MOLECULAR DIAGNOSTICS FOR PNEUMONIA

Who, What, When, Why, Where, and How (much)?

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Disclosures

Research support

- **BioFire Diagnostics**
- **Curetis**
- **Accelerate**
- Quidel
- ChromaCode
- Luminex
- Genmark
- Alere

Meeting/travel support

- **BioFire Diagnostics**
- ChromaCode
- Alere
- iCubate

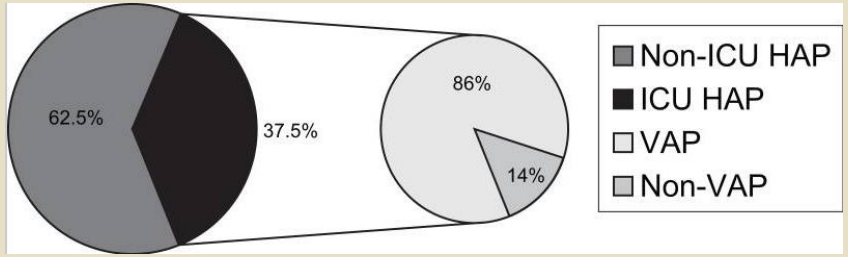
Consulting/honoraria

- **BioFire Diagnostics**
- ChromaCode
- Luminex
- Genmark

BOLD: Products discussed today

Lower Respiratory Illness

- Community acquired infections
  - Leading reason for unscheduled <u>outpatient</u> healthcare visit
    - *S. pneumoniae, H. influenzae*, "atypical bacteria", viral
  - Empirically treated, Lab Dx often not done
- Hospital acquired infections
  - 2nd leading cause of HAI (HAP/VAP)
    - GNRs (MDR), MRSA, rarely viral
  - Impact
    - Increased length of stay (4d vs 16d)
    - Increased all cause mortality (10% vs 25%)
    - Account for **<u>50% of all Abx</u>** prescribed in ICUs



Organism	%
P. aeruginosa	24.4
S. aureus	20.4
Enterobacteriaceae	14.1
Streptococcus spp	12.1
Haemophilus	9.8
Acinetobacter	7.9
Neisseria spp	2.6
S. maltophilia	1.7
Others	4.7

Loffelholz et al. *Int. J. Microbiol. 2010,* Naghavi et al. *Lancet. 2015 Jan 10;385(9963):117-71,* Hing et al. *National Health Statistics Report. 2010,* Vincent et al.. *JAMA Dec 302(21) 2323-29*

Practice and Consequences

- Prognosis/Mortality
  - Dependent on severity index, early empiric antibiotics, **laboratory diagnosis**

Table 5. Variables associated with early (≤2 days), late (≥3 days) and total mortality[a]

	% mortality			Multivariate OR (95% CI)			Multivariate p value		
Variable	**Early**	**Late**	**Total**	**Early**	**Late**	**Total**	**Early**	**Late**	**Total**
PSI risk class									
Low-risk (classes I, II, III)	0.2	1.9	2.1	13.0 (4.0–42.6)	6.1 (4.0–9.3)	7.3 (4.9–10.9)	<0.01	<0.01	<0.01
High-risk (classes IV, V)	3.1	12.1	15.2						
Empirical antibiotic									
No IDSA/ATS first choice	1.2	8.1	9.3	–	0.6 (0.5–0.8)	0.7 (0.5–0.9)	NS	<0.01	0.02
IDSA/ATS first choice	1.7	5.8	7.4						
Aetiological diagnosis									
No	1.8	7.2	8.9	–	0.5 (0.3–0.8)	0.5 (0.3–0.8)	NS	<0.01	<0.01
Yes	1.3	6.4	7.7						

Garau J et al. Clin Microbiol Infect. 2008 Apr;14(4):322-9

Laboratory Challenges – Diagnosis of LRTI

- Bacterial "pathogens"
  - Common pathogens are often upper respiratory flora/colonizers
- Specimen
  - Sputum/ETA
    - More likely to contain URT/oral flora → poor PPV
    - Difficult to manipulate → viscosity impacts plating reliability/quantitation
  - BAL
    - "Clean" LRT specimen but susceptible to sampling bias, invasive

≥48-72 h for ID/AST

- Challenges in interpretation an reporting
  - Conditional – *S. pneumoniae, H. influenzae, M. catarrhalis, P. aeruginosa, S. aureus, Enterobacteriaceae*
    - Quant: BAL$\geq 10^4$, SPU/ETA $\geq 10^5$-10^6
    - Semi-quant: "Significant" quantity (2+), predominance, > NOF, ≥ 3 pathogens is MPI

Laboratory Results – Goal

- Improved patients outcomes
  - Reduce time to optimal targeted therapy
    - Rapid result, **High PPV**
      - Accurate ID - multiple pathogens
      - Quantitative results?
      - Resistance profile
  - Reduction in unnecessary therapy
    - Rapid result, **High NPV**
      - Single high prevalence/consequence pathogen
      - Select resistance markers

Can molecular diagnostics help?

Question #1: Method of Dx

- Are laboratories offering MolDx for Lower Respiratory Tract Infections?
  - What tests?
    - Viral panels? LDTs? Anything for **bacterial** pneumonia?
  - What specimens…off label?
  - What population?
    - Inpatient? Outpatient? No restriction?
  - Any measured outcomes?

Molecular Options (Targeted)

- MRSA
  - ~60% of ICU patients meet IDSA criteria for empiric anti-MRSA therapy
    - ~5% culture prevalence, ~8% NAAT prevalence

