A PEEK BEHIND THE CURTAIN

A DESCRIPTION OF TESTING MODALITIES INCLUDING STRENGTHS, WEAKNESSES, AND APPROPRIATE UTILIZATION FOR BACTERIAL IDENTIFICATION IN THE CLINICAL MICROBIOLOGY LABORATORY

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DISCLOSURES

- No relevant disclosures

OBJECTIVES

- Provide an overview of the diversity and complexity of laboratory testing
- Describe current culture-based and culture-independent methods used for bacterial identification
- Understand strengths and weakness of each approach

AN ANALOGY

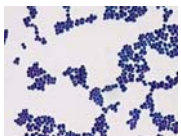
WHAT ARE OUR "UTENSILS"

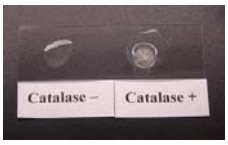
Culture-dependent

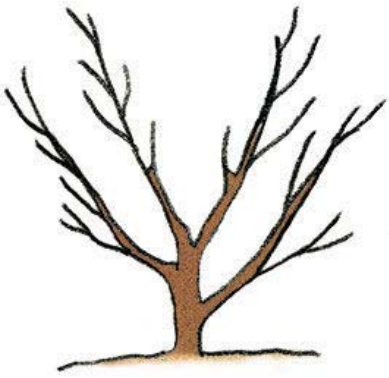
- Require pure isolate (viable)
- Non-targeted (hypothesis-free)

Biochemical tests

- Oldest approach
  - ID based on physiologic or metabolic characteristics of an organism







Culture Independent

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Biochemical tests

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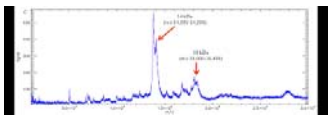
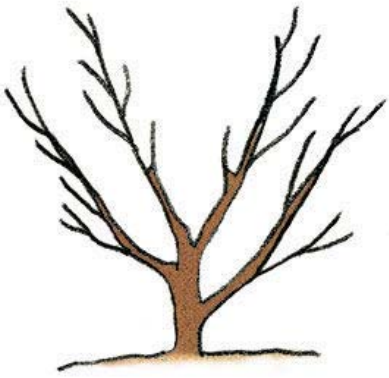
  - ID based on physiologic or metabolic characteristics of an organism

Mass spectrometry (MALDI-ToF MS)

- Adopted within last decade
  - ID based on analysis of cellular peptides





Culture Independent

- Performed directly on specimen
- Targeted (hypothesis-based)

Nucleic acid amplification tests (NAATs)

- Targeted detection of short genetic sequence unique to specific organism
  - Singleplex & Multiplex PCR, Microarray
  - "Hypothesis-based"

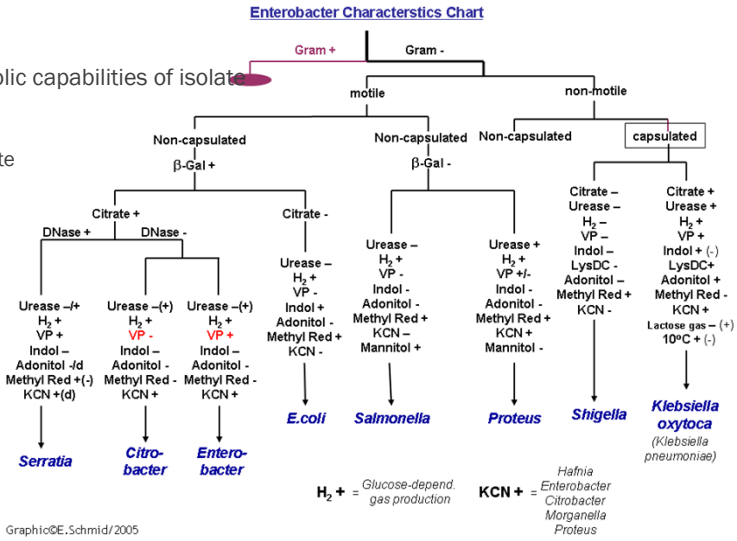
Nucleic acid sequencing tests

- Targeted (16s rRNA)
- Non-targeted (mNGS)
  - "Hypothesis-free"

CULTURE-DEPENDENT

Biochemical identification

- Identification based on "global" metabolic capabilities of isolate
  - Individual tubes, single substrate (+/-)
  - Requires pure, metabolically active isolate



CULTURE-DEPENDENT

Biochemical identification

- Identification based on "global" metabolic capabilities of isolate
  - Individual tubes, single substrate (+/-)
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CULTURE-DEPENDENT

Biochemical identification

- Identification based on “global” metabolic capabilities of isolate

bioMérieux Customer: Micro Lab — **Microbiology Chart Report** — Printed May 11, 2019 20:03 PKT

Patient Name: Patient ID:
Location: Physician:
Lab ID: 0091 Isolate Number: 1

Organism Quantity:
Selected Organism : Francisella tularensis

Identification Information	Analysis Time:	9.95 hours	Status:	Final
Selected Organism	92% Probability	Francisella tularensis		
	Bionumber:	0002000100001200		
ID Analysis Messages	Confirm by serological tests Highly pathogenic organism			

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	-	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	+	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

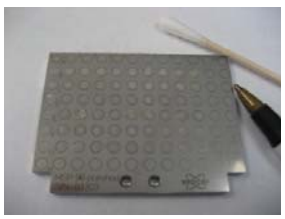
Limitations

- Non-reactive organisms
- Fastidious organisms
- Limited “reference” library

CULTURE-DEPENDENT

Mass spectrometry (MALDI-ToF MS)

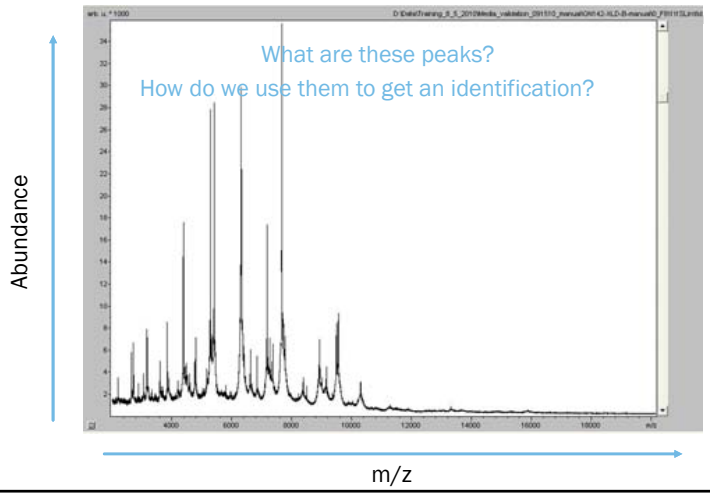
- Identification based on **specific** (ribosomal) protein signature
  - Introduced ~2010 as FDA-cleared bacterial identification system
  - Requires pure isolate

