Integration of Molecular Techniques in Clinical Microbiology

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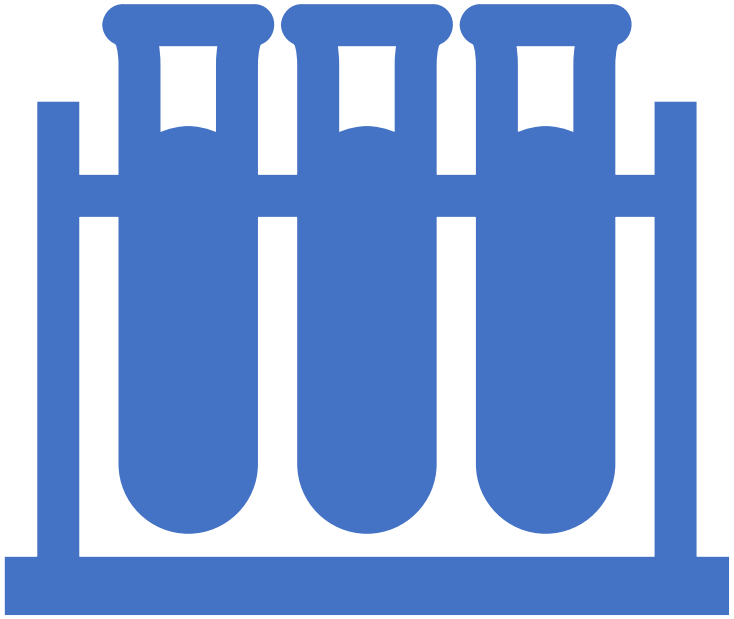
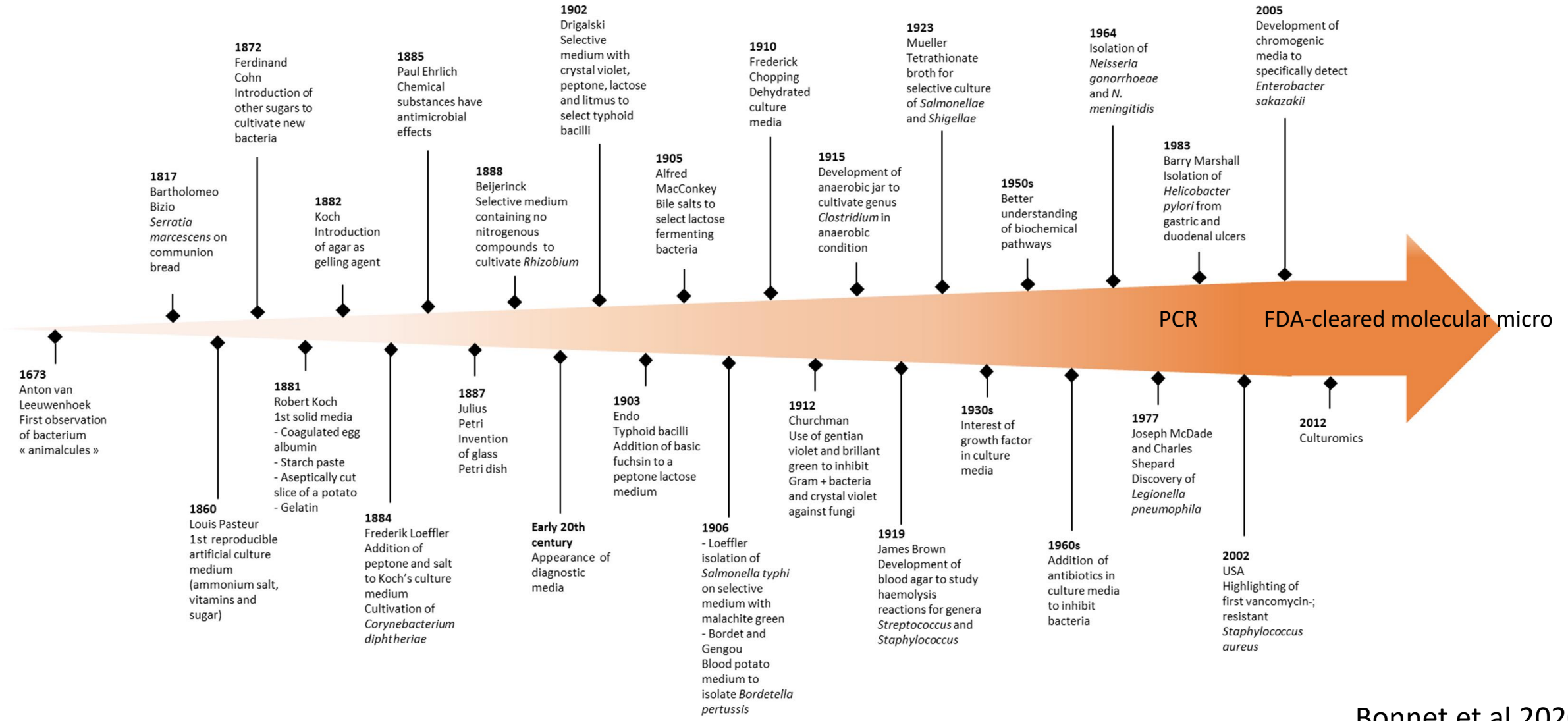
Disclosures

- Nothing to disclose
- **We are not promoting ANY manufacturer!!**
- While we discuss particular assays to illustrate examples or to relay our experience, the choice of a “best” assay is dependent on many factors unique to a laboratory



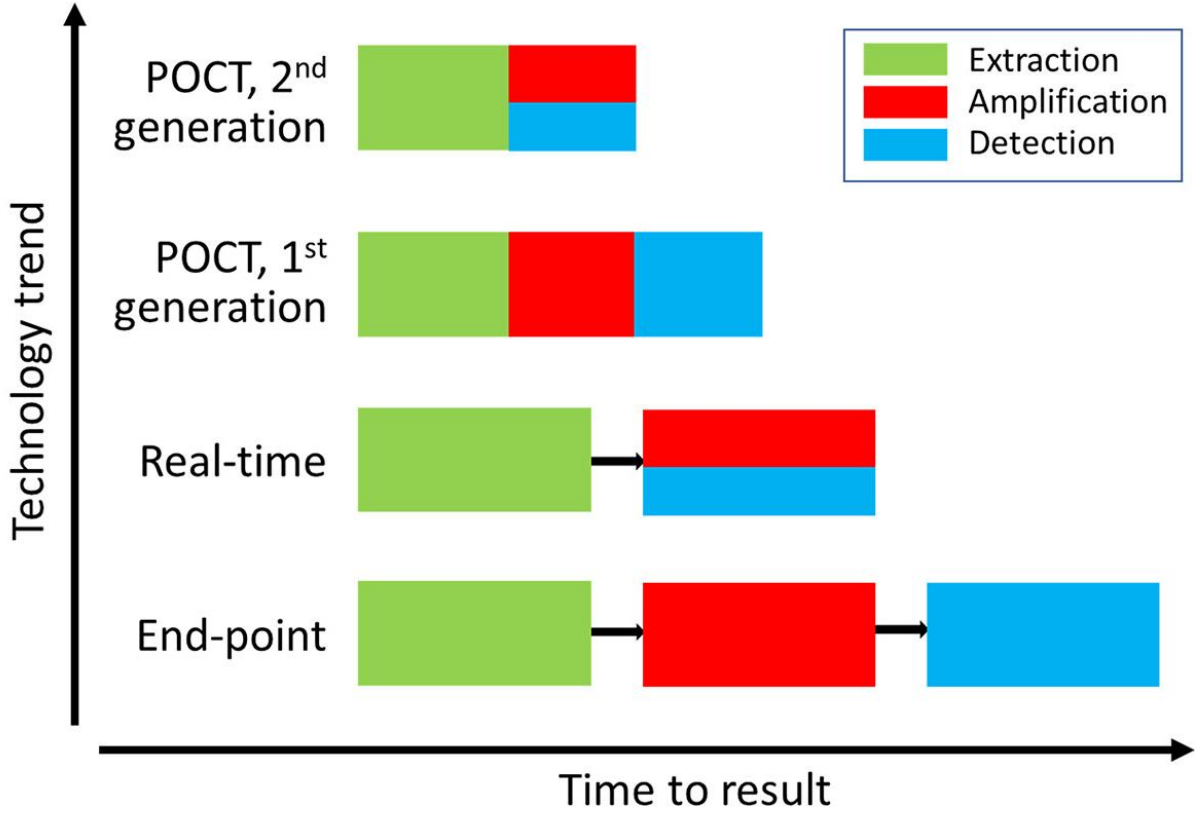
Timeline of Clinical Microbiology



Bonnet et al 2020

Molecular techniques have transformed ID diagnostics over the past 20 years

- Early assays focused on STI testing, respiratory viral infections, and viral loads
- Recent assays have focused on syndromic approaches and cover wide phylogenetic ground
- There has been an evolution from end-point tests with separate workflows to high-throughput contained assays
- General pros and cons of molecular testing
    - Sensitivity/specificity
    - TAT
    - Training time
    - Standardized resulting