S. aureus		Culture	
		Positive	Negative
NAAT	Positive	71	73
	Negative	1	438

Sensitivity: 98.6% **NPV: 99.8%**
Specificity: 85.7% PPV: 49.3%

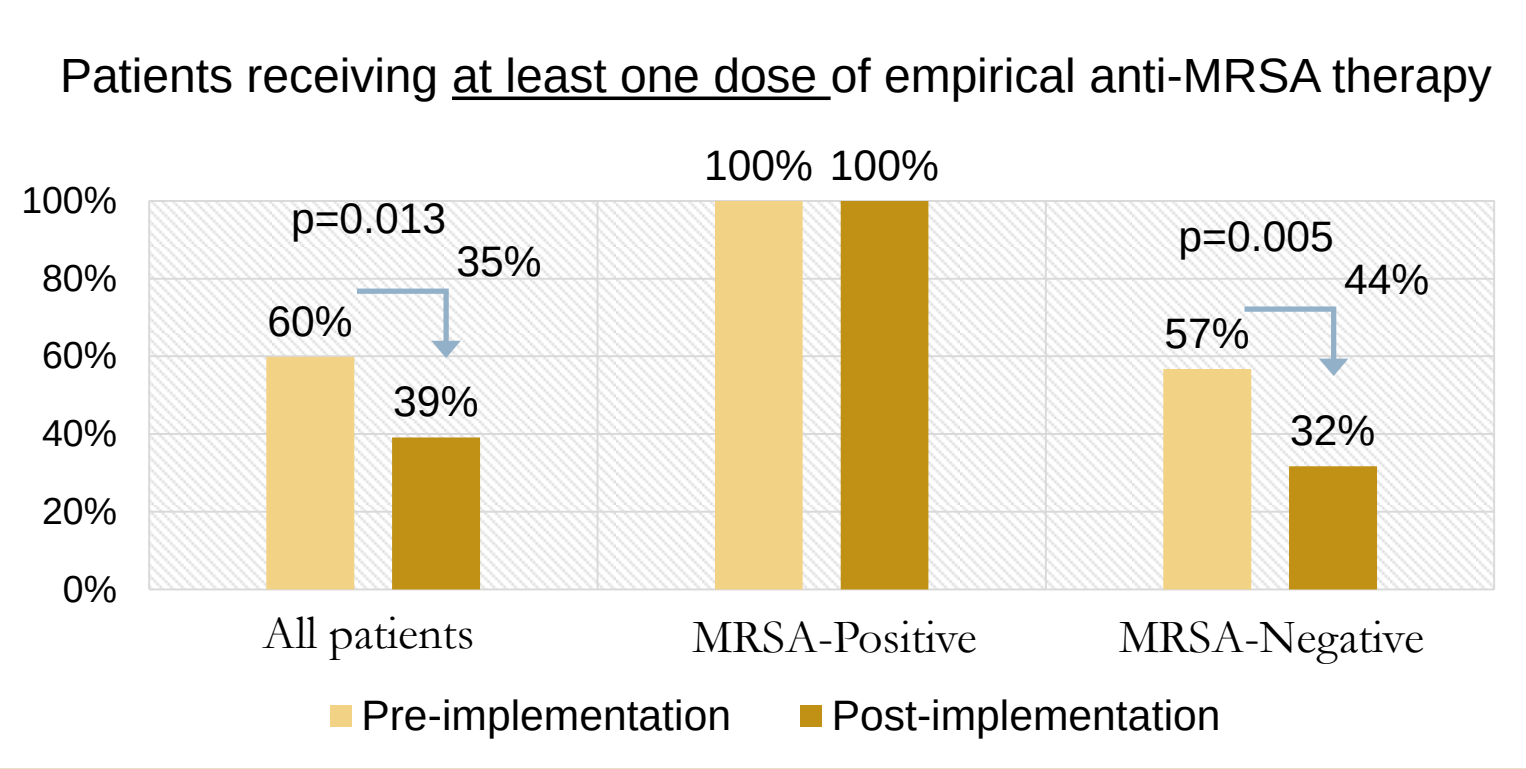
MRSA		Culture	
		Positive	Negative
NAAT	Positive	28	20
	Negative	1	534

Sensitivity: 96.6% **NPV: 99.8%**
Specificity: 96.3% PPV: 58.3%

~65% of "false positive" specimens had *S. aureus* on culture plate, not reported due to laboratory policy
- ~50% of patients had *S. aureus* reported in culture from subsequent specimen

Molecular Options (Targeted)

- MRSA
  - Impact of NAAT on anti-MRSA therapy



Why not 100% reduction in MRSA (-)?

Patient met "major" risk factors
-Anti-MRSA abx given immediately

Concern for other focus of infection

Passive reporting
-Result not reviewed/acted upon until daily rounds

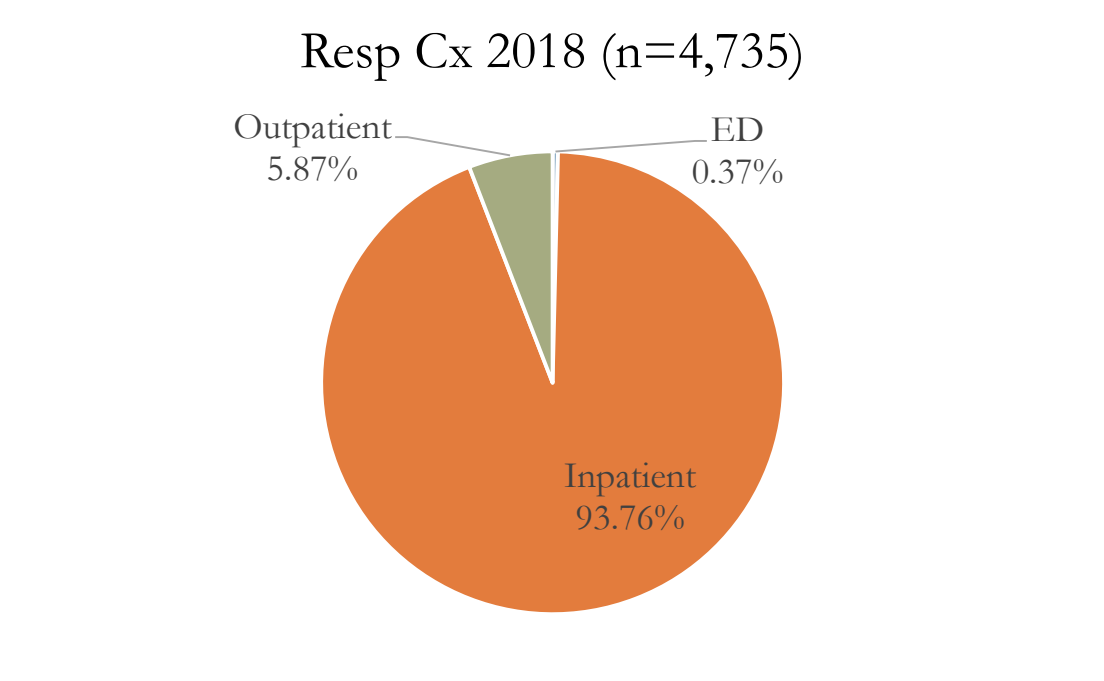
-Pharmacy/ASP intervention **essential** to increase benefit of rapid MolDx

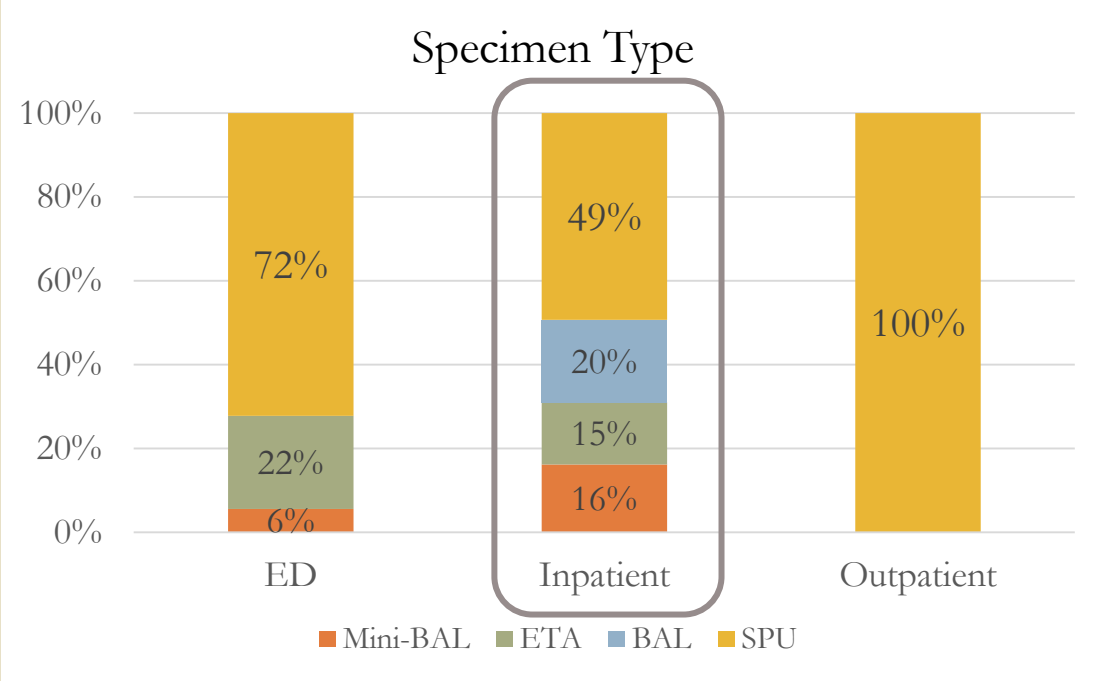
Molecular Options (Panels)