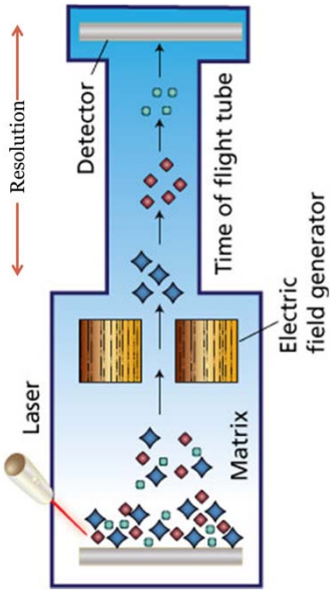


CULTURE-DEPENDENT

Mass spectrometry (MALDI-ToF MS)

- Identification based on **specific** (ribosomal) protein signature

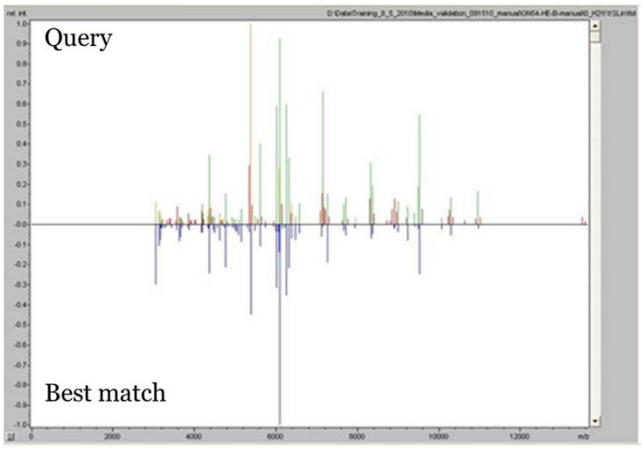




CULTURE-DEPENDENT

Mass spectrometry (Bruker)

- Identification based on **specific** (ribosomal) protein signature



Comparison of peaks
1. Reference vs. sample
2. Sample vs reference
3. Amplitude

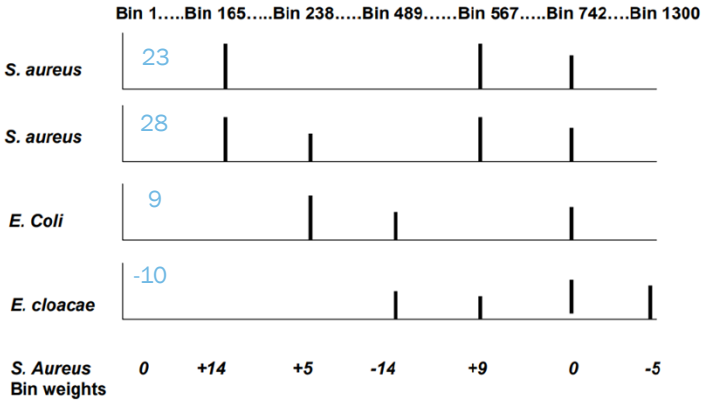
Score
1. Numeric 1-10
2. Multiply
3. Log convert

Criteria
1. >2.0 → Species ID
2. 1.7-2.0 → Genus ID
3. <1.7 → Unreliable

CULTURE-DEPENDENT

Mass spectrometry (Vitek)

- Identification based on **specific** (ribosomal) protein signature



Comparison of peaks
1. Reference vs. sample

Score
1. Each peak assigned "bin"
2. Bins weighted
3. Add total score

Criteria
1. Score/Max score
2. Report as "% confidence"

Depth of library (more isolates) captures diversity → better scores

CULTURE-DEPENDENT

- Vitek MS
  - Floor instrument
  - Single use disposable plate
    - 48- spot (3 plates /run)
  - CPU with spectra analysis software
    - "% confidence score"
    - Easily integrated with Vitek 2 AST
  - FDA-cleared for **~400** microbe species
    - 207 mold/yeast, 16 Nocardia, 39 mycobacteria
    - Average of **40 spectra/species**
      - E. coli: 437 strains, 681 spectra
      - S. aureus 348 strains, 456 spectra
      - Weirdobacter spp?

CULTURE-DEPENDENT

- Maldi Biotyper CA
  - Benchtop instrument
  - Reusable steel target plate
    - 48- or 96-spot
  - CPU with spectra analysis software
    - Requires interface with AST system
    - Collapse/cross-walk of IDs
  - FDA-cleared for **~350** microbes
    - 40 yeasts, 5 Nocardia, 0 Mycobacteria
    - Separate libraries for AFB, Mold, BT agents

"RUO" library double in size

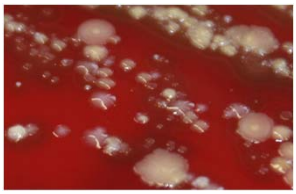
CULTURE-DEPENDENT

Workflow comparison

- Biochemical vs MALDI-ToF MS

Wound, throat, urine, stool

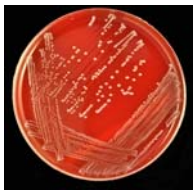
24h



Primary culture

What's significant?

24h



Subculture

Adequate inoculum
Gram stain

12-24h

Phenoptypic ID

GN, GP, Strep, Ana

60-72 h

Non-reactive bacteria? Miss-read Gram stain? Cost?

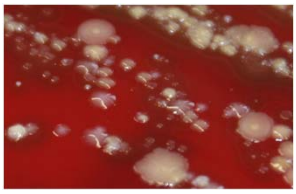
CULTURE-DEPENDENT

Workflow comparison

- Biochemical vs MALDI-ToF MS

Wound, throat, urine, stool

24h



Primary culture

What's significant?

2 min

30 sec

24 h

Non-reactive bacteria? Miss-read Gram stain? Cost?

CULTURE-DEPENDENT

Performance/accuracy (n = 980 isolates)

Organism group and identification parameter (isolate data)	MALDI-TOF MS identification (no. of isolates [%])	Routine biochemical phenotypic identification (no. of isolates [%])[d]	*P* value[e]
All isolates (n = 980; 42 genera, 92 species)			
Genus correct	968 (98.8)	960 (98.0)	NS
Species correct	902 (92.0)	814 (83.1)	<0.01
Major error	1 (0.1)	16 (1.6)	<0.01
Minor error	16 (1.6)	14 (1.4)	NS
No identification	8 (0.8)	5 (0.5)	NS
Enterobacteriaceae (n = 311; 14 genera, 21 species)			
Genus correct	311 (100)	311 (100)	NS
Species correct	304 (97.7)	304 (97.7)	NS
Major error	(0)	(0)	
Minor error	1 (0.3)	7 (2.3)	0.05
No identification	(0)	(0)	
Nonfermentative Gram-negative rods (n = 88; 10 genera, 17 species)			
Genus correct	83 (94.3)	82 (93.2)	NS
Species correct	81 (92.0)	77 (87.5)	NS
Major error	1 (1.1)	2 (2.3)	NS
Minor error	(0)	1 (1.1)	NS
No identification	2 (2.3)	4 (4.5)	NS