Schmitz, *J. Clin Micro* 2022

Objectives

- Understand the challenges of validating molecular methods against traditional (non-molecular) gold standards
- Consider ways to integrate molecular and traditional test results in technologist training and clinical reporting
  - Workflow
  - Reporting & target coverage (LIS dependent resulting modules, keeping results cohesive)
  - Mixed workflows
  - Molecular literacy (staffing groups)
- Appreciate the use of integrated quality monitors to longitudinally assess performance
- **What we will NOT focus on:**
  - Comparative offerings between manufacturers
  - Technical details of the platforms
  - Isolate sequencing and metagenomic assays

Common Verification Challenge #1: Comparator method is less sensitive than the new method

Challenges

- Discrepancies between culture data and molecular data
- Falsely lowered specificity
- May require additional work/money to verify by another method

Recommendations

- Review the raw data (i.e Ct values)
- Communicate with your vendor FAS
- Send specimens to a reference lab for verification
- ***Ask a neighboring lab to help verify result***

Common Verification Challenge #2: Positive samples are difficult to acquire

Challenges

- New target(s) for your laboratory
- Low prevalence target(s)
- Difficult to cover all organisms on a multi-target panel
- Collection device is specific to the molecular platform

Recommendations

- Ask the vendor for assistance with sourcing specimens
- Purchase from a reference laboratory
- Determine when and how QC material can be used to supplement clinical specimens
- Plan ahead! Create your own positive specimen repository
- ***Ask a neighboring lab if they can provide specimens***
- Note: Track the specimens you send to other labs for discrepancy analysis!

Common Verification Challenge #3: Multiplex assays

Challenges

- New target(s) for your laboratory
- Presence of clinical samples with multiple targets
- Sensitivity differences amongst assays

Recommendations

- Review the literature
- ***Discuss with a neighboring lab that is running the same assay***

Common Verification Challenge #4: Multi-cycler instruments

Challenges

- How to cover all cyclers without overburdening the process
- Verification specimens
- QC
- Precision

Recommendations

- Determine how many individual "instruments" your system comprises
- Rotate amongst the cyclers
- Determine your lab’s comfort level
- ***Discuss with a neighboring lab!***

Common Verification Challenge #5: Understanding contamination risks

Challenges

- Laboratory workflow
- Platform setup
- Level of containment

Recommendations

- Single-use reagents wherever possible
- Consider sample pathways through the laboratory from accessioning to resulting (periodic self-audit)
- Environmental testing
- ***Discuss with a neighboring lab!***

How can NACMID help?

Side note:
Can we create and foster a community of shared resources?

Quality monitors

- A strong QA program will highlight:
  - Test volumes
  - Positivity rates (with levels for quantitative tests)
  - Correlation with associated tests:
    - Culture, Gram stain, and other chemistries
    - Molecular and Culture species ID
    - Molecular and phenotypic susceptibility results
  - BWH is actively building a quality program to produce regular monitors across the menu.
    - Responsiveness to results
    - Adaptation of SOPs
    - Communication with clinical stakeholders

https://www.sustained-quality.com/quality-creating-good-product/

Cases

Sexual and Women's health

- Mostly outpatient
- Includes some of the earliest and some of the most recently adopted molecular assays
- Some clear causative targets, some nuanced targets
- No strong traditional gold standard to verify against
- Quality monitors don't generally include culture correlation (tests are relatively siloed)

Molecular stool testing

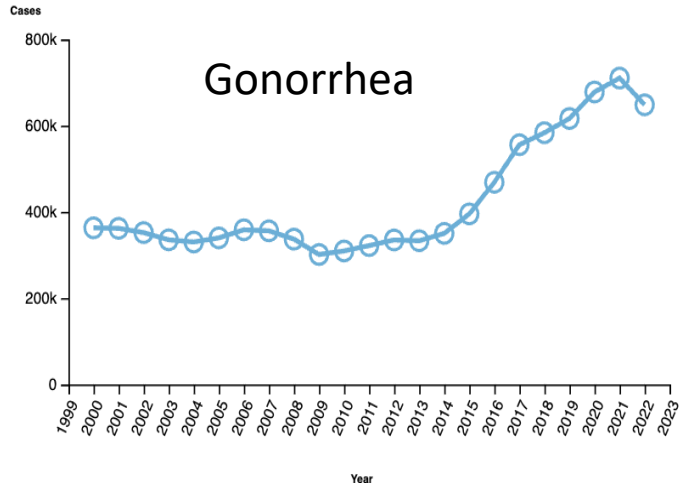
- Mostly outpatient
- Phylogenetically diverse pathogens with a mix of gold standard tests (or lack thereof)
- Targets are generally clear
- Some targets have strong traditional gold standard
- Quality monitors include correlation for some targets

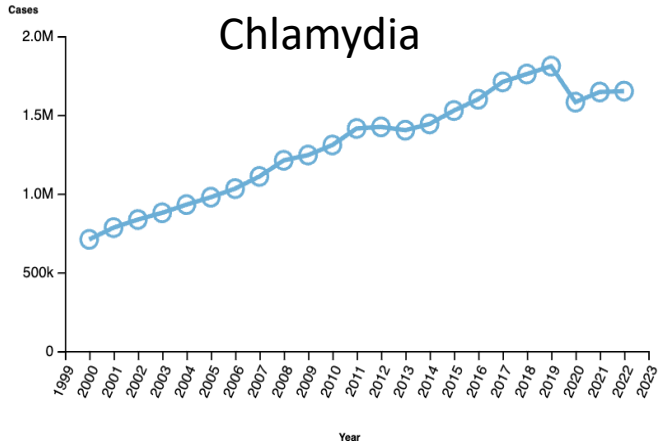
Molecular testing of positive blood cultures

- High impact, clinically visible test (mostly inpatient)
- Targets are clear
- Most have strong traditional gold standard
- Quality monitors reflect highly interwoven molecular, culture, and susceptibility results

Case 1: Women's/ Sexual Health Testing

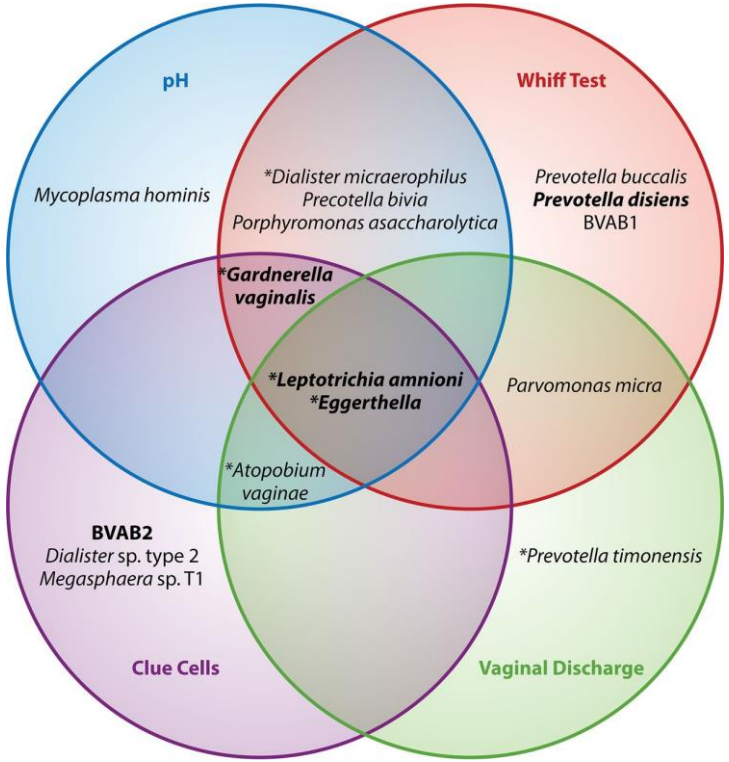
- Sexually transmitted disease incidence (CT and NG) has been rising in the US (1:5 has an STI)
- Untreated STIs can lead to pelvic inflammatory disease (PID), increased risk of HIV, certain cancers, and infertility
- Direct cost in the billions
- Difficulties in culturing *N. gonorrhoeae* drove the development of many of the transport media that we know today: Stuarts and Amies
- Chlamydia requires stringent cell culture conditions
- Unsurprisingly, testing for NG and CT was one of the first widely adopted molecular tests driven by Hologic (TMA), BD (Viper, SDA), and Roche & Cepheid (PCR) – BWH 2007
- Minimal need to correlate with other methods as culture is done only for NG (infrequently for susceptibility testing)





Case 1: Sexual and Women's health Testing

- Vaginosis is a dysbiosis – a shift of *Lactobacillus* spp to *Gardnerella* and others (*Prevotella* spp., *Atopobium vaginae*, *Sneathia* spp., *Megasphaera* spp., etc.)
- The most common cause of vaginal discharge in the US
- Associated with obstetric and gynecological complications as well as increased risk for acquisition of HIV and STIs
- Genital cultures lack specificity and are NOT recommended for the diagnosis of BV (*G. vaginalis* detected in 50-60% of asymptomatic women)
- Molecular assays that compute likelihood of BV based on ratios of multiple targets are gaining popularity and are increasingly covered by insurance as they become standard of care



Coleman et al 2018

Gardnerella vaginalis as a Cause of Bacterial Vaginosis: Appraisal of the Evidence From _in vivo_ Models