	FilmArray Pneumo	Unyvero LRT	Accelerate Respiratory
Regulatory	FDA-cleared	FDA-cleared	Development
Technology	NAAT	NAAT/array	Microscopy/FISH
Specimen	Sputum, ETA, BAL	Sputum, ETA	?
Bacterial targets	18	20	?
Viral targets	8	0	0
Resistance	7	17 (10 FDA-cleared)	Phenotypic
Result	Semi-quantitative	Qualitative	Quantitative?
Workflow	Sample-Result	2-step (Lysis, Analysis)	2 step (Lysis, Analysis)
Time to result	~ 1 hour	4.5-5.5 h	8-12 h ?

Question #2a: Choosing a test

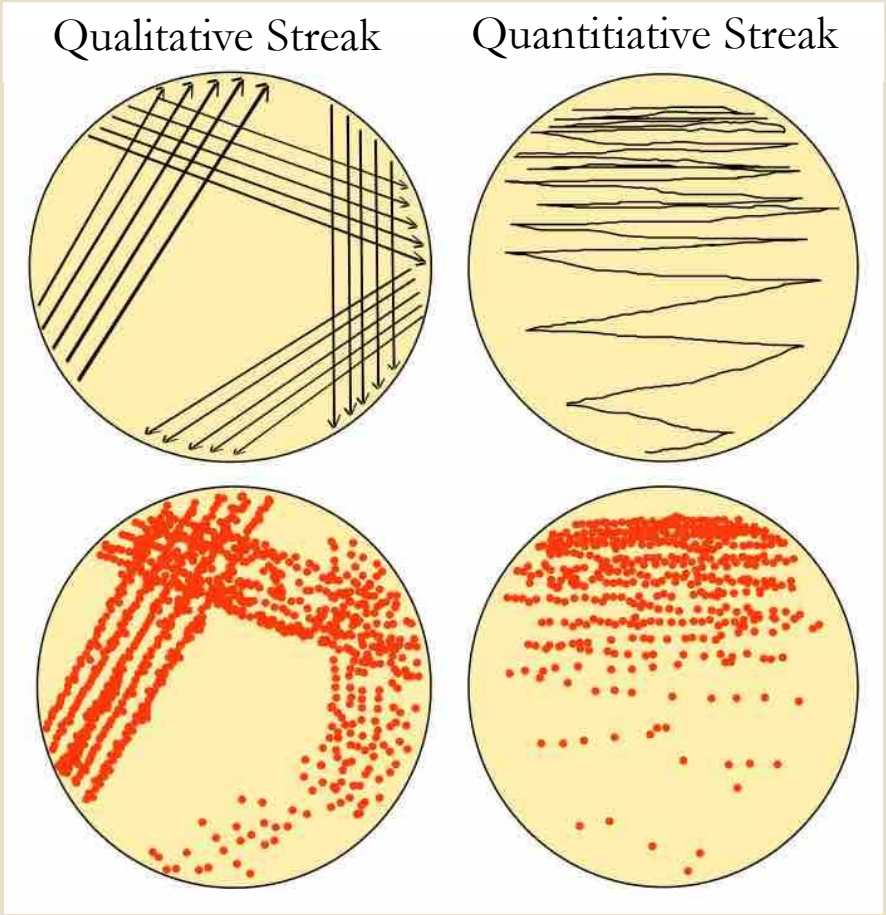
- What specimens do you receive for analysis?
  - Resp Cx volume inpatient vs. outpatient vs. ED?
  - What type of specimen do you receive?





Question #2b: Choosing a test

- How do you work up/report results from respiratory cultures?
  - Quantitative or qualitative?
    - 10^4 *E. coli*, 10^7 *P. aeruginosa* vs. 1+ *E. coli*, 4+ *P. aeruginosa*
  - Is this specimen dependent?
    - BAL vs Sputum?
  - Specific thresholds for reporting pathogens?
    - Absolute or relative?
  - Are viral NAATs ordered on inpatient HAP/VAP?
    - Frequency?



IDSA/ATS Guidelines 2016

Goal: Minimize unnecessary abx exposure through de-escalation and shorter abx courses for LRTIs

- Laboratory Dx guidelines focus on **sensitivity** over specificity → High NPV
  - Recommend non-invasive (sputum)
  - **Semi-quantitative culture**

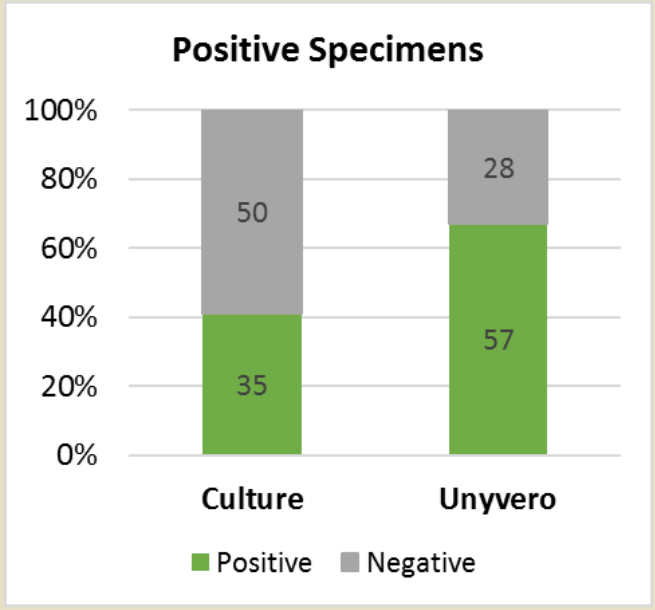
- If invasive BAL/mini-BAL collected, recommend use of **quantitative** thresholds
  - No difference in mortality between patients whose Abx withheld based on Q-culture thresholds vs. treated
  - Patients treated based on thresholds reduced total time on Abx, reduced rate of superinfection w/ MDRO