Major error = incorrect genus
Minor error = incorrect species

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2010, p. 900–907

CULTURE-DEPENDENT

Performance/accuracy (n = 980 isolates)

Organism group and identification parameter (isolate data)	MALDI-TOF MS identification (no. of isolates [%])	Routine biochemical phenotypic identification (no. of isolates [%])[d]	*P* value[e]
Gram-positive cocci in cluster (n = 261; 2 genera, 9 species)[b] **Staph, Rothia**			
Genus correct	261 (100)	259 (99.2)	NS
Species correct	246 (94.3)	165 (63.2)	<0.01
Major error	(0)	2 (0.8)	NS
Minor error	1 (0.4)	(0)	NS
No identification	(0)	(0)	
Gram-positive cocci in chains (n = 165; 2 genera 16 species)[c]			
Genus correct	163 (98.8)	165 (100)	NS
Species correct	140 (84.8)	145 (87.9)	NS
Major error	(0)	(0)	
Minor error	12 (7.3)	3 (1.8)	0.03
No identification	2 (1.2)	(0)	NS
Miscellaneous bacteria (n = 94; 12 genera, 17 species)			
Genus correct	91 (96.8)	83 (88.3)	0.03
Species correct	79 (84.0)	76 (80.9)	NS
Major error	(0)	11 (11.7)	<0.01
Minor error	(0)	1 (1.1)	NS
No identification	3 (3.2)	1 (1.1)	NS

Major error = incorrect genus
Minor error = incorrect species

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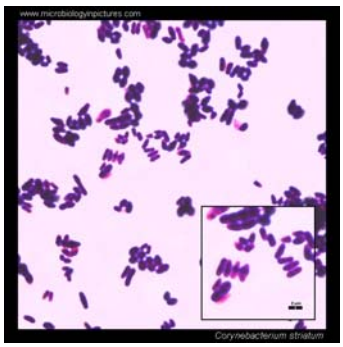
MALDI-TOF MS

"*Corynebacterium* spp."



- *Gram-positive, catalase positive bacilli*
  - *~100 species of Corynebacterium*
  - *Many other "coryneform" genera with similar appearance*
    - *(Dermabacter, Arthrobacter, Brevibacterium)*
- *Common skin commensal*
  - *Rarely associated with infection → frequently considered skin contaminant*
  - ***Not included** in phenotypic libraries*
  - ***Difficult to discriminate** species based on spot biochemicals*

MALDI-TOF MS

"*Corynebacterium* spp."

- *MALDI enabled easy reporting and accurate ID → recognition of important associations*
  - *C. macginleyi → conjunctivitis*
  - *C. urealyticum* → urinary tract infection (stones)
  - *C. kroppenstedtii* → granulomatous mastitis
  - C. *tuberculostearicum* → wound infection
  - *Turicella otididis* → otitis media
- *Policy change to auto report these species and AST when isolated from appropriate sources*
  - *Other Corynebacterium spp reported at "normal skin flora"*

CULTURE-DEPENDENT

Do we still need biochemical tests?

	MALDI	Biochemical
Breadth of IDs	>1,000	200-300
Accuracy (species)	>95%	85%
Time to result	30 sec.	12-24 h
Cost	$0.25	$8.00
Gram-stain dependence	No	Yes

MALDI-TOF MS

Over-reliance

Warning

- PT survey – Simulated urine culture containing GNR
- MALDI-ToF result

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Proteus hauseri CC_2695 MCW	2.50	183417
2 (+++)	Proteus hauseri NY_1373 MCW	2.20	183417
3 (+++)	Proteus hauseri CC_2400 MCW	2.20	183417
4 (+++)	Proteus vulgaris DSM 13387_QC DSM	2.25	585
5 (+++)	Proteus vulgaris LMG 5586 LMG	2.20	585
6 (+++)	Proteus vulgaris DSM 30119 DSM	2.22	585
7 (+++)	Proteus vulgaris B156 UFL	2.20	585
8 (+++)	Proteus vulgaris (PX) 22086129 MLD	2.21	585
9 (+++)	Proteus penneri CIP 105117 CIP	2.20	102862
10 (+++)	Proteus hauseri NY_1346 MCW	2.20	183417

All "top 10" results have confidence scores >2.0 (high confidence)

We reported "*P. hauseri*"

MALDI-TOF MS

Over-reliance

Warning

- PT survey – Simulated urine culture containing GNR
- Intended answer: *Proteus vulgaris*

Table 1. Bacterial Identification

Total Responses Identification	Referees (73) LABS	%	Participants (2043) LABS	%
Proteus vulgaris	50	68.5	1066	52.2
Proteus sp.	17	23.3	510	25.0
Gram-negative bacilli, Enterobacteriaceae	-	-	2	0.1
Gram-negative bacilli, aerobic	-	-	11	0.5
Consensus for correct identification of organism	67	91.8	1589	77.8
Unintended:				
Proteus penneri	2	2.7	116	5.7
Proteus hauseri	4	5.5	324	15.9

MALDI-TOF MS

Warning

Over-reliance

- PT survey – Simulated urine culture containing GNR
- Intended answer: *Proteus vulgaris*

Table 2. Result by Method

System	LABS	% of Laboratory Designation *Proteus vulgaris*	*Proteus* sp.	*Proteus penneri*[b]	*Proteus hauseri*[b]
API	44	59.1	31.8	6.8	-
BD Phoenix	103	77.7	20.4	1.0	-
Biochemical Methods	41	34.1	29.3	-	4.9
Bruker MALDI	341	65.1	21.1	0.3	12.9
MicroScan	413	92.3	5.6	1.9	-
Vitek 2	697	3.0	44.0	13.3	38.2
Vitek MS MALDI	317	91.2	6.9	0.9	0.6

MALDI-TOF MS

Warning

Over-reliance

- PT survey – Simulated urine culture containing GNR
- Acknowledged limitations (the "fine print")

Proteus hauseri NY_1373 MCW	Species hauseri / penneri / vulgaris of the genus Proteus have very similar patterns: Therefore distinguishing their species is difficult.
Proteus penneri CIP 105117 CIP	Species hauseri / penneri / vulgaris of the genus Proteus have very similar patterns: Therefore distinguishing their species is difficult.
Proteus vulgaris (PX) 22086129 MLD	Species hauseri / penneri / vulgaris of the genus Proteus have very similar patterns: Therefore distinguishing their species is difficult.

Escherichia coli DSM 682 DSM	closely related to Shigella / Escherichia fergusonii and not definitely distinguishable at the moment

Streptococcus oralis NRZ 40923	Streptococcus mitis / oralis / peroris / pneumoniae / pseudopneumoniae are closely related! The result may be confirmed by a further test, e.g. bile test or optochin test, according to standard clinical microbiological practice.