*Sydney Morrill[1,2], Nicole M. Gilbert[2,3,4] and Amanda L. Lewis[1,2,3]**

Case 1: Sexual and Women's health Testing

- IDSA/ASM Lab utilization guidelines 2024:
  - Multiplex molecular assays for detection of several organisms associated with bacterial vaginosis are more specific and sensitive than syndromic assessment alone (Amsel's), Nugent Gram stain or hybridization probe testing that only includes *G. vaginalis.*
  - In patients being tested for vaginitis, adding testing for CT/ NG identifies approximately 25% more infections in high-risk populations.
  - Aerobic vaginitis is a unique pathologic entity different from bacterial vaginosis that may require Gram stain and vaginal culture. Often labs will classify this specimen as wound to provide the appropriate work-up.
- Utility of performing on the same platform (same collection device) as STI testing provides comprehensive testing and logistical ease (BD, Cepheid, Hologic)
- As with STI – difficulty in performing lab level validation against the gold standard

Verification and workflow

- Vaginal culture replaced with BV and CV/TV (Candida/Trichomonas) assays
- Challenging to introduce new collection device to a large system
- Unable to do side by side comparison with culture
- Change in reporting:
  - Culture = normal vaginal flora, *Gardnerella vaginalis*, yeast, other organisms if pure in culture
  - Molecular = positive or negative for bacterial vaginosis, Candida, and Trichomonas
- Verifications specimens acquired from OSHs performing the same molecular assay
- Our lab's biggest challenge – specimen management
- How to best promote molecular testing while maintaining culture for specific clinical scenarios

BWH experience with molecular BV/CV/TV testing on the Hologic Aptima platform

- Last year we performed ~12000 BV/CV/TV assays
- Positivity rate is high (~30%) but in line with expected rates from epidemiological studies
- Difficult transition from culture to molecular in terms of communication and collection devices
  - Multiple discussions about the use of wound culture orders to allow for some culture-based testing – educational comments
  - Need to collect additional specimens for yeast susceptibility
- Overall positive feedback from providers
- Improved reimbursement climate
- Major labor savings in the laboratory (half the FTE, straightforward training)

Target	POS	% POS
BV	3935	34.06
CV	3341	28.92
C. glabrata	295	2.55
TV	182	1.58
TOTAL	11,554	

Data from 2023

Case2: Molecular Stool testing

- **Pros:**
  - More sensitive than culture for common bacterial pathogens
  - Cover a wide range of pathogens - including targets that often must be sent to reference labs
  - Shorter TAT
  - Less labor-intensive and require, overall, less training

TABLE 1 Comparative sensitivity of culture to the BD MAX EBP assay

Isolate type (from prepared stool samples)	Sensitivity (%) by organism concentration and measurement method									
	10^7 CFU/ml		10^6 CFU/ml		10^5 CFU/ml		10^4 CFU/ml		10^3 CFU/ml	
	BD MAX	Culture	BD MAX	Culture	BD MAX	Culture	BD MAX	Culture	BD MAX	Culture
Campylobacter[a]	NA[b]	NA[b]	100	100	100	100	100	63.8	100	43.8
EHEC (O157)[c]	100	100	100	75	100	75	87.5	12.5	13.3	0
Salmonella[d]	100	100	100	100	100	81.3	68.8	31.3	43.8	0
Shigella[d]	100	100	100	100	100	75	100	38	81	25

Anderson, JCM, 2014

- **Cons:**
  - Platform availability
  - Reagents costs
  - Recovering for EPI and susceptibility
  - Panel restrictions (loss of some targets, need to deal with less preferred targets)
  - **Less literature for viral and parasitic pathogens: gold standard methods vary from microscopy to antigen tests to PCR**

Case2: Molecular Stool testing

- A variety of FDA-cleared platform choices are available with different pros and cons (and more are on the way)
- Factors to consider include:
  - Test volume (large volume with intention to batch? Small volume with intention to run upon receipt?)
  - Target range
    - Option to offer sub-panels, i.e. viral?
    - Keep some targets as culture on smaller panels?
  - Integration with current platforms, test menu, and workflows
  - Billing (frequently used in outpatient setting)

BWH Stool Bacterial Panel Verification

Target	# Positives	Comments
Campylobacter	26 clinical from BWH and OSH	1 sample negative by BWH
Salmonella	21 clinical from BWH and OSH	Repeated positive with late Ct value. Specimen previously frozen
Shiga toxin	6 – clinical 17 – contrived	• 2 samples negative by BWH sent to OSH for confirmation. 1 positive, 1 negative • Contrived – pooled neg stool spiked with 3 dilution levels each of O111:H8 and O157:H7 run in triplicate.
Shigella/EIEC	9 clinical from OSH	Two specimens also pos for Campy which were negative by BWH
Bacterial negative	20 from BWH	All negative as expected

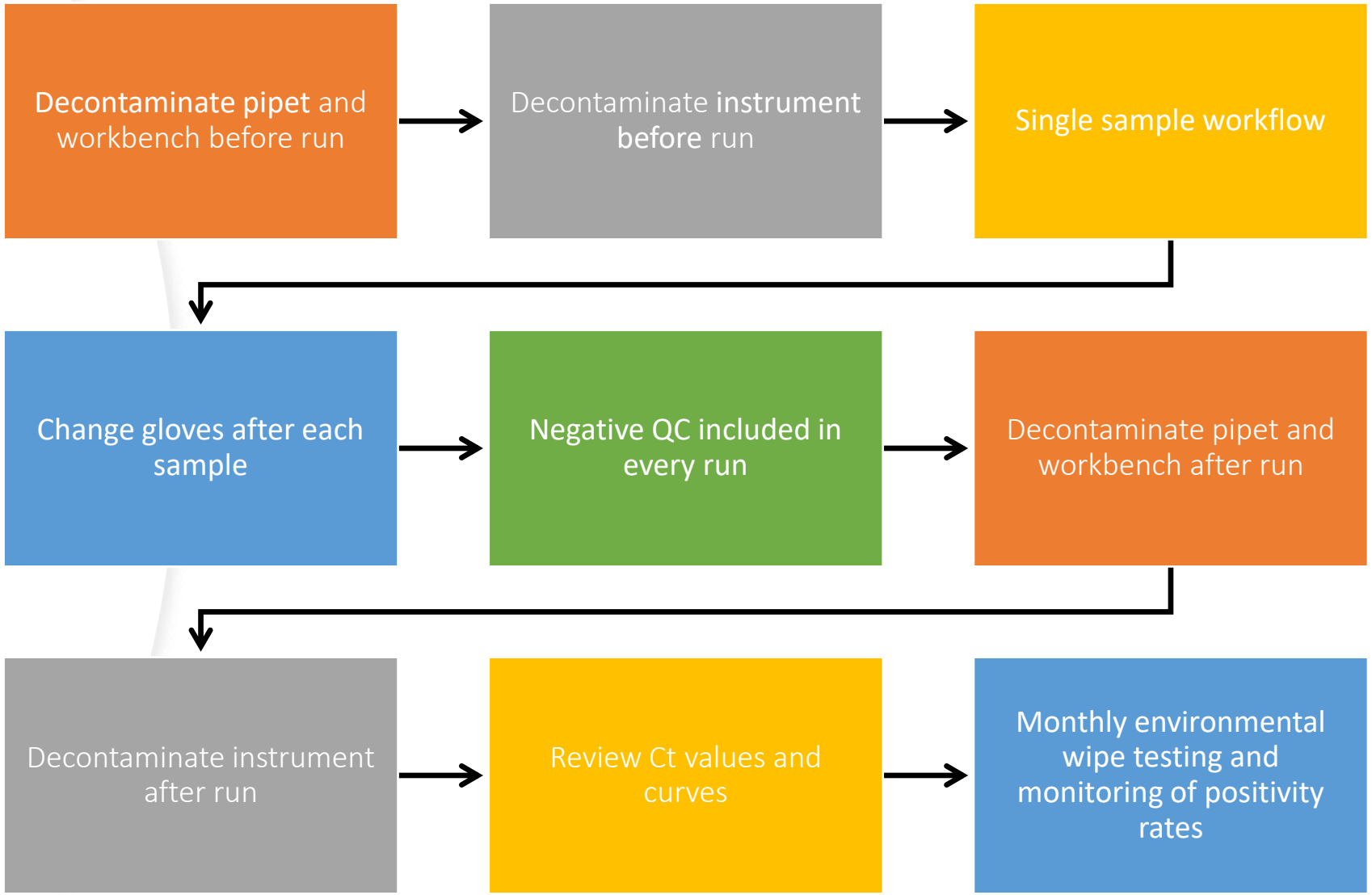
BWH Stool Viral Panel Verification

Target	# Positives	Comments
Norovirus	13 from OSH	• 3 samples were negative at BWH. Sent to 3rd lab which reported negative for all samples. • 1 sample reported with Noro/Sapovirus. Pos for noro only by BWH. Sent to 3rd lab and positive for Noro/Rotavirus
Rotavirus	5 from OSH	Rota only by OSH, Rota and Norovirus at BWH. Sent to 3rd lab for Noro only assay – positive for Noro.
Adenovirus	2 from OSH	Both positive for Adeno. One specimen also positive for Rotavirus. Repeat result of Adeno, Rota, Noro positive. Sent to 3rd lab – positive for Adeno, Rota, Noro.
Astrovirus/ Sapovirus	0	No clinical samples available. Verified by QC material.
Viral negative	10 from OSH	Phew!