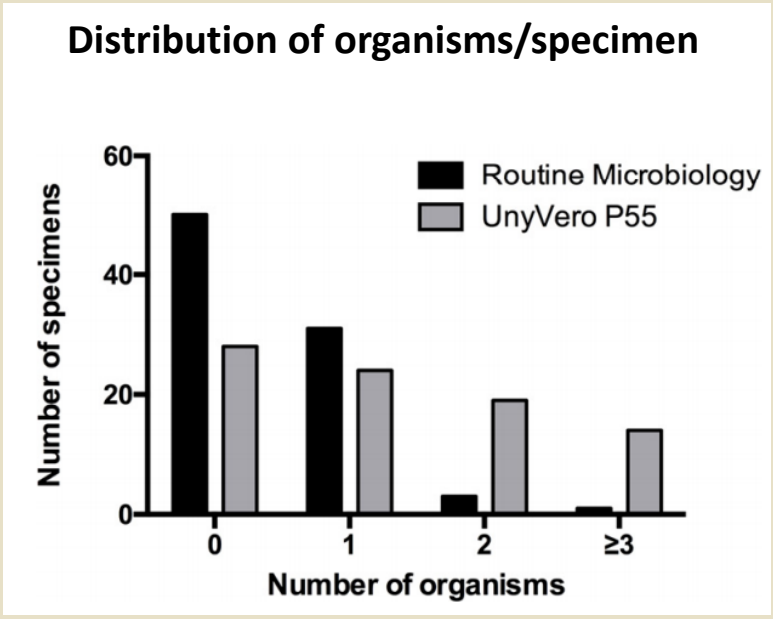
Molecular test could support this goal through **rapid result** and **high sensitivity/NPV**

Quantitative test essential to aid interpretation of complex specimens and provide maximal benefit for invasive specimens

Unyvero: Overview

- **Qualitative** multiplex MolDx
  - Study #1: 85 Sputum or ETA specimens (HAP, VAP, CAP)
    - Results compared to semi-quant SOC culture report





Positive: ≥ 1 "pathogen"
Negative: No growth, **NOF**, mixed growth/doubtful significance

C. Ozongwu et al. Biomolecular Detection and Quantification 13 (2017) 1–6

Unyvero: Overview

Target Organism	Routine laboratory	True Positive (Routine and Unyvero P55)	False Positive (Unyvero P55 only)	False Negative (Routine only)	Sensitivity (%)	Specificity (%)
S. aureus	4	4	5	0	100.0	93.8
S. pneumoniae	3	1	2	2	33.3	97.6
A. baumannii	0	0	3	0	–	96.5
P. aeruginosa	11	11	12	0	100.0	83.8
H. influenzae	7	6	6	1	85.7	92.3
M. catarrhalis	1	1	2	0	100.0	97.6
S. maltophilia	5	4	16	1	80.0	80.0
E. cloacae cplex	0	0	4	0	–	95.3
E. aerogenes	0	0	1	0	–	98.8
E. coli	3	3	12	0	100.0	85.4
K. pneumoniae	3	3	4	0	100.0	95.1
K. oxytoca	0	0	2	0	–	97.6
K. variicola	0	0	0	0	–	100.0
M. morganii	0	0	0	0	–	100.0
Proteus spp	0	0	2	0	–	97.6
S. marcescens	1	1	6	0	100.0	92.9
C. freundii	0	0	1	0	–	98.8
L. pneumophila	0	0	0	0	–	100.0
M. pneumoniae	0	0	0	0	–	100.0
Overall (average)		34	78	4	88.8	94.9

- Sensitivity 89%
- Low overall concordance:
  - 67% (57/85) of specimens
- Discordance
  - 86% (24/28) of discordant specimens reported as
    - NOF
    - Non-significant growth
    - Mixed growth/doubtful significance

Abx? Low abundance?

Clinically significant?

C. Ozongwu et al. Biomolecular Detection and Quantification 13 (2017) 1–6

Unyvero: Overview

- **Analysis of 14 randomly selected specimens**
  - Compare Cx, Unyvero, quantitative 16S NGS
    - Single pathogen reported by Cx → also reported by Unyvero and accounted for majority of sequencing reads
    - NOF reported by Cx → Unyvero positive, NGS demonstrated a mixture of reads without a predominating organism

Specimen number & type	Routine Culture Result	Unyvero P55 Result	16S rRNA Sequencing Results
347 ETT	*P. aeruginosa*	*P. aeruginosa* (+++)	*Pseudomonas* spp. 79.6% *Others 20.4%*
346 ETT	*P. aeruginosa*	*P. aeruginosa* (+++) *S. marcescens* (+++) *S. aureus* (++) *S. maltophilia* (++)	*Pseudomonas* spp. 38.4% *Streptococcus spp. (not including S. pneumoniae) 17.1%* *Neisseria* spp. 16.4% *Serratia* spp. 12.5% *Stenotrophomonas spp* 1.6% *Others 14.0%*
360 ETT	Normal respiratory flora	*S. maltophilia* (++)	*Acinetobacter* spp. 3.4% *Pseudomonas* spp. 1.2% *Mixed commensal genera*

"These data suggest broad agreement of NGS with routine culture rather than Unyvero"

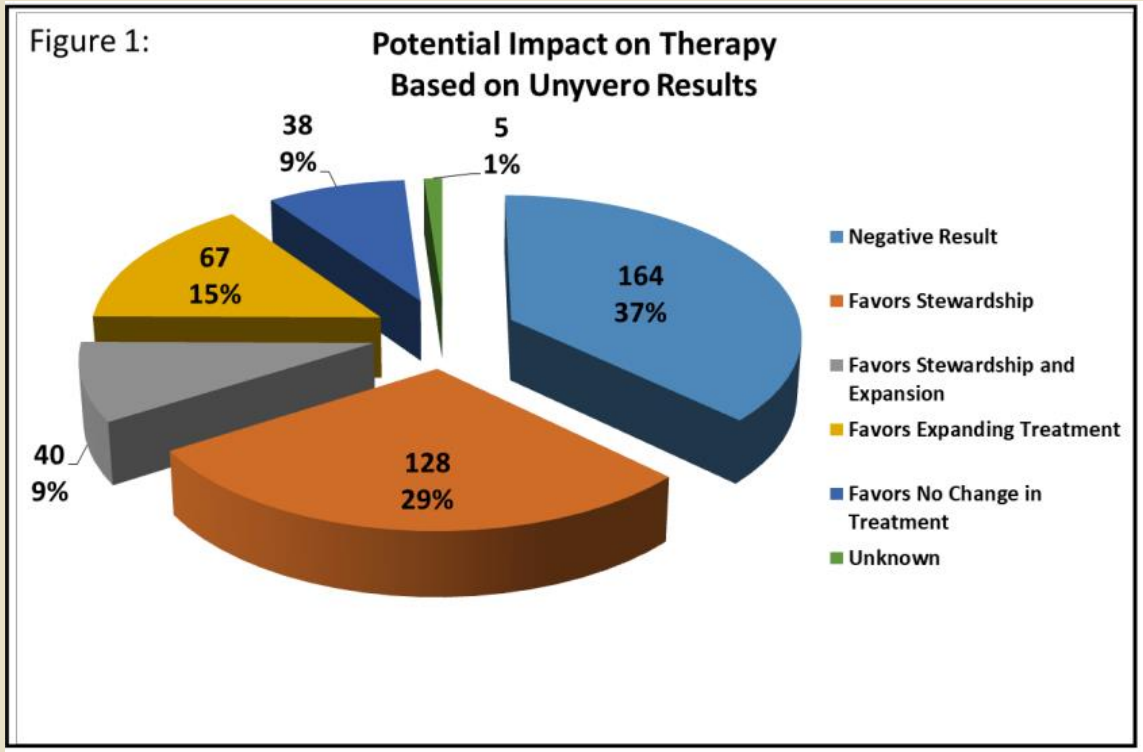
Limited ability to de-escalate antibiotics → Can **not** differentiate colonization vs. infection