MALDI-TOF MS

Pseudo-outbreak

- *Mycobacterium chimera*
  - NTM related to *M. avium/intracellulare*
  - Rarely recognized as cause of human infection (poorly differentiated form MAC)
  - 2015 → Identified as cause of indolent infections following open chest surgeries
    - Linked to heater-cooler units used during surgery

Spring 2021 - IPAC identified several patients with "*M. chimera*" infection at community hospital

Age	Primary Problem	Specimen Type	Collected	Collection Department	Pathogen (A)
73 Y	Pulmonary nodules (Principal Hospital Problem)	BAL (Bronchial Alveolar Lavage)	04/21/2021 0929	SJH OR	Mycobacterium chimaera (m. intracellular group)
83 Y	COPD exacerbation (*) (Admission Diagnosis)	BAL (Bronchial Alveolar Lavage)	04/08/2021 0836	SJH OR	Mycobacterium chimaera (m. intracellular group)
Deceased (81 Y)	Microscopic polyangiitis (*) (Principal Hospital Problem)	BAL (Bronchial Alveolar Lavage)	03/15/2021 0959	SJH INTENSIVE CARE	Mycobacterium chimaera (m. intracellular group)

MALDI-TOF MS

Addressing the changes

Modify reporting to:

- Accommodate strengths and limitations of MALDI-ToF
- Help providers interpret *"new"* organisms

Undifferentiated species: *"M. intracellulare/chimera"*

Increased accuracy: *Bacterioides ovatus* (*Bacterioides fragilis* group)

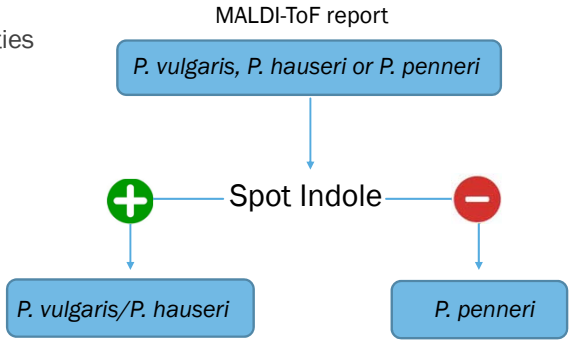
Updated taxonomy: *Cutibacterium acnes* (*Propionibacterium acnes*)

MALDI-TOF MS

Conclusion

- Be aware of limitations!
- Align your LIS reportables with MALDI-ToF capabilities
  - Don't report species that cannot be differentiated
- Build algorithms to include biochemical tests

Clinical picture MALDI Biochems Epidemiology



BACK FOR A SNACK

Pure isolate

"Is this BBQ or teriyaki?"

Primary specimen

"What is in this?"

"Does this contain onions?"

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Molecular tests

- Targeted
  - Amplified "PCR" tests
    - Single-target (SARS-CoV-2, MRSA)
    - Multiplex (Respiratory virus panel)
    - Quantitative (HIV, CMV, etc.)

FAST, Sensitive, Inexpensive (relatively)

Biased!

- Non-targeted
  - 16S rRNA sequencing
  - NGS

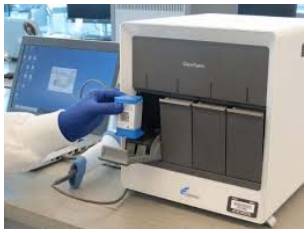
Slow, less sensitive, Expensive (relatively)

*Un*biased!

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Targeted

- Gene Xpert (Qualitative RT-PCR)
  - 1-4 targets, 40-90 min TAT.
    - SA/MRSA
    - Cdiff
    - Sars/Flu
    - MTB



On-demand, Sample-to-answer, Detected/Not detected

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Xpert benefits

- Cdiff
  - The need: Rapid result, high NPV to guide specific intervention

Rapid Antigen tests

	Assay	Sensitivity (%) (95% CI)[a]	Specificity (%) (95% CI)[a]
Toxin	Remel Xpect	68.8 (59.9–76.8)	99.4 (98.2–99.9)
Toxin	Techlab Tox A/B Quik Chek	74.4 (65.8–81.78)	98.9 (97.6–99.7)
Toxin	Premier Immunocard A + B	68.8 (59.9–76.8)	93.0 (90.4–95.2)
GDH	Techlab C. diff Chek-60	87.6 (72.4–93.0)	94.3 (91.7–96.2)

Eastwood et. al, JCM 2009

Comparison of molecular tests to for detection of *C. difficile*[a]

Test	No. of specimens with result TP	FP	TN	FN	% sensitivity (CI)	% specificity (CI)
Portrait	109	31	398	2	98.2 (93–99)	92.8 (89–95)
Gene Xpert	58	18	199	0	100 (93–100)	91.7 (87–95)
GeneOhm	37	2	129	1	97.4 (86–99)	98.5 (94–99)
Illumigene	14	4	77	1	93.3 (68–99)	95.1 (87–98)

Buchan et. al, JCM 2012

*14/18 FP positive by alternative PCR test

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Xpert benefits

- Cdiff
  - Positive impact of high sensitivity – *"I believe the result!"*

***Clostridium difficile* Outcomes at Froedtert Hospital**

	EIA, n=79	PCR, n=87	P-value
Duration of antibiotic therapy in days, mean (SD)	2.31 (4.45)	0.88 (2.48)	0.007
Diagnostic test performed per patient, mean (SD)	2.73 (0.52)	1.16 (0.67)	<0.001
Duration of special isolation in days, mean (SD)	1.46 (3.81)	0.62 (3.30)	0.13

With 2 h TAT, *C. difficile*-specific therapy often held until result is available

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Xpert benefits

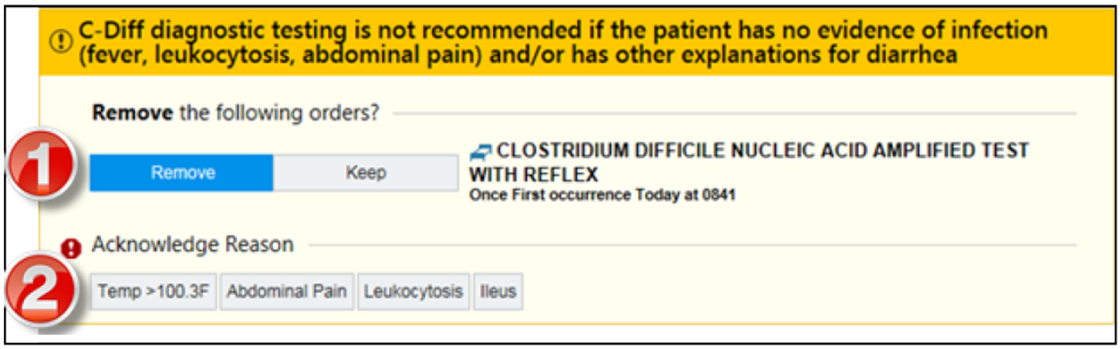
- Cdiff
  - Negative impact of high sensitivity – *"Is this a clinically significant finding"*
- Evidence
  - Colonization: 5%-15% asymptomatic carriage (up to 57% in LTAC patients)
    - Therapy not effective at eliminating spores, negative impact of unnecessary abx
  - Test of Cure: Detection of residual Cdiff DNA following treatment/resolution of symptoms
  - Quality metrics: Reported as Hospital Acquired Infection (HAI) if initial detection >3 days from admission

How do we reap the benefits of high sensitivity and mitigate the harm?
(*Right patient, Right time, Right test*)

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Xpert Cdiff utilization

- Right patient: decision support
  - Automatic screen for common contraindications
  - *BPA for patients who have received laxatives or enema in past 24 h*



CULTURE-INDEPENDENT IDENTIFICATION METHODS

Xpert Cdiff utilization

- Right time: Laboratory utilization
  - Cancel repeat test orders (7-day positive, 14-day negative)

Summary of *C. difficile* PCR tests performed.