*Precision – two positive and two negative samples performed in triplicate over three days. Checkerboarded

**Rotavirus QC produced false positive Adeno result

Stool Panel Workflow: Importance of Molecular Hygiene



Stool Testing: Integrating Molecular and Culture Techniques

- Positive Salmonella and Shigella reflexed to culture
- Indeterminate samples reflexed to culture
- Reflex procedure performed by molecular tech
- Reason for culture reflex communicated within stool

S8478516 Collect D/T: 09/21/2024 1502 **Receive D/T: 09/21/2024 1602**

Order account #: **Order location: BWTRNMC**

Order physician: UNKNOWN,UNKNOWN

STOOL CULTURE

Setup D/T: 09/21/2024 1602

SPECIMEN DESCRIPTION	STOOL	(4596) [BW]
SPECIAL REQUESTS	Reflexed from indeterminant PCR	(4596) [BW]
CULTURE / TEST	PEND	
REPORT STATUS	PEND	

Stool Testing: Integrating Molecular and Culture Techniques

- Communication between molecular and bacteriology technologists
- Language developed for reporting a negative culture
- LIS issues integrating molecular and culture results

S8478503 Collect D/T: 09/21/2024 1500 Receive D/T: 09/21/2024 1600
Order account #: Order location: BWTRNMC
Order physician: UNKNOWN,UNKNOWN

STOOL CULTURE
Setup D/T: 09/21/2024 1600

SPECIMEN DESCRIPTION	STOOL	(4596) [BW]
SPECIAL REQUESTS	Reflexed from positive PCR	(4596) [BW]
CULTURE / TEST	Culture has been reflexed from Bacterial PCR due to detection of Salmonella or Shigella which have not grown in culture. This may be due to the greater sensitivity of PCR.	(4596) [BW]
REPORT STATUS	FINAL 09/21/2024	(4596) [BW]

BWH experience with molecular stool testing on the BD Max

Bacterial Panel

Target	POS PCR 2023-2024	% POS	POS Culture (STX antigen) 2022-2023	% POS	fold change POS rate
CAMPYLOBACTER PCR	152	3.00	59	1.28	2.3
SALMONELLA PCR	63	1.25	48	1.04	1.2
SHIGA TOXIN PCR	26	0.51	4	0.41	1.3
SHIGELLA PCR	48	0.95	11	0.24	4.0
TOTAL	5060	Bacterial PCR	4597	Bacterial culture	
			978	Shiga toxin antigen	

Viral Panel

Target	POS PCR	% POS
ADENOVIRUS PCR	16	0.66
HUMAN ASTROVIRUS PCR	34	1.41
NOROVIRUS PCR	102	4.22
ROTAVIRUS PCR	33	1.37
SAPOVIRUS PCR	48	1.99
TOTAL	2417	Viral PCR

- Compared to the previous year, test volume increased ~10%
- Positivity rates increased for all targets, particularly Campy
- Positivity rate increase in Shigella may also reflect presence of EIEC (Ct values in quality monitors)
- ~50% as many viral panels ordered (reagent savings by using a 2 cartridge system)

Case 3: Molecular assays for bloodstream infections

- Direct-from-blood assays continue to be elusive
- Molecular assays on positive blood cultures are becoming (are?) standard of care
  - Multiple platforms available
  - Some have Gram-stain specific panels
- Literature is varied, but they are widely associated with a decreased time to appropriate therapy
  - Unclear impact on length of stay (may vary by context)
- BWH recently adopted the BCID-2

BWH BCID Verification

Gram Positives	Total # ID'd	# FA	# FN
Enterococcus faecalis	3	1	2
Enterococcus faecium	3	0	3
Listeria monocytogenes	0	0	0
Staphylococcus spp.	21	12	9
Staphylococcus aureus	10	6	4
Staphylococcus epidermidis	9	7	2
Staphylococcus lugdunensis	2	0	2
Streptococcus spp.	7	6	1
Streptococcus agalactiae (group B)	1	1	0
Streptococcus pneumoniae	1	1	0
Streptococcus pyogenes (group A)	2	2	0

No Growth	Total # ID'd	# FA	# FN
	22	11	11

Gram Negatives	Total # ID'd	# FA	# FN
Acinetobacter calcoaceticus-baumannii complex	1	1	0
Bacteroides fragilis	1	0	1
Haemophilus influenzae	2	1	1
Neisseria meningitidis	0	0	0
Pseudomonas aeruginosa	3	3	0
Stenotrophomonas maltophilia	1	1	0
Enterobacterales	38	21	17
Enterobacter cloacae complex	4	1	3
Escherichia coli	12	8	4
Klebsiella aerogenes	1	0	1
Klebsiella oxytoca	2	1	1
Klebsiella pneumoniae group	11	6	5
Proteus spp.	4	2	2
Salmonella spp.	3	1	2
Serratia marcescens	2	1	1

Yeast	Total # ID'd	# FA	# FN
Candida albicans	5	4	1
Candida auris	0	0	0
Candida glabrata	1	1	0
Candida krusei	1	1	0
Candida parapsilosis	1	1	0
Candida tropicalis	1	1	0
Cryptococcus neoformans/gattii	1	1	0

Culture Pos, Panel Neg	Organism
1	Priestia megaterium
1	Sphingomonas paucimoblis
1	Bacillus cereus group
1	Clostridium septicum
1	Lactobacillus casei/paracasei/rhamnosus
1	Corynebaterium spp
1	Bacteroides thetaiotaomicron
1	Actinomyces odontolyticus
1	Chryseobacterium Indologenes, Arthrobacter spp.
1	GNR, unable to ID
1	Enterococcus gallinarum
1	Candida dubliniensis

Resistance Genes	Total # ID'd	# FA	# FN
CTX-M	13	7	6
IMP	0	0	0
KPC	0	0	0
mcr-1	0	0	0
mecA/C	6	4	2
mecA/C and MREJ	4	1	3
NDM	0	0	0
OXA-48-like	0	0	0
vanA/B	1	0	1
VIM	0	0	0

Performed spike-ins with strains characterized by WGS:

- Members of *Enterobacter cloacae* complex and *Klebsiella pneumoniae* group
- KPC
- NDM
- IMP
- OXA
- CTX

BWH BCID Verification – Precision with 8 modules

Pool 1
Acinetobacter calcoaceticus-baumannii complex
Candida albicans
Enterococcus faecalis (vanB)
Enterococcus faecium (vanA)
Staphylococcus spp.
Staphylococcus aureus (*mecA* and MREJ)
Streptococcus spp.
Streptococcus agalactiae (group B)
Streptococcus pyogenes (group A)

Pool 2
Candida glabrata
Candida krusei
Enterobacterales
Enterobacter cloacae complex
Haemophilus influenzae
Klebsiella oxytoca
Listeria monocytogenes
Staphylococcus spp.
Staphylococcus epidermidis

Pool 3
Bacteroides fragilis
Candida parapsilosis
Candida tropicalis
Enterobacterales
Klebsiella pneumoniae group (KPC)
Pseudomonas aeruginosa (VIM)
Serratia marcescens
Streptococcus spp.
Streptococcus pneumoniae

Pool 4
Candida auris
Enterobacterales
Escherichia coli Z521 *(mcr-1)*
Escherichia coli Z297 (IMP)
Klebsiella aerogenes
Neisseria meningitidis
Proteus mirabilis
Stenotrophomonas maltophilia

Pool 5
Cryptococcus gattii
Cryptococcus neoformans
Enterobacterales
Klebsiella pneumoniae group Z138 (OXA-48-like)
Klebsiella pneumoniae group Z460 (CTX-M and NDM)
Salmonella enterica typhimurium
Staphylococcus spp.
Staphylococcus lugdunensis

Precision plan:

- 5 pools of organisms/AMR
- Run in quadruplicate
- Performed on different dates by different technologists on different modules

Staff Training and Workflow

- What is your staff's background knowledge?
- What is your staff's molecular experience?
- What is your staff's bacteriology experience?
- How many shifts need to be trained?
- How to assist with result review in a timely fashion?

