If you haven't implemented these technologies yet



- Work with your microbiology lab to decide which technologies make sense for your institution
- Work with IT to optimize EMR for results cascading, building reports, and linking results to isolation orders and/or antibiotic stewardship recommendations
- Be familiar with limitations of tests, and implications for institutional IC protocols as well as state/federal reporting
- Be comfortable with discrepant results and have a plan for adjudicating them

- Thank you-
- Any Questions?