Test category	Number of tests	%
Total tests	20,526	100.0
Repeat tests[a]	1637/20,526	8.0
Initial test positive	554/1637	33.8
Repeat test positive	541/554	97.7
Repeat test negative	13/554	2.3
Initial test negative	970/1637	59.3
Repeat test positive	44/970	4.5
Repeat test negative	926/970	95.5

[a] Tests repeated within 7 days of a previous valid test result.

Buchan et al. Diagnostic Microbiology and Infectious Disease 90 (2018) 307–310

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Xpert Cdiff utilization

- Right time: Laboratory utilization
  - Cancel repeat test orders (7-day positive, 14-day negative)

Table 2
Univariate logistic regression analysis of factors contributing to discordant initial and repeat test results.

Clinicopathologic feature	Repeat negative	Repeat positive	*P*	Odds ratio for repeat positive test (95% CI)
Number	248	24	NA	NA
Age, average	59	55	0.37	0.99 (0.96–1.01)
Gender, Male	130/248 (52.4%)	12/24 (50%)	0.82	0.91 (0.39–2.1)
History of *C. difficile* (PCR confirmed) in 60 days preceding test	8/248 (3.2%)	10/24 (41.7%)	<0.001	18.97 (6.64–54.17)
Presence of diarrhea at time of test	220/248 (88.7%)	22/24 (91.7%)	0.66	1.40 (0.31–6.27)
Fever (>38 °C) at time of test	39/248 (15.7%)	5/24 (20.8%)	0.35	1.65 (0.57–4.77)
Leukocytosis (>11,000 leukocytes/μL) at time of test	106/248 (42.7%)	9/24 (37.5%)	0.80	1.12 (0.46–2.69)
Received any antibiotic therapy in 14 days preceding test	215/248 (86.7%)	15/24 (62.5%)	0.003	0.255 (0.10–0.63)
Received empiric therapy[a] for *C. difficile* in 7 days preceding test	34/248 (13.7%)	3/24 (12.5%)	0.85	0.88 (0.25–3.12)
History of laxative use within the last week (%)	100/248 (40.3%)	9/24 (37.5%)	0.76	0.88 (0.37–2.08)
Average length of stay in days (range)	8.67 (0–67)	4.14 (0–13)	0.007	0.86 (0.78–0.96)

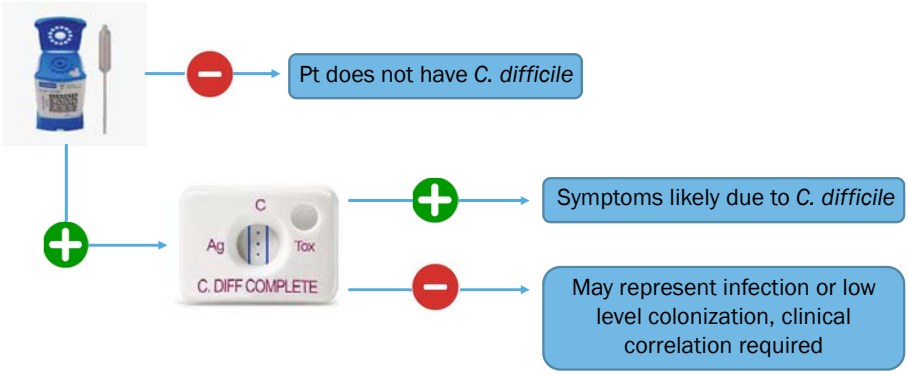
[a] Metronidazole or oral vancomycin.

Buchan et al. Diagnostic Microbiology and Infectious Disease 90 (2018) 307–310

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Xpert Cdiff utilization

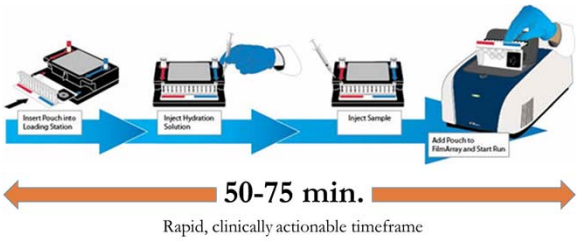
- Right test(s): Algorithmic approach
  - Use of sensitive initial "screen" followed by specific "confirmation"
  - Aids in reporting only cases with high likelihood of clinical significance

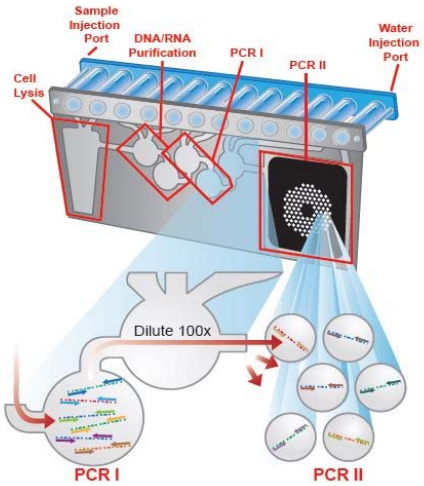


CULTURE-INDEPENDENT IDENTIFICATION METHODS

Targeted

- BioFire (Amplified, miniturized-singleplex)
  - 18-35 targets, 45-75 min
    - Meningitis/Encephalitis
    - Respiratory virus
    - *new* Pneumonia (bacterial and viral)





CULTURE-INDEPENDENT IDENTIFICATION METHODS

BioFire ME Panel

- Tested 751 CSF – 57 (7.5% positive)

Bacterial n=13 (22.8%)

- Pathogens

	Total	Stain(+)
E. coli:	2	0 (0%)
H. influenzae:	2	1 (50%)
S. pneumoniae:	6	4 (66%)
N. meningitidis:	2	1 (50%)
Grp B streptococcus:	1	0 (0%)

- Gram Stain
  - **46.2% sensitive** (6/13)
    - 0/4 *S. pneumo* grew in CSF culture
- CSF culture
  - **15.4% sensitive** (2/13)
    - 1/2 *N. meningitidis*, 1/2 *H. influenzae*

Viral n=39 (68.4%)

- Pathogens

	Total
HSV-1	5
HSV-2:	7
CMV:	1
VZV:	8
Enterovirus:	10
HHV-6	9

- Dual-positive
  - CMV + HHV-6
    - Untreated AIDS
    - CMV retinitis/encephalitis

Fungal n=5 (8.8%)