BWH BCID Staff Training and Workflow

BioFire Blood Cul

BioFire Blood Culture

BioFire Blo

Identification

Introductio

Correlating and

BioFire Blood Culture

Identification 2 (BCID2) Panel

Introduction Session 4: Scenarios

HOOL

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BIOFIRE BY BIOMÉRIEUX

BIOFIRE BY BIOMÉRIEUX

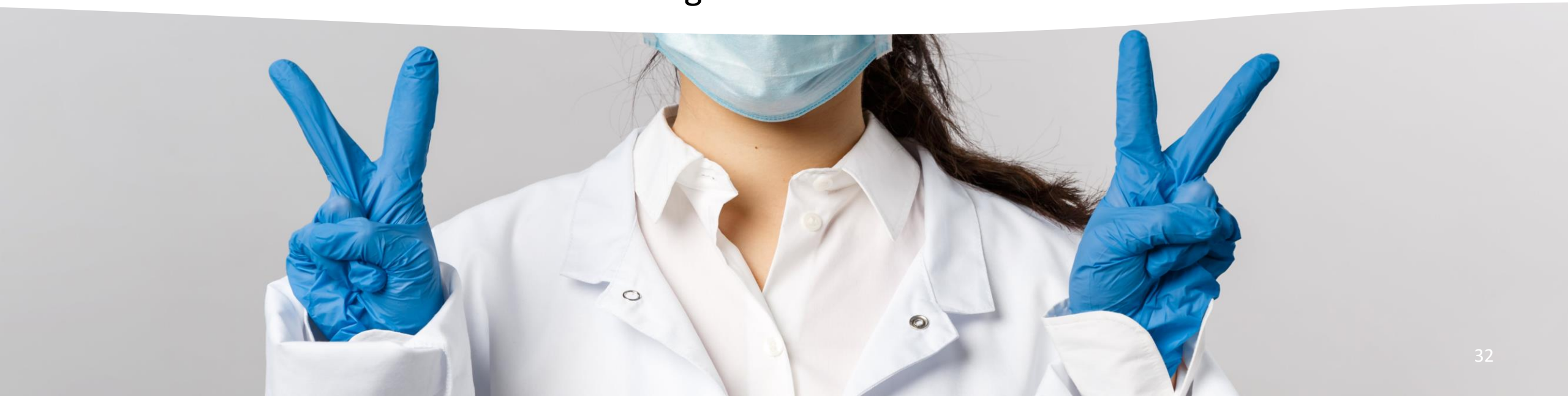
BRIGHAM HEALTH

BRIGHAM AND WOMEN'S HOSPITAL

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

Procedure – Set Up

- **STERILITY IS CRUCIAL!**
  - Billions of copies from just 1 target → **EASY TO CONTAMINATE!**
- **Only 1 person in the hood**
- **Only 1 bottle in the hood**
- **Always clean before/after procedure AND between patient samples**
- Clean with bleach Sani-Cloth followed by DI Water:
  - Hood surface
  - Pouch Loading Station



Positive Blood Culture Workflow

START

Positive bottle: GRAM STAIN

- Enter gram stain in Sunquest, **CALL GRAM STAIN RESULT**
- Always enter ETC code **BCIDGS** into last line

  Note: If this is the 2nd bottle of the set, delete previous code BCIDGS and re-enter on last observation line after 2nd bottle GS result
- Should a BCID be run?

  Rule: One BCID per 7 days (from collect date to collect date) with the **same Gram stain result**

Organisms Seen

NO

No BCID2. Add ETC code **BCID7D** under gram stain and suppress.

No Organisms Seen

- **No BCID2**
- Follow NOS protocol

YES

- Order BCID2.
- Using large CID label for panel, print 5 small CID labels.
- Label bottle, cartridge, sterile tube, and binder with small CID stickers. Save last sticker for results.
- Add BCID2 accession # to BC54 culture workup notes.

Run BCID2. **CORRELATE** with Gram stain.

Results agree

- Release to chart
- Record results in positive blood log
- Label printout with BC54 and BCID2 stickers
- Save printout in the BCID2 binder
- Do NOT call BCID2 results

Results DO NOT agree

- **Follow Resulting and Troubleshooting Guides before proceeding!!!**
- Review results with Supervisor before reporting
- If no supervisor available, **DO NOT** report BCID2 result. Add ETC code **BCIDPN** below gram stain results.
- Email Micro Special Requests for a follow up.
- Document results in blood binder and save printout.
- If results have been reviewed and released, replace code BCIDPN with code **BCIDRR**.
- If results have been reviewed and NOT released, replace code BCIDPN with code **BCIDHC**. Credit BCID2.

Procedure – Set Up

Good molecular practice:
- ✓ Use clean gloves
- ✓ Don't reach lab coat sleeves into supply bags

- **Clean → Change gloves → Gather supplies**
- Gather supplies:
  - Vacuum-sealed pouch
  - 1 sample buffer ampoule (do not touch tip!)
  - 1 hydration injection vial (blue)
  - 1 sample injection vial (red)
  - 1 transfer pipette

  } Included in BCID2 kit (kept at room temp)

  - 1 sterile 5.0 mL secondary tube
  - 3.0 mL syringe
  - Saf-T Holder Blood Culture Device with Female Luer Adapter
  - BCID2 patient labels (5)

BioFire®
Blood Culture Identification 2 (BCID2) Panel - IVD

BIOFIRE BY BIOMÉRIEUX

www.BioFireDx.com

Run Summary

Sample ID:	703NSICMOD8	**Run Date:**	01 Dec 2022 11:42 AM
Organisms Detected:	None	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes Detected:	None		

⚠ **Note:** Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate

Result Summary

Antimicrobial Resistance Genes

⊘	N/A	CTX-M
⊘	N/A	IMP
⊘	N/A	KPC
⊘	N/A	*mcr-1*
⊘	N/A	*mecA/C*
⊘	N/A	*mecA/C* and MREJ (MRSA)
⊘	N/A	NDM
⊘	N/A	OXA-48-like
⊘	N/A	*vanA/B*
⊘	N/A	VIM

Gram Positive Bacteria

Not Detected	*Enterococcus faecalis*
Not Detected	*Enterococcus faecium*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus* spp.
Not Detected	*Staphylococcus aureus*
Not Detected	*Staphylococcus epidermidis*
Not Detected	*Staphylococcus lugdunensis*
Not Detected	*Streptococcus* spp.
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)

Gram Negative Bacteria

Not Detected	*Acinetob… baceticus-baumannii* complex
Not Detected	*Bact… fragilis*
Not Detected	*…bacterales*
	Enterobacter cloacae complex
	Escherichia coli
	Klebsiella aerogenes
	Klebsiella oxytoca
	Klebsiella pneumoniae group
	Proteus spp.
Not Detected	*Salmonella* spp.
Not Detected	*…ratia marcescens*
Not Detected	*Haemo… influenzae*
Not Detected	*Neisseria m… itidis*
Not Detected	*Pseudomonas … osa*
Not Detected	*Stenotrophomonas … ilia*

Yeast

Not Detected	*Candida albicans*
Not Detected	*Candida auris*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*
Not Detected	*Cryptococcus neoformans/gattii*

Results are divided into sections

Run Details

Pouch:	BCID2 Panel v1.0	**Protocol:**	BC2 v3.0
Run Status:	Completed	**Operator:**	dongjing gao (dg730)
Serial No.:	56099680	**Instrument:**	TM03935
Lot No.:	2APF22		

BioFire®
Blood Culture Identification 2 (BCID2) Panel - IVD

BIOFIRE BY BIOMÉRIEUX

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Run Summary

Sample ID: 703NSICMOD8 | Run Date: 01 Dec 2022 11:42 AM
Organisms Detected: None | Controls: Passed
Applicable Antimicrobial Resistance Genes Detected: None

⚠ Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate

Result Summary

Gram Negative Bacteria

Not Detected *Acinetob… baceticus-baumannii* complex
Not Detected *Bact… fragilis*
Not Detected *…bacterales*
Enterobacter cloacae complex
Escherichia coli
Klebsiella aerogenes
Klebsiella oxytoca
Klebsiella pneumoniae group
Proteus spp.
Not Detected *Salmonella* spp.
Not Detected *…ratia marcescens*
Not Detected *Haemo… influenzae*
Not Detected *Neisseria m… itidis*
Not Detected *Pseudomonas … osa*
Not Detected *Stenotrophomonas … ilia*

Yeast

Not Detected *Candida albicans*
Not Detected *Candida auris*
Not Detected *Candida glabrata*
Not Detected *Candida krusei*
Not Detected *Candida parapsilosis*
Not Detected *Candida tropicalis*
Not Detected *Cryptococcus neoformans/gattii*

Run Details

Pouch: BCID2 Panel v1.0 | Protocol: BC2 v3.0
Run Status: Completed | Operator: dongjing gao (dg730)
Serial No.: 56099680 | Instrument: TM03935
Lot No.: 2APF22

BioFire®
Blood Culture Identification 2 (BCID2) Panel - IVD

BIOFIRE by BIOMÉRIEUX

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Run Summary

Sample ID:	703NSICMOD8	**Run Date:**	01 Dec 2022 11:42 AM
Organisms Detected:	None	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes Detected:	None		

⚠ **Note:** Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Result Summary

Antimicrobial Resistance Genes

⊘	N/A	CTX-M
⊘	N/A	IMP
⊘	N/A	KPC
⊘	N/A	*mcr-1*
⊘	N/A	*mecA/C*
⊘	N/A	*mecA/C* and MREJ (MRSA)
⊘	N/A	NDM
⊘	N/A	OXA-48-like
⊘	N/A	*vanA/B*
⊘	N/A	VIM

Gram Positive Bacteria

Not Detected	*Enterococcus faecalis*
Not Detected	*Enterococcus faecium*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus* spp.
Not Detected	*Staphylococcus aureus*
Not Detected	*Staphylococcus epidermidis*
Not Detected	*Staphylococcus lugdunensis*
Not Detected	*Streptococcus* spp.
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)