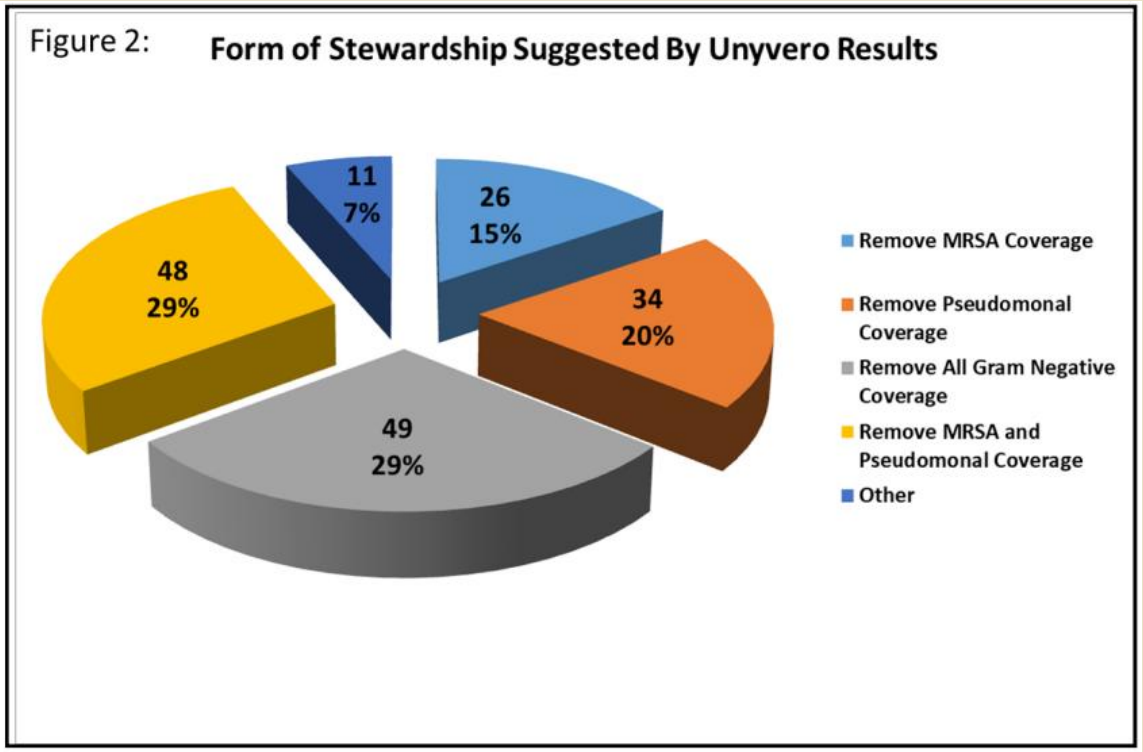
Potential for over-utilization of antibiotics to treat "insignificant" bacteria

C. Ozongwu et al. Biomolecular Detection and Quantification 13 (2017) 1–6

Unyvero: Impact

- Study #2
  - 442 patients, 93% sensitive compared to culture





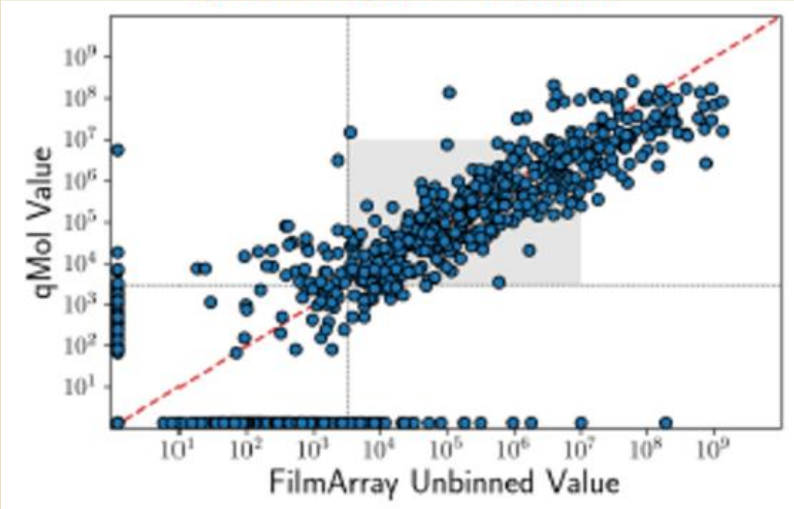
  - **29% narrow** therapy, **15% escalate** therapy based on Unyvero result
    - Escalation most commonly based on detection of *S. maltophilia* → no data on relative abundance in culture

Mopuru et al, ASM Microbe 2018. Abstract

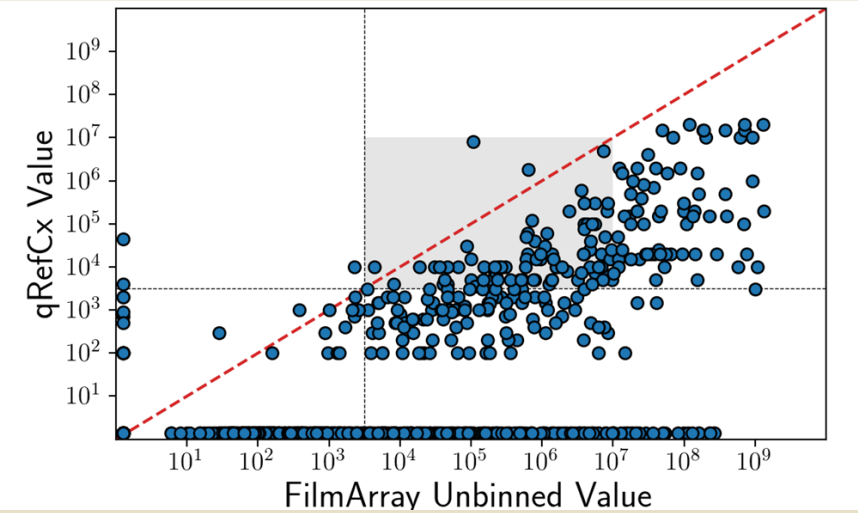
FA-Pneumo: Overview

- Semi-quantitative multiplex MolDx
  - 18 bacterial agents (15 reported semi-quantitatively) in addition to 9 viral agents
- Addresses problem of relative abundance for bacterial targets in resp. specimens
- AMR
  - $10^{3.5}$ to $>10^{6.5}$, reported semi-quantitatively in "bins"
  - Accuracy of molecular quantification?