- Pathogens

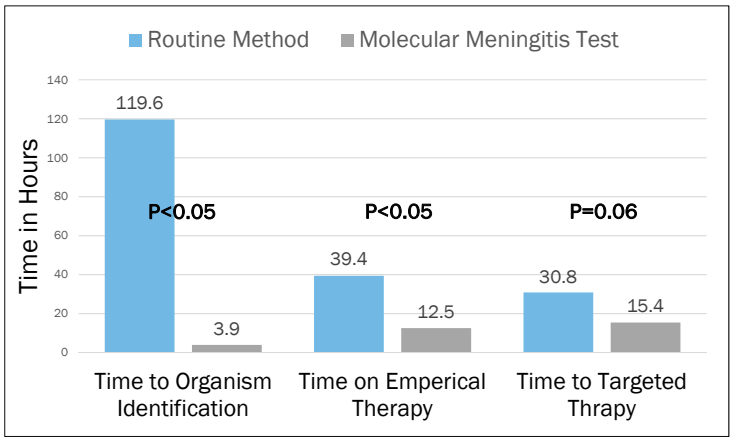
	Total	Stain(+)
Cryptococcus sp:	5	4 (80%)

- CSF culture
  - **80.0% sensitive** (4/5)
- CSF antigen
  - **100% sensitive** (5/5)

CULTURE-INDEPENDENT IDENTIFICATION METHODS

BioFire ME Panel

- Positive impact



- Reduced exposure to unnecessary abx
  - Toxicity, MDR, *C. difficile* disease
- Reduce admission rate for Enterovirus
  - Common, self-limited cause of CA meningitis
  - Save $3,000 per positive patient **not admitted**
    - Robinson et al. *Pediatr Infect Dis J 2002*
- Cost neutral
  - $239.63 (SOC) vs. $239.14 (FA-ME)
    - SOC includes multiple Dx tests (Culture, multiple viral PCR), labor, unnecessary Abx
    - Soucek et al. *J. Pharm Practice* 2017

CULTURE-INDEPENDENT IDENTIFICATION METHODS

BioFire ME Panel

- Drawbacks – What's missing?

Not on ME Panel

- *S. aureus*
- *CoNS*
- *Enterococcus*
- *P. aeruginosa*
- *A. baumannii*
- *Enterobacterales other than E. coli K1*
- *C. acnes*

Characteristic features of common causes of bacterial meningitis

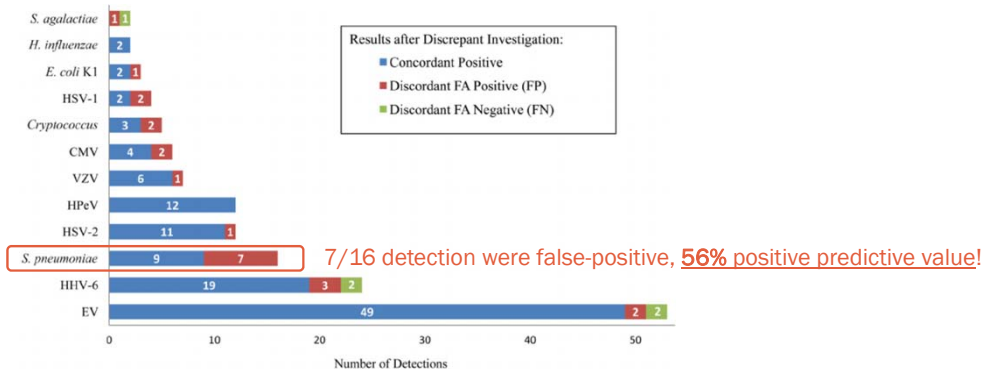
Organism	Site of entry	Predisposing conditions
Neisseria meningitidis	Nasopharynx	Usually none, rarely complement deficiency
Streptococcus pneumoniae	Nasopharynx, direct extension across skull fracture, or from contiguous or distant foci of infection	All conditions that predispose to pneumococcal bacteremia, fracture of cribriform plate, cochlear implants, defects of the ear ossicle (Mondini defect)
Listeria monocytogenes	Gastrointestinal tract, placenta	Defects in cell-mediated immunity pregnancy, liver disease, alcoholism, malignancy
Coagulase-negative staphylococci	Foreign body	Surgery and foreign body, especially ventricular drains
Staphylococcus aureus	Bacteremia, foreign body, skin	Endocarditis, surgery and foreign body, especially ventricular drains
Gram-negative bacilli	Various	Advanced medical illness, neurosurgery, ventricular drains, disseminated strongyloidiasis
Haemophilus influenzae	Nasopharynx, contiguous spread from local infection	Diminished humoral immunity

- Not recommended for traumatic or surgical infections
- Not recommended for infections with indwelling hardware

CULTURE-INDEPENDENT IDENTIFICATION METHODS

BioFire ME Panel

- Drawbacks
  - High sensitivity – Susceptible to contamination
    - *S. pneumoniae* common in upper respiratory tract specimens *and individuals*

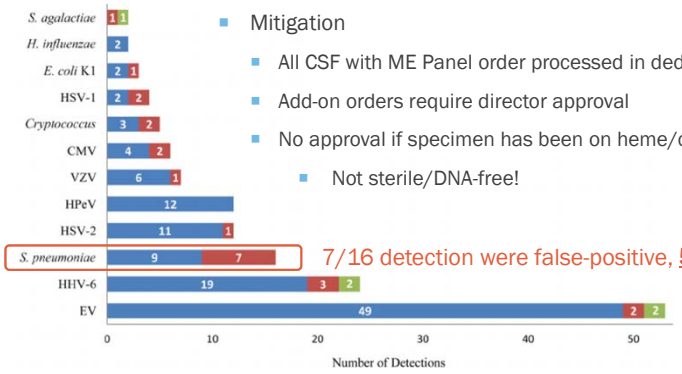


7/16 detection were false-positive, 56% positive predictive value!

CULTURE-INDEPENDENT IDENTIFICATION METHODS

BioFire ME Panel

- Drawbacks
  - High sensitivity – Susceptible to contamination
    - *S. pneumoniae* common in upper respiratory tract specimens *and individuals*



- Mitigation
  - All CSF with ME Panel order processed in dedicated hood **prior to** other orders e.g. culture
  - Add-on orders require director approval
  - No approval if specimen has been on heme/chem/cytology automation
    - Not sterile/DNA-free!

7/16 detection were false-positive, **56%** positive predictive value!