Genus-level identification for species that are not included for Staph and Strep

Run Details

Pouch:	BCID2 Panel v1.0	**Protocol:**	BC2 v3.0
Run Status:	Completed	**Operator:**	dongjing gao (dg730)
Serial No.:	56099680	**Instrument:**	TM03935
Lot No.:	2APF22		

BioFire®
Blood Culture Identification 2 (BCID2) Panel - IVD

BIOFIRE by BIOMÉRIEUX

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Run Summary

Sample ID:	703NSICMOD8	**Run Date:**	01 Dec 2022 11:42 AM
Organisms Detected:	None	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes Detected:	None		

⚠ **Note:** Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Result Summary

Gram Negative Bacteria

Not Detected	*Acinetobacter calcoaceticus-baumannii* complex
Not Detected	*Bacteroides fragilis*
Not Detected	*Enterobacterales*
Not Detected	*Enterobacter cloacae* complex
Not Detected	*Escherichia coli*
Not Detected	*Klebsiella aerogenes*
Not Detected	*Klebsiella oxytoca*
Not Detected	*Klebsiella pneumoniae* group
Not Detected	*Proteus* spp.
Not Detected	*Salmonella* spp.
Not Detected	*Serratia marcescens*
Not Detected	*Haemophilus influenzae*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*
Not Detected	*Stenotrophomonas maltophilia*

Order-level identification for species that are not included for Enterobacterales

Yeast

Not Detected	*Candida albicans*
Not Detected	*Candida auris*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*
Not Detected	*Cryptococcus neoformans/gattii*

Run Details

Pouch:	BCID2 Panel v1.0	**Protocol:**	BC2 v3.0
Run Status:	Completed	**Operator:**	dongjing gao (dg730)
Serial No.:	56099680	**Instrument:**	TM03935
Lot No.:	2APF22		

BioFire®
Blood Culture Identification 2 (BCID2) Panel - IVD

BIOFIRE BY BIOMÉRIEUX

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Run Summary

Sample ID:	452 S AUR MOD 6	**Run Date:**	30 Nov 2022 11:36 AM
Organisms Detected:	*Staphylococcus* spp.	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes Detected:	None		

⚠ Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Result Summary

Antimicrobial Resistance Genes

⊘	N/A	CTX-M
⊘	N/A	IMP
⊘	N/A	KPC
⊘	N/A	*mcr-1*
⊘	N/A	*mecA/C*
⊘	N/A	*mecA/C* and MREJ (MRSA)
⊘	N/A	NDM
⊘	N/A	OXA-48-like
⊘	N/A	*vanA/B*
⊘	N/A	VIM

Gram Positive Bacteria

	Not Detected	*Enterococcus faecalis*
	Not Detected	*Enterococcus faecium*
	Not Detected	*Listeria monocytogenes*
✓	Detected	*Staphylococcus* spp.
	Not Detected	*Staphylococcus aureus*
	Not Detected	*Staphylococcus epidermidis*
	Not Detected	*Staphylococcus lugdunensis*
	Not Detected	*Streptococcus* spp.
	Not Detected	*Streptococcus agalactiae* (Group B)
	Not Detected	*Streptococcus pneumoniae*
	Not Detected	*Streptococcus pyogenes* (Group A)

Gram Negative Bacteria

Not Detected	*Acinetobacter calcoaceticus-baumannii* complex
Not Detected	*Bacteroides fragilis*
Not Detected	*Enterobacterales*
Not Detected	*Enterobacter cloacae* complex
Not Detected	*Escherichia coli*
Not Detected	*Klebsiella aerogenes*
Not Detected	*Klebsiella oxytoca*
Not Detected	*Klebsiella pneumoniae* group
Not Detected	*Proteus* spp.
Not Detected	*Salmonella* spp.
Not Detected	*Serratia marcescens*
Not Detected	*Haemophilus influenzae*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*
Not Detected	*Stenotrophomonas maltophilia*

Yeast

Not Detected	*Candida albicans*
Not Detected	*Candida auris*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*
Not Detected	*Cryptococcus neoformans/gattii*

This sample has a species of Staph that is ***not*** aureus, epidermidis, or lugdunensis.

Run Details

Pouch:	BCID2 Panel v1.0	**Protocol:**	BC2 v3.0
Run Status:	Completed	**Operator:**	paola villarroel (pav)
Serial No.:	56100451	**Instrument:**	TM0D356
Lot No.:	2APF22		

BioFire®
Blood Culture Identification 2 (BCID2) Panel - IVD

BIOFIRE BY BIOMÉRIEUX

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Run Summary

Sample ID:	452 S AUR MOD 6	**Run Date:**	30 Nov 2022 11:36 AM
Organisms Detected:	*Staphylococcus* spp.	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes Detected:	None		

⚠ Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Result Summary

Gram Negative Bacteria

Not Detected	*Acinetobacter calcoaceticus-baumannii* complex
Not Detected	*Bacteroides fragilis*
Not Detected	*Enterobacterales*
Not Detected	*Enterobacter cloacae* complex
Not Detected	*Escherichia coli*
Not Detected	*Klebsiella aerogenes*
Not Detected	*Klebsiella oxytoca*
Not Detected	*Klebsiella pneumoniae* group
Not Detected	*Proteus* spp.
Not Detected	*Salmonella* spp.
Not Detected	*Serratia marcescens*
Not Detected	*Haemophilus influenzae*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*
Not Detected	*Stenotrophomonas maltophilia*

Yeast

Not Detected	*Candida albicans*
Not Detected	*Candida auris*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*
Not Detected	*Cryptococcus neoformans/gattii*

Run Details

Pouch:	BCID2 Panel v1.0	**Protocol:**	BC2 v3.0
Run Status:	Completed	**Operator:**	paola villarroel (pav)
Serial No.:	56100451	**Instrument:**	TM0D356
Lot No.:	2APF22		

Blood Culture Identification 2 (BCID2) Panel - IVD

BIOFIRE by bioMérieux

www.BioFireDx.com

Run Summary

Sample ID:	SPIKE 208 MOD 3
Organisms Detected:	*Enterobacterales* *Klebsiella pneumoniae* group
Applicable Antimicrobial Resistance Genes Detected:	CTX-M KPC

This sample has a species that **is** included in the Enterobacterales list, so results are positive for ***BOTH*** **Enterobacterales** and **Klebsiella pneumoniae group**.

This organism is also positive for 2 different resistance genes.

Result Summary

✓	Detected	CTX-M
	Not Detected	IMP
✓	Detected	KPC
	Not Detected	*mcr-1*
⊘	N/A	*mecA/C*
⊘	N/A	*mecA/C* and MREJ (MRSA)
	Not Detected	NDM
	Not Detected	OXA-48-like
⊘	N/A	*vanA/B*
	Not Detected	VIM

Gram Positive Bacteria

Not Detected	*Enterococcus faecalis*
Not Detected	*Enterococcus faecium*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus* spp.
Not Detected	*Staphylococcus aureus*
Not Detected	*Staphylococcus epidermidis*
Not Detected	*Staphylococcus lugdunensis*
Not Detected	*Streptococcus* spp.
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)

Pouch:	BCID2 Panel v1.0	**Protocol:**	BC2 v3.0
Run Status:	Completed	**Operator:**	Arielle Holtz (AH)
Serial No.:	83041926	**Instrument:**	TM16198
Lot No.:	2ZE523		

Blood Culture Identification 2 (BCID2) Panel - IVD

BIOFIRE by bioMérieux

www.BioFireDx.com

D:	SPIKE 208 MOD 3
d:	*Enterobacterales* *Klebsiella pneumoniae* group
al d:	CTX-M KPC

Run Date:	17 Oct 2023 8:09 AM
Controls:	Passed

an occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate

Result Summary

Gram Negative Bacteria

Not Detected	*Acinetobacter calcoaceticus-baumannii* complex
Not Detected	*Bacteroides fragilis*
✓ Detected	*Enterobacterales*
Not Detected	*Enterobacter cloacae* complex
Not Detected	*Escherichia coli*
Not Detected	*Klebsiella aerogenes*
Not Detected	*Klebsiella oxytoca*
✓ Detected	*Klebsiella pneumoniae* group
Not Detected	*Proteus* spp.
Not Detected	*Salmonella* spp.
Not Detected	*Serratia marcescens*
Not Detected	*Haemophilus influenzae*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*
Not Detected	*Stenotrophomonas maltophilia*

Yeast

Not Detected	*Candida albicans*
Not Detected	*Candida auris*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*
Not Detected	*Cryptococcus neoformans/gattii*

Pouch:	BCID2 Panel v1.0	**Protocol:**	BC2 v3.0
Run Status:	Completed	**Operator:**	Arielle Holtz (AH)
Serial No.:	83041926	**Instrument:**	TM16198
Lot No.:	2ZE523		

Blood Culture Identification 2 (BCID2) Panel - IVD

BIOFIRE BY BIOMÉRIEUX

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Run Summary

Sample ID:	SPIKE 208 MOD 3	**Run Date:**	17 Oct 2023 8:09 AM
Organisms Detected:	*Enterobacterales* *Klebsiella pneumoniae* group	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes Detected:	CTX-M KPC		

Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for antimicrobial resistance gene(s) does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Result Summary