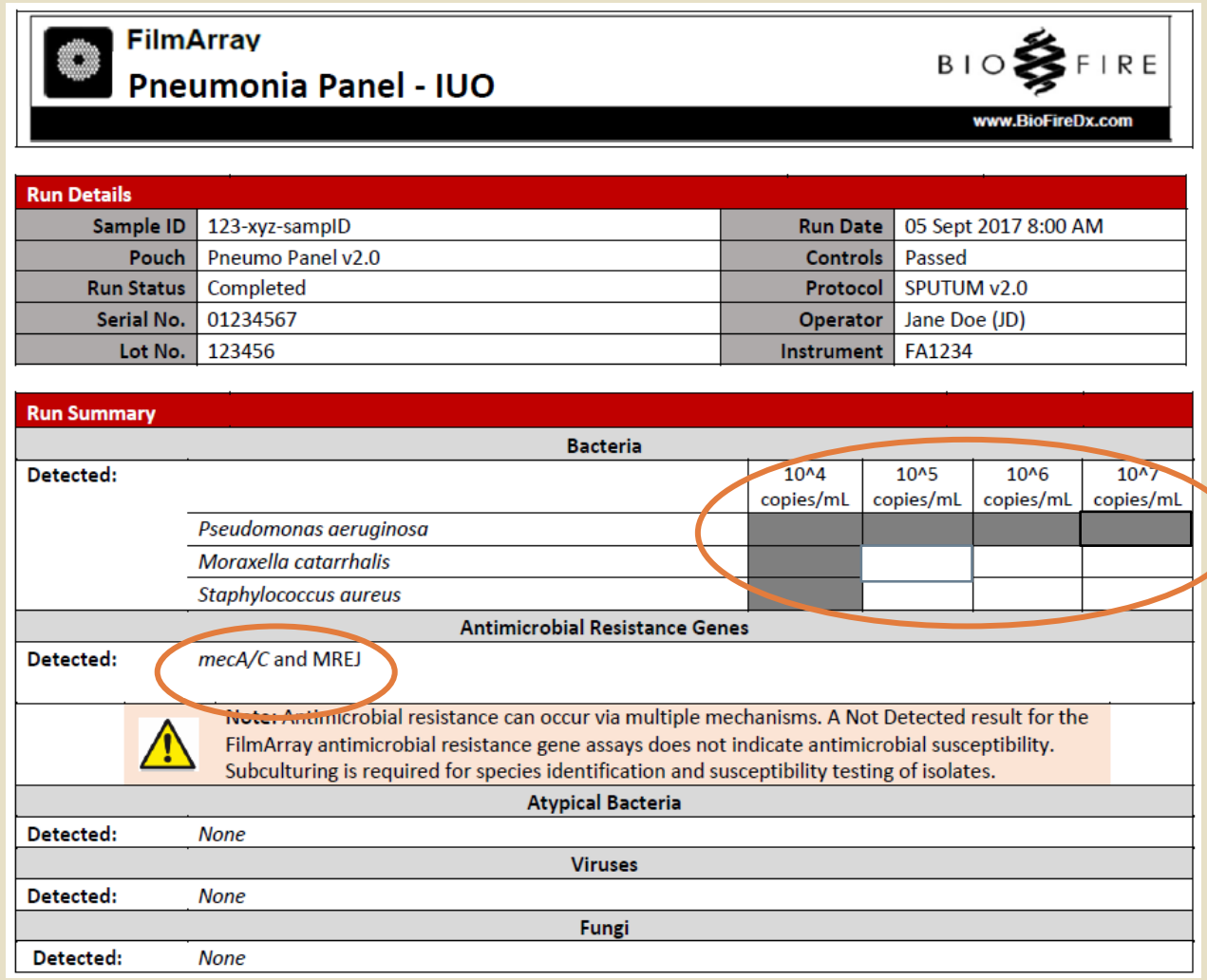
Singleplex qPCR vs FilmArray



qCulture vs. FilmArray



FA-Pneumo: Report



FilmArray
Pneumonia Panel - IUO
BIOFIRE
www.BioFireDx.com

Run Details			
Sample ID	123-xyz-sampID	Run Date	05 Sept 2017 8:00 AM
Pouch	Pneumo Panel v2.0	Controls	Passed
Run Status	Completed	Protocol	SPUTUM v2.0
Serial No.	01234567	Operator	Jane Doe (JD)
Lot No.	123456	Instrument	FA1234

Run Summary

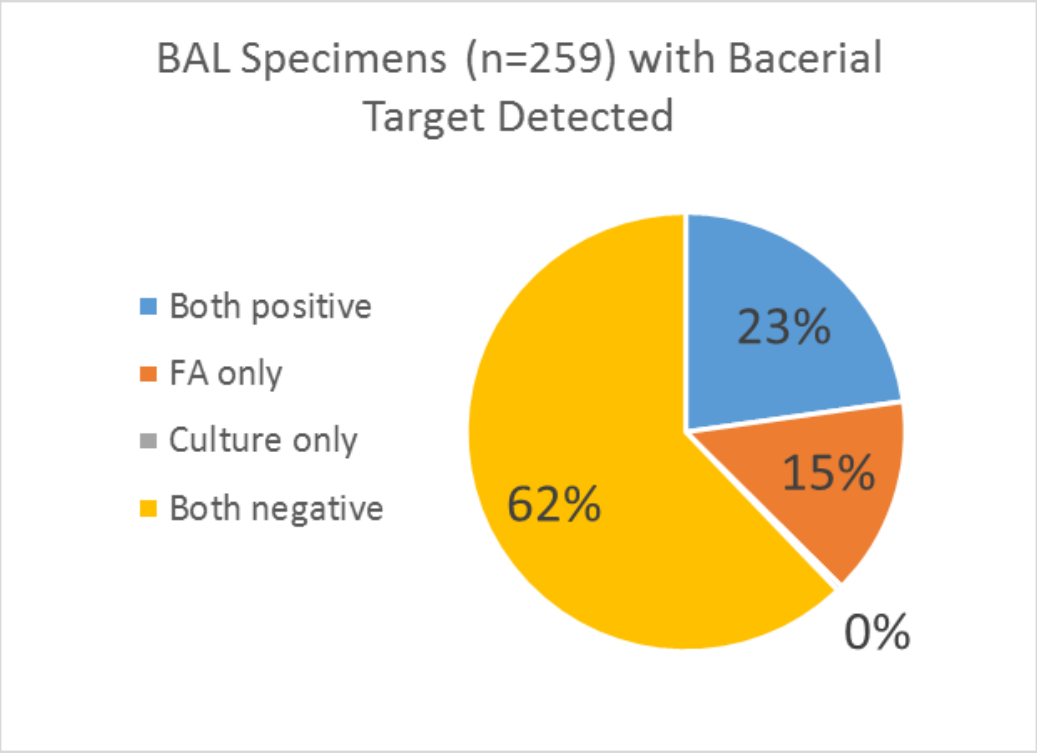
Bacteria					
Detected:		10^4 copies/mL	10^5 copies/mL	10^6 copies/mL	10^7 copies/mL
	Pseudomonas aeruginosa				
	Moraxella catarrhalis				
	Staphylococcus aureus				
Antimicrobial Resistance Genes					
Detected:	*mecA/C* and MREJ				
Note: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.					
Atypical Bacteria					
Detected:	*None*				
Viruses					
Detected:	*None*				
Fungi					
Detected:	*None*				