CULTURE-INDEPENDENT IDENTIFICATION METHODS

BioFire ME Panel

- Drawbacks
  - Qualitative, analytic result…*is detection clinically significant?*
    - HHV-6 → Most common viral target detected (20-25%)
      - **Latency** in oligodendrocytes **in CNS**, monocytes, macrophage
      - **Sub-clinical** reactivation in **53%** of critically ill patients
      - **Ci-HHV-6** in ~1% of human population
      - HHV-6 encephalitis well described in HSCT, but **rare elsewhere**

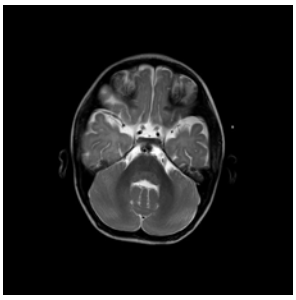
Up to 80% of HHV-6 detections in CSF deemed to be of unlikely clinical significance

Similar may be true of other integrated herpesviruses e.g. VZV, CMV, HSV

Green et al. CID 2018:67 (1 October)

CULTURE-INDEPENDENT IDENTIFICATION METHODS

BioFire ME Panel



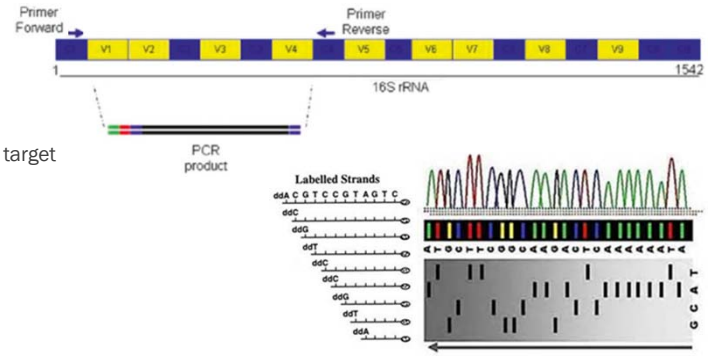
- Drawbacks
  - Mitigation – Clinical and laboratory correlation
    - Assess patient risk factors (HSCT vs "community acquired" meningitis/encephalitis)
    - Cranial imaging for consistent MRI findings (bilateral hyperintensity of medial lobes)
    - Rule out ci-HHV-6 (dPCR)
    - Plasma HHV-6 viral load
      - <10,000 copies/mL encephalitis extremely rare

Green et al. CID 2018:67 (1 October)

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Non-Targeted (sequencing)

- "16s"
  - Target bacterial 16s rRNA
    - PCR-based amplification of 500-700 bp target
    - Sanger sequence analysis of amplicon



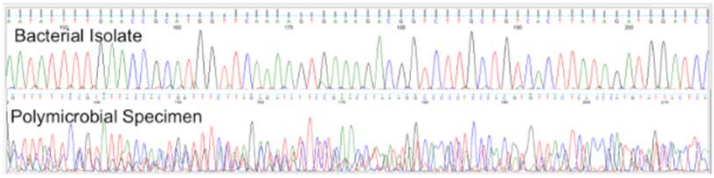
In *theory*, this enables…

- Unbiased "hypothesis-free" identification of bacteria in a specimen
  - Independent of culture viability (antibiotic-exposed, fastidious)
- Sensitivity similar to targeted PCR
- "Rule out" an infectious etiology

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Non-Targeted (sequencing)

- Caveats a plenty!
  - Specimen
    - Monomicrobial (sterile tissue/fluid)
    - Fresh (non-FFPE)



  - Sensitivity
    - Targeted PCR > 16s
    - Culture?

Broad-Range 16S rRNA PCR Compared With Culture

N=394 sterile fluids		Culture +	Culture −
16S	+	86 (21.8%)	18 (4.6%)
	−	19 (4.8%)	271 (68.8%)

Species in culture-positive, 16S-negative specimens[b]	No.
Propionibacterium acnes	11[c]
Staphylococcus lugdunensis	1
Staphylococcus aureus	2
Staphylococcus capitis	1
Staphylococcus epidermidis	3
Staphylococcus sp	1
Total	19

[c] Four cultures became positive after >10 days

Rampini et al. CID 2011:53 (15 December)

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Non-Targeted (sequencing)

- Caveats a plenty!
  - Culture-negative specimens
    - Sterile fluids/tissues → 42% sensitive
    - PJI synovial fluid → 35% sensitive

Low organism burden → 16s has relatively high LoD
Can not be used to rule out infection!!!!!!!

Culture negative, high index of suspicion for bacterial infection

Specimen type	Number of positive specimens	Number of negative specimens	Percent positive	Total number of specimens
Fresh tissue	Sequence result			
Microscopy positive[a]	9	5	64.3%	14
Microscopy negative	18	88	17.0%	106
No microscopy result	6	22	21.4%	28
Total	33	115	22.3%	148

29%

Rampini et al. *CID* 2011:53; Bemer et al. *J Clin Microbiol* 2014:52 (10); Payne et al. *Can J Infect Dis and Med Microb* 2016

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Non-Targeted (sequencing)

- "real world" impact
  - Unregulated ordering: 163 specimens over 10 months → 16s and culture

RESULT	NUMBER	PERCENT OF TOTAL	CLINICALLY VALUABLE RESULT FROM SEQUENCING
Specimens tested by sequencing and culture	163	100%	N/A
Negative by both tests	86	52.8%	No
Culture and sequencing positive for same organism (culture result available before sequencing)	34	20.9%	No, culture result was obtained first
Sequencing positive only for a previously diagnosed infection (patient was being treated, which prevented growth on culture)	7	4.3%	No, infection had already been identified and effective treatment initiated
Specimens with multiple organisms identified by culture and sequencing was indeterminate	5	3.1%	No, sequencing does not work if multiple organisms are present
Culture positive, sequencing negative	22	13.5%	No
Sequencing positive, culture negative (patient was on antibiotics that would prevent bacterial growth in culture)	7	4.3%	Yes Maybe? Narrow Abx based on ID
Sequencing positive, culture negative	1	0.6%	Yes

- Conclusions
  - <5% of cases provide clinically useful/actionable information
  - Does **not** provide susceptibility result e.g. MRSA vs MSSA
  - Long TAT, may report results after completing empiric therapy
  - Additional cost to hospital is $75-100k

CULTURE-INDEPENDENT IDENTIFICATION METHODS

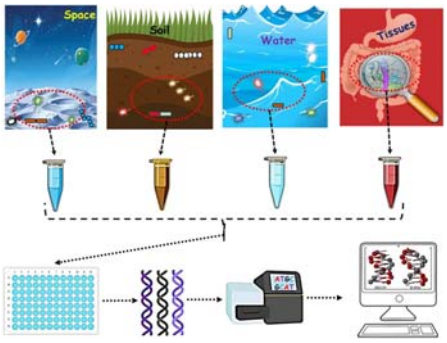
Non-Targeted (sequencing)

- Test utilization – Maximize benefits of expensive and low yield test
  - "Freeze and hold"
    - Freeze portion of tissue/fluid until culture completed
  - Preference for stain positive (gram or histology)
    - If culture-negative, these are good candidates for 16s
  - Consideration for source (sterile vs non-sterile)
    - Non-sterile source, polymicrobial stain will **not** generate useful information
  - Recommend specific PCR rather than general 16S
    - If specific concern for *S. aureus, Toxoplasma, Pneumocystis*, MTB, etc. consider specific PCR
    - Increase sensitivity and specificity!