Antimicrobial Resistance Genes

✓	Detected	CTX-M
	Not Detected	IMP
✓	Detected	KPC
	Not Detected	*mcr-1*
⊘	N/A	*mecA/C*
⊘	N/A	*mecA/C* and MREJ (MRSA)
	Not Detected	NDM
	Not Detected	OXA-48-like
⊘	N/A	*vanA/B*
	Not Detected	VIM

Gram Positive Bacteria

Not Detected	*Enterococcus faecalis*
Not Detected	*Enterococcus faecium*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus* spp.
Not Detected	*Staphylococcus aureus*
Not Detected	*Staphylococcus epidermidis*
Not Detected	*Staphylococcus lugdunensis*
Not Detected	*Streptococcus* spp.
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)

Run Details

Pouch:	BCID2 Panel v1.0	**Protocol:**	BC2 v3.0
Run Status:	Completed	**Operator:**	Arielle Holtz (AH)
Serial No.:	83041926	**Instrument:**	TM16198
Lot No.:	2ZE523		

- Possible results for AMR:
  - **Detected**
  - **Not Detected**
  - **Not Applicable (N/A)**
- BCID2 will only result positive AMR results for organisms that the gene applies to
- In this example:
  - Genes relevant to Klebsiella pneumoniae (gram negative) are resulted as **Detected**/**Not Detected**
  - Genes relevant to gram positive bacteria are resulted as **N/A**

Gram Positive Correlation Table

Gram Stain		Organism	Comments
Gram Positives	**Gram Positive Cocci in Pairs/Clusters**	***Staphylococcus* spp.**	
		Staphylococcus aureus	
		Staphylococcus epidermidis	
		Staphylococcus lugdunensis	
	Gram Positive Cocci in Pairs/Chains	*Enterococcus faecalis*	
		Enterococcus faecium	
	Gram Positive Cocci in Pairs/Chains	***Streptococcus* spp.**	
		Streptococcus pyogenes (group A)	
		Streptococcus agalactiae (group B)	
	Gram Positive Cocci in Pairs/Short Chains	*Streptococcus pneumoniae*	
	Gram Positive Rods	*Listeria monocytogenes*	
	Yeast/Budding Yeast	*Candida albicans*	*For any type of yeast detected on GS or BCID2 panel, set up ChromAgar plate and add to culture workup*
		Candida auris	
		Candida glabrata	
		Candida krusei	
		Candida parapsilosis	
		Candida tropicalis	
	Yeast/Round Yeast Cells	*Cryptococcus neoformans/gattii*	**Round cells**

Gram Negative Correlation Table

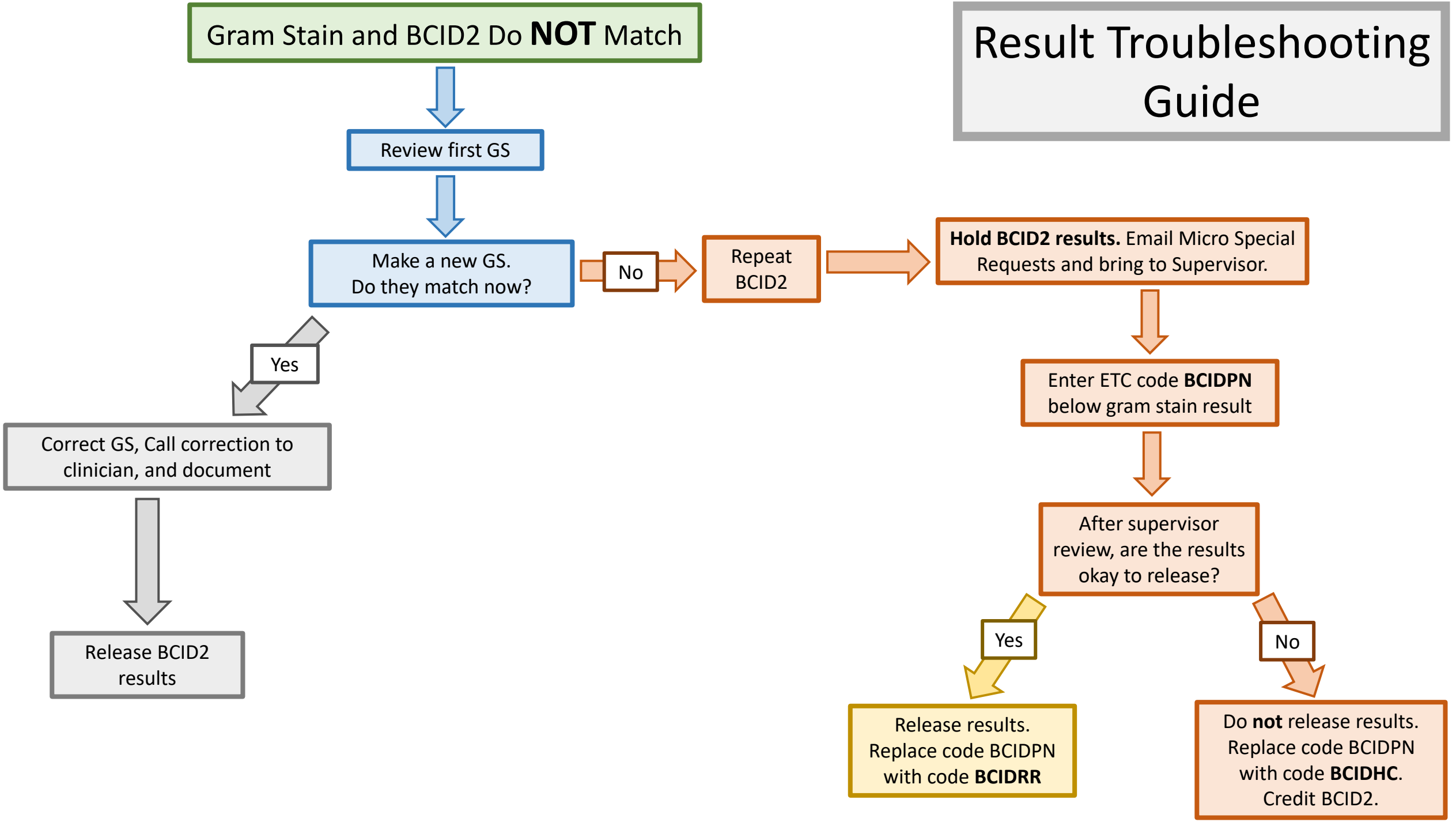
Gram Stain		Organism	Comments
Gram Negatives	**Gram Negative Diplococci (GNDC)**	*Neisseria meningitidis*	**Seal Plates/Email Micro Special Requests**
	Gram Negative Coccobacilli (GNCB)	*Haemophilus influenzae*	For other GNCB that are **NOT** identified as *H. influenzae*: **Seal Plates/Email Micro Special Requests**
	Gram Negative Rods	**Enterobacterales**	
		Enterobacter cloacae complex	
		Escherichia coli	
		Klebsiella aerogenes	
		Klebsiella oxytoca	
		Klebsiella pneumoniae group	
		Proteus spp.	
		Salmonella spp.	
		Serratia marcescens	
		Pseudomonas aeruginosa	
		Acinetobacter calcoaceticus-baumannii complex	GNR but may appear as GNCB or GNDC
		Stenotrophomonas maltophilia	
		Bacteroides fragilis	Small GNR. Set up anaerobic plates Brucella and LKV.

Resulting Decision Guide

Scenario	Gram Stain Result*	BCID2 Result	Do They Correlate?	What To Do
1	1 morphotype	1 organism	Yes	Okay to Result
2	1 morphotype	1 organism	No	See Troubleshooting Guide
3	1 morphotype	2 or more organisms	No or Partial (*i.e*. correlation between Gram stain and only one of the BCID2 targets)	See Troubleshooting Guide
4	1 morphotype	2 or more organisms	Yes	Okay to Result
5	2 or more morphotypes	1 or more organism	If **each BCID2** result matches a Gram Stain morphotype	Okay to Result
6	2 morphotypes	2 organisms	Yes	Okay to Result
7	1 or more morphotypes	None	Not applicable	Okay to Result
8	GVR	None	Not applicable	Okay to Result
9	GVR	GNR	No	See GVR Guide
10	GVR	Listeria	No	See GVR Guide
11	NOS	Do not run. Follow NOS protocol.	Not applicable	Not applicable

* Morphotype refers to distinct Gram stain pattern of microorganism, such as Gram positive cocci in clusters, Gram positive cocci in chains, Gram negative rods, Yeast, etc..