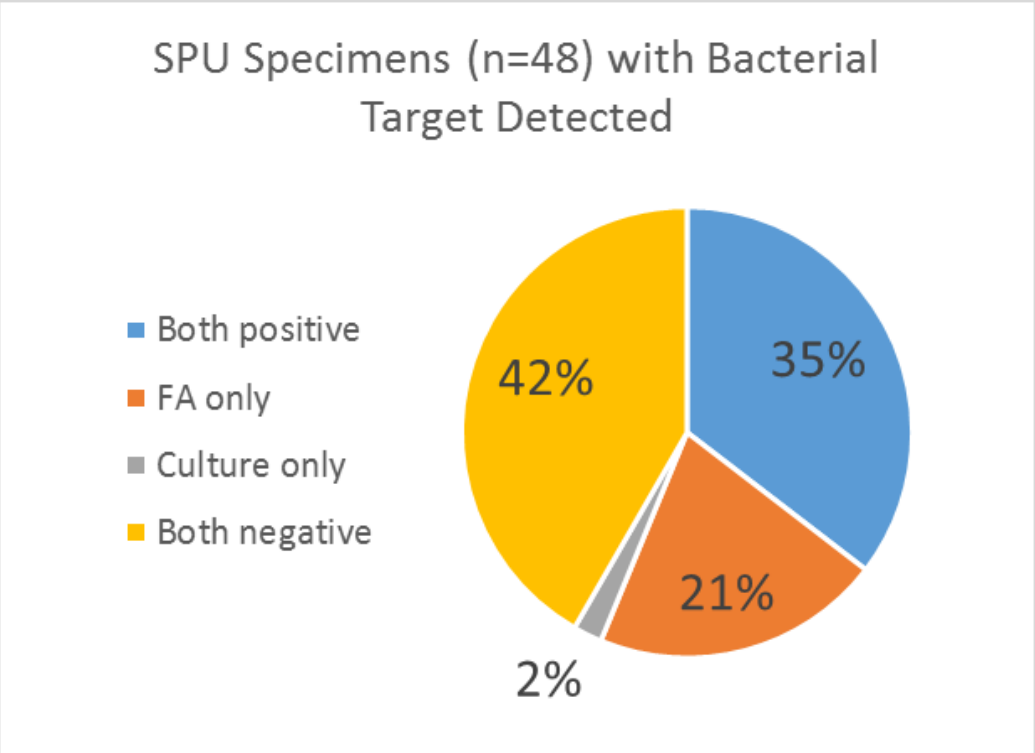
- Summary of bacteria detected
- Relative abundance
  - Report broadly applicable
    - Quantitative vs. semi quantitative reporting
    - Specimen-specific thresholds
  - Sensitive detection of resistance markers
    - Early Abx escalation/deescalation
    - Infection control/isolation

E.g. *Moraxella & S. aureus* may not clinically significant but MRSA still infection control concern

Bacterial Targets: Overview

- Number of specimens with on-panel target detected





FilmArray detected a bacterial target in 65% more BAL and 57% more SPU specimens than routine culture

Bacterial Targets: Performance

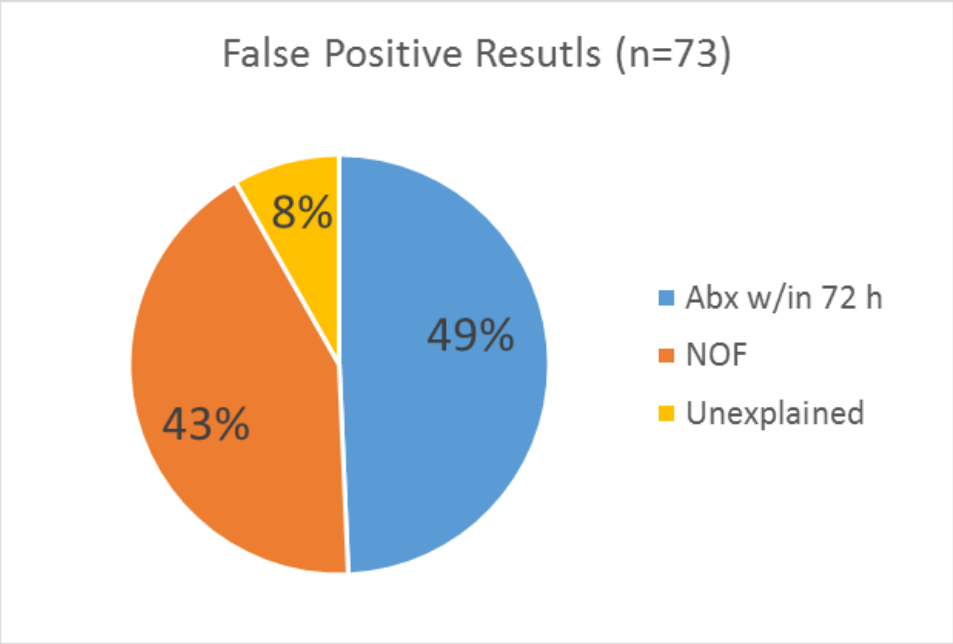
- FA vs. SOC culture report (BAL)

"sensitivity" "specificity"

Target	SOC+/FA+	SOC+/FA-	SOC-/FA+	SOC-/FA-	Total	PPA	NPA
A. baumannii	1	0	0	258	259	100%	100%
E. cloacae cplx	7	0	4	248	259	100%	98.4%
E. aerogenes	3	0	1	255	259	100%	99.6%
E. coli	1	1	1	254	259	50.0%	99.6%
H. influenzae	4 20%	0	16	236	259	100%	93.7%
K. oxytoca	2	0	3	251	259	100%	98.8%
K. pneumoniae	8	0	3	247	259	100%	98.8%
M. catarrhalis	2 25%	0	6	249	259	100%	96.9%
Proteus sp	2	0	2	254	259	100%	99.2%
P. aeruginosa	18	1	6	234	259	94.7%	97.5%
S. marcescens	3	0	0	256	259	100%	100%
S. agalactiae	1	0	6	253	259	100%	97.6%
S. pneumoniae	2	0	3	254	259	100%	98.8%
S. pyogenes	0	0	1	258	259	100%	99.6%
S. aureus	21 50%	1	21	216	259	95.5%	91.1%

Bacterial Targets: Discordance

- FA vs. SOC culture report (BAL)



- Unexplained → 3/6 (50%) were *S. aureus* quantified at 10^4/mL
  - ~10^3 in Cx – Not detected or not reported/below threshold
  - (2) *P. aeruginosa* 10^5, abx **not** recorded; (1) *H. inlfluenzae* 10^6
- NOF
  - 10/31 (32%) quantified at 10^4/mL
  - 13/31 (42%) contained ≥ 1 more predominant target(s) recovered in Cx → not reported per lab policy?
- Abx → Useful to detect these?
  - Prevent premature discontinuation of Abx based on negative Cx
  - Allow appropriate de-escalation (e.g. pip/tazo to amox for *H. flu*)