CULTURE-INDEPENDENT IDENTIFICATION METHODS

Non-Targeted (sequencing)

- Metagenomic NGS (mNGS), the "Whole enchilada"
  - Truly unbiased sequencing approach
    - bacterial, viral, fungal, human



In *theory*, this enables…

- Unbiased "hypothesis-free" identification of any/all organisms in a specimen
- Sensitivity similar to targeted PCR
- "Rule out" an infectious etiology

CULTURE-INDEPENDENT IDENTIFICATION METHODS

mNGS

- Caveats a plenty!
  - Interpretation
    - Non-quantitiative
    - Relatively sensitive
    - *Non-targeted*
  - Interfering signals
    - Human DNA accounts for >90% of nucleic acid in blood & tissue specimens
      - Reduces sensitivity for low abundance microorganisms
      - Too few microorganism reads to map → poor ID or unable to ID

"Cast a wide net and you catch a lot of fish"

CULTURE-INDEPENDENT IDENTIFICATION

mNGS

- Caveats a plenty!
  - Lymph node – culture-negative abscess
    - Sanger 16S failed, multiple bacterial sequences present

Can bugs with predominant reads be assumed to be predominant?

Are the low concentration reads significant? Contaminant?

Deep Sequencing Results

Species name	% of total Reads	Number of Reads
Veillonella parvula/dispar/atypica	23.6	2742
No match ≥99%	22.36	2599
*Fusobacterium periodonticum**	17.16	1994
Veillonella dispar/parvula/denticariosi*	10.55	1226
Streptococcus oralis	5.65	657
*Prevotella nanceiensis**	5.22	607
Campylobacter concisus	2.95	343
Streptococcus parasanguinis	2.62	304
Peptostreptococcus stomatis	2.36	274
Streptococcus salivarius/vestibularis/thermophilus	2	232
Veillonella dispar/parvula**	1.59	185
Streptococcus pseudopneumoniae/pneumoniae/mitis/[illegible]lis	0.69	80
Rothia mucilaginosa	0.64	74
Haemophilus parainfluenzae	0.46	54
Gemella haemolysans	0.31	36
Streptococcus constellatus/intermedius*	0.31	36
Oribacterium sinus	0.25	29
Veillonella atypica	0.24	28
Gemella sanguinis	0.22	25
Fusobacterium periodonticum/nucleatum	0.22	25
Capnocytophaga sputigena	0.22	25
Prevotella melaninogenica	0.2	23
Streptococcus infantis	0.2	23

CULTURE-INDEPENDENT IDENTIFICATION METHODS

mNGS

- Caveats a plenty!
  - Read prevalence vs true prevalence
    - What is the LoD of mNGS for various microorganisms

Table 1. Performance characteristics for the mNGS assay

Performance metric	Method		Results
Limits of detection (LOD)[a]	Qualitative detection of PC dilution replicates by probit analysis		
	Pathogen type	Representative organism	LOD
	DNA virus	CMV	14 copies/mL
	RNA virus	HIV	313 copies/mL
	Bacterium, gram-positive	*Streptococcus agalactiae*	10 CFU/mL
	Bacterium, gram-negative	*Klebsiella pneumoniae*	8 CFU/mL
	Fungus, mold	*Aspergillus niger*	220 CFU/mL
	Fungus, yeast	*Cryptococcus neoformans*	0.2 CFU/mL
	Parasite	*Toxoplasma gondii*	81 organisms/mL

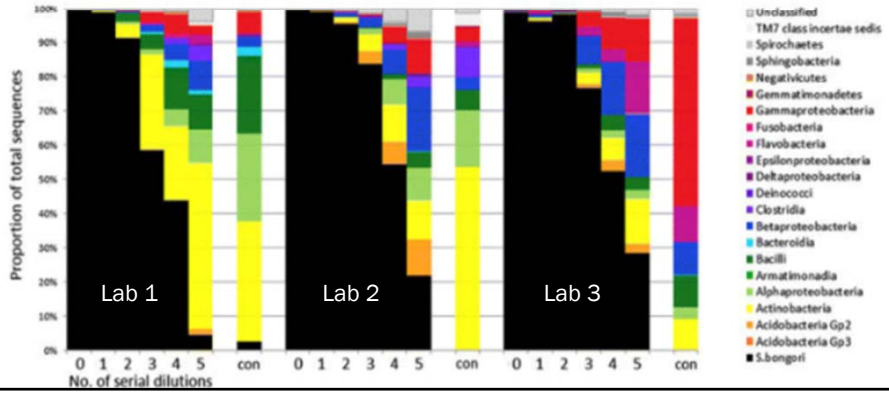
Factors influencing sensitivity…genome size, susceptibility to lysis, complete genome availability

CULTURE-INDEPENDENT IDENTIFICATION METHODS

mNGS

- Caveats a plenty!
  - Low level organism…*or low level contamination*?

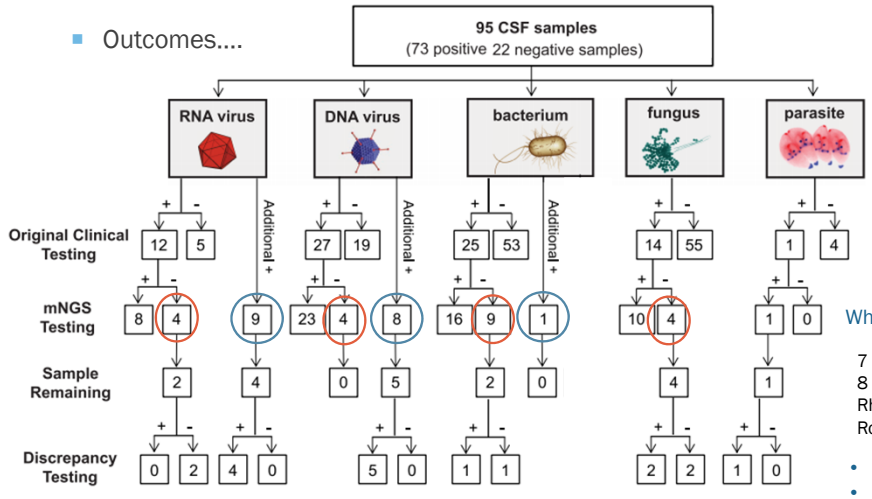
– Specimens spiked with *S. bongori*, serially diluted (10^8-10^3 CFU/mL), sent to 3 independent labs



CULTURE-INDEPENDENT IDENTIFICATION METHODS

mNGS

- Outcomes….



What about the 18 additional detections?

7 HIV (all patients seropositive)
8 herpesviruses (EBV, CMV, HSV, HHV-6)
Rhinovirus
Rotavirus

- Questionable clinical significance
- Targeted PCR tests available

CONCLUSIONS

- Advances in technology has provided the laboratory with fantastic tools for identification of microorganisms (isolates and direct specimen)
- Maximal benefit relies on
  - Appropriate utilization – right patient , right test, right time
  - Understanding the strengths and limitation of each approach
  - Considering all test results in the context of the clinical picture
- Clinical microbiologists have specific training and expertise in laboratory diagnostics
  - While hidden behind the curtain, we are happy to provide guidance to help provide the best patient care