Result Troubleshooting Guide

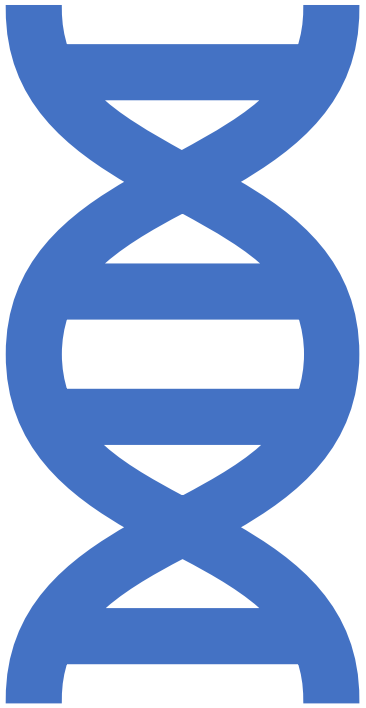


Case 3: Molecular assays for bloodstream infections (Clinical and quality challenges)

- For optimal impact, bloodstream molecular panels have to be tightly coordinated with infectious disease and pharmacy groups for actionability
- Guidance for interpretation and actions needs to be clearly presented and linked with the result, i.e. how many clinicians understand the significance of a CTX? How it differs from an AmpC? Know which species fall into the Enterobacterales?
- Link to site specific guidance document within the BCID test result
  - Range of organisms on the assay
  - Explanation of genus vs. species level results
  - Significance of species and resistance genes with definitions and clinical recommendations for treatment
  - Explanation of assay workflow
- Results are actively monitored by ID pharmacy

Case 3: Molecular assays for bloodstream infections (Clinical and quality challenges)

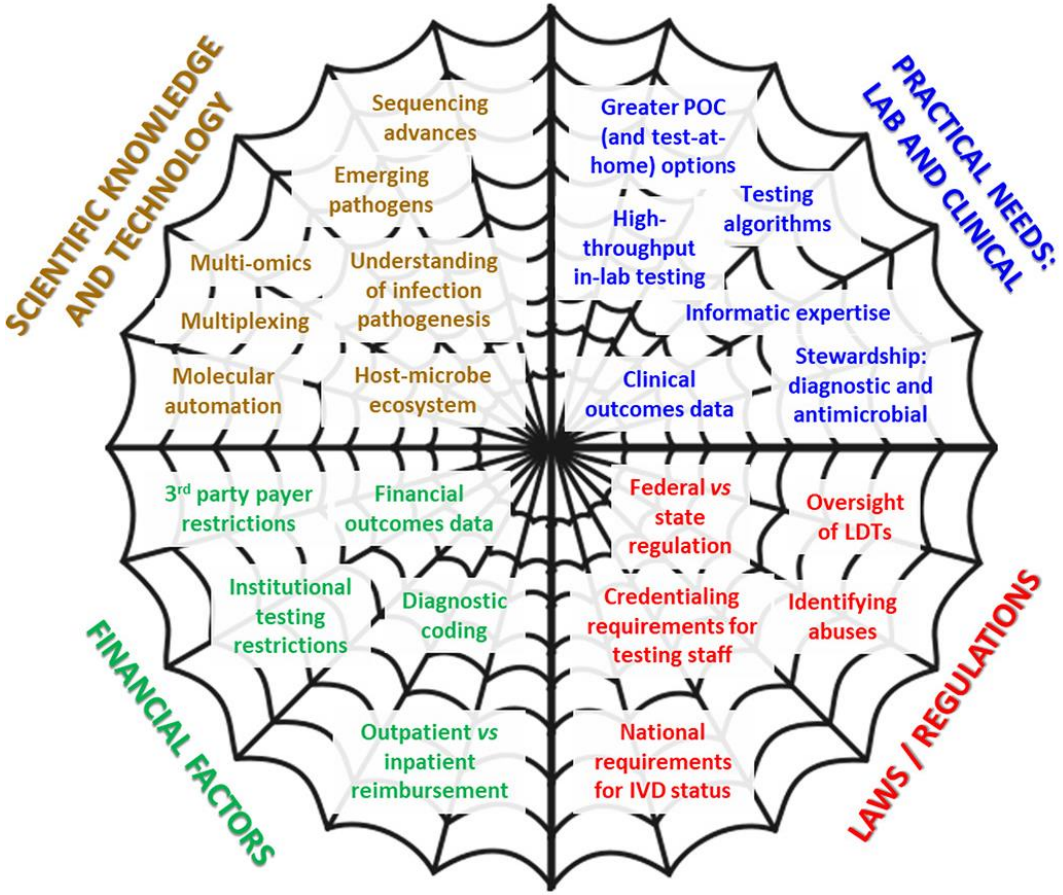
- We have been live for 10 months and have run ~1800 panels
- Our larger quality program has focused on integrating BCID results with our Gram stain accuracy assessments
- BCID performance assessments have been focused on correlation with culture
  - Overall sensitivity and specificity
  - Sensitivity in polymicrobial infections
  - Correlation of susceptibility genotype with phenotype
- Common questions
  - BCID:culture concordance on mixed CONS infections
  - Presence of ceftriaxone resistance in the absence of a CTX enzyme



Summary

- Molecular assays continue to change the shape of the clinical microbiology lab
  - New technologies
  - Different staffing and training models
  - Molecular literacy and hygiene
  - Communication with clinical teams
- Increasingly not run as isolated tests, but integrated with traditional culture and susceptibility assays
- Optimal workflow and staffing approaches are laboratory-dependent, but even those groups with defined culture-based staff groups need to educate those about molecular assays and how to incorporate results

Summary



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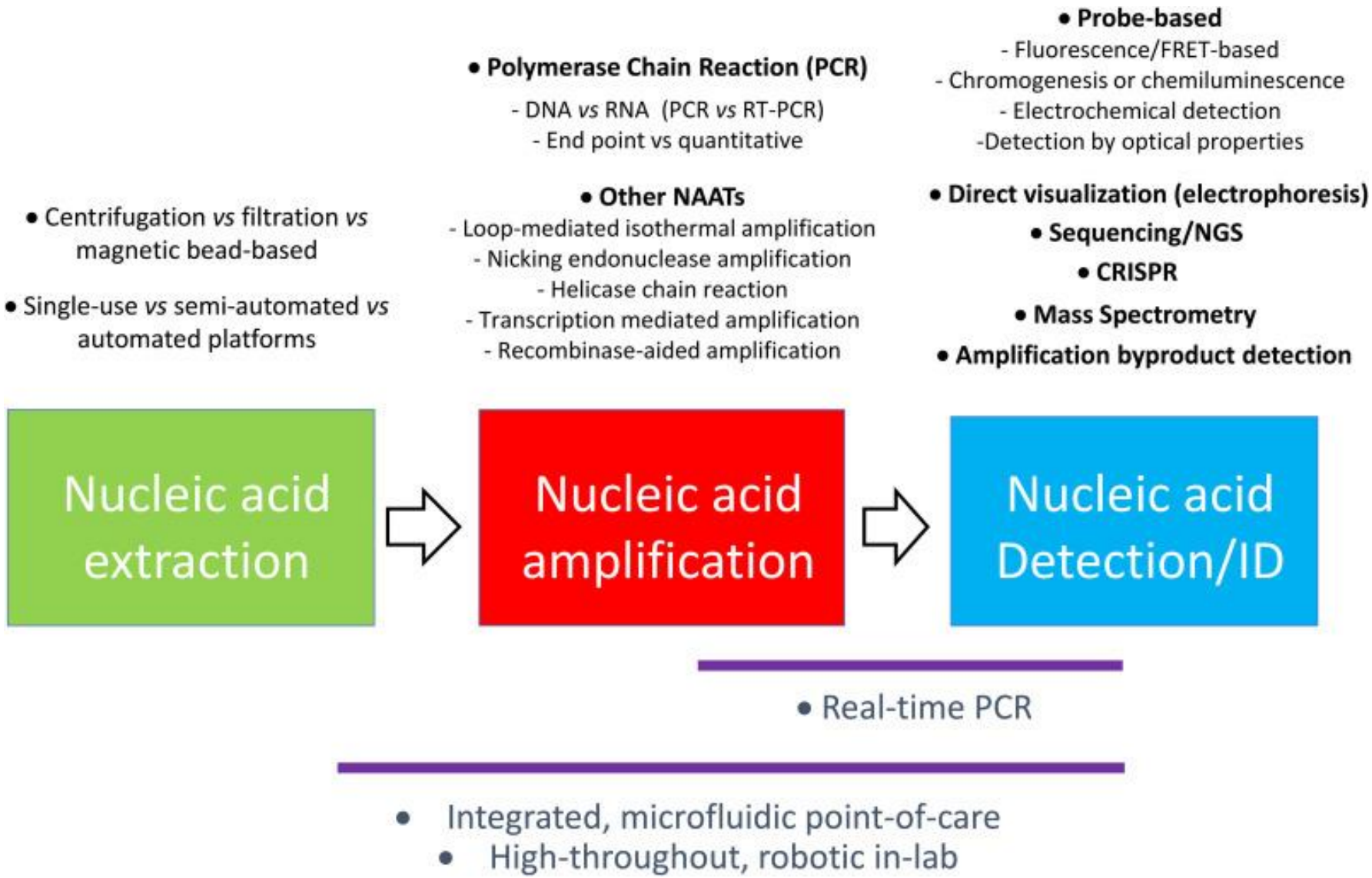
Thank you!!

- Staff of the BWH Clinical Microbiology Laboratory!
  - Victoria Hamrahi (Molecular supervisor)
  - Arielle Gentile (Technical Specialist)
- Colleagues at NWH and BWHFH for working with us to build harmonized testing/reporting
- Colleagues at Clin Micro labs locally and nationally for taking the time they don’t have to share experience and specimens



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