Bacterial Targets: Correlation

- Composition of positive specimens – Correlation of <u>predominant</u> target detection

60

97

Targets	SOC 0	SOC 1	SOC 2	SOC 3	SOC 4
FA 1	31	29/29 (100%)	-	-	-
FA 2	5	11/13 (85%)	8/9 (89%)	1/1 (100%)	-
FA 3	-	1/1 (100%)	2/2 (100%)	-	-
FA 4	1	1/1 (100%)	1/1 (100%)	-	0/1 (0%)
FA 5	-	-	-	-	
FA 6	-	1/1 (100%)	-	-	-

<u>Predominant organism agreement</u>

- At least 1 target detected FA+Cx
  - 55/59 (93%) of specimens
- > 1 target detected by FA or SOC
  - 26/30 (87%) of specimens

FA: Staaur 10^6, Entclo 10^5 → Cx: Entclo 10^5
FA: Staaur 10^5, Entclo 10^4 → Cx: Entclo 10^4

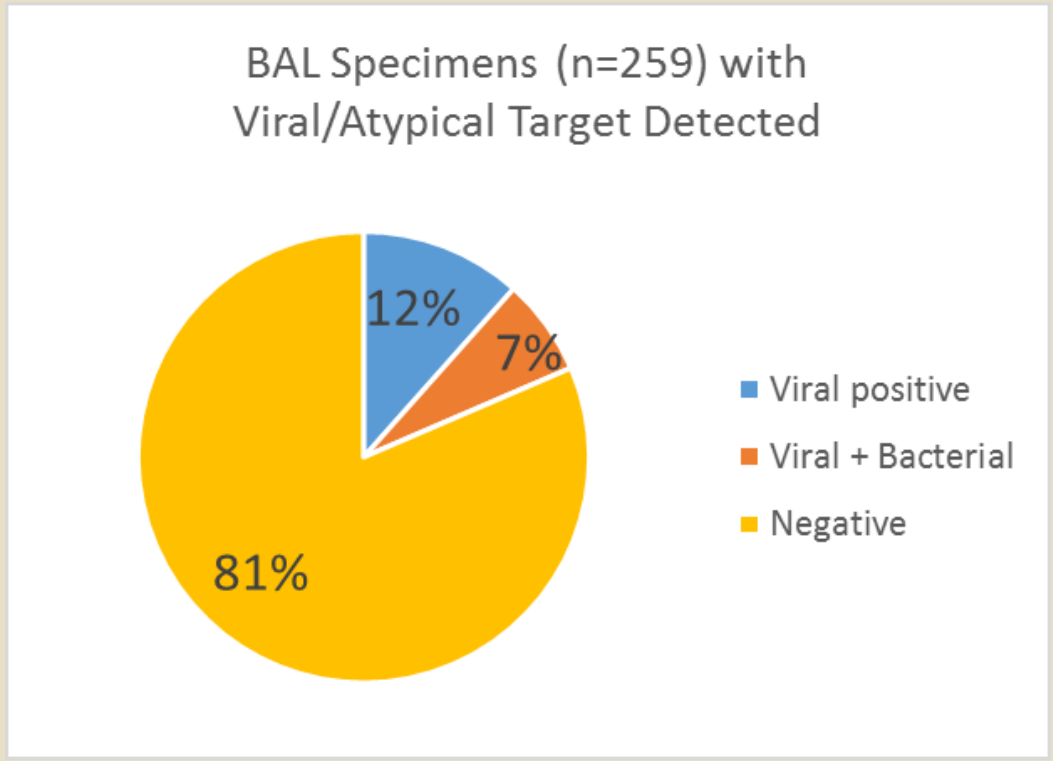
Both patients on anti-staphylococcal abx at time of collection

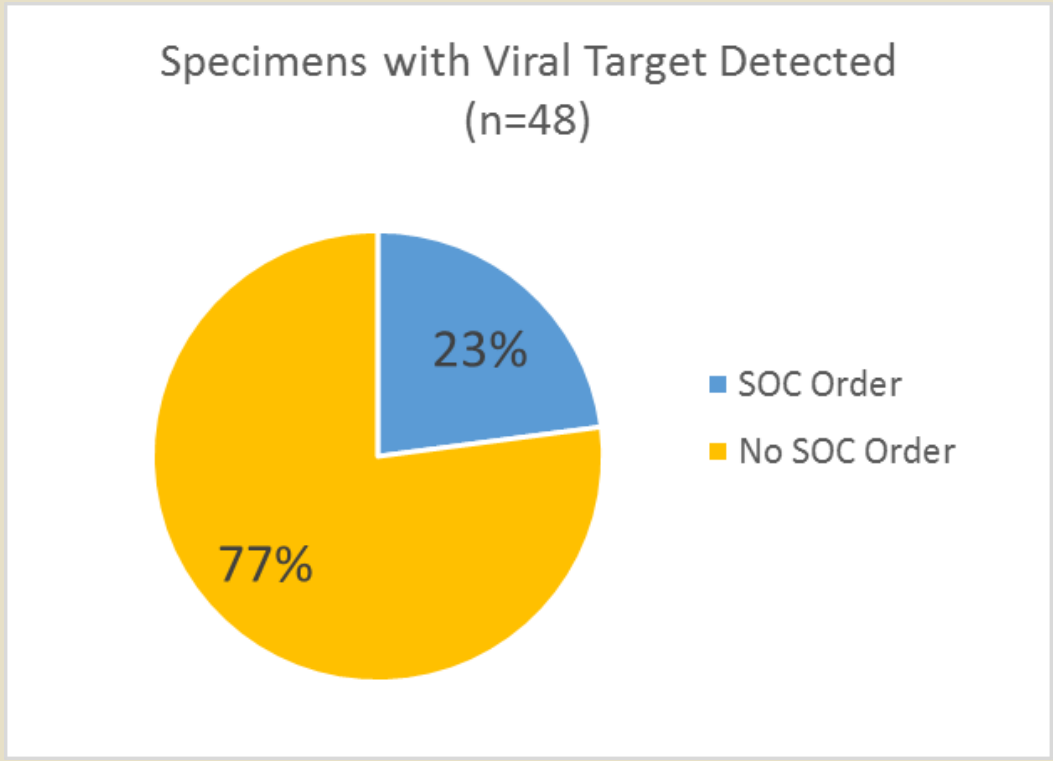
FA: Strpne 10^7, Pseaer 10^5 → Cx: Pseaer 10^4, Strpne "few"

No NOF, No Abx…abundance of Pseaer outcompete?

Viral Targets: Overview

- Number of specimens with on-panel target detected





FilmArray detected a viral target in 19% of BAL.

77% of positive specimens did not have SOC order

Viral Targets: Performance

- Number of specimens with on-panel target detected

Target	FA+	SOC Order	SOC Agree	FA No Bacteria
hRV/EV	17	6/17 (35%)	6/6 (100%)	7/17 (41%)
CoV	9	2/9 (22%)	2/2 (100%)	7/9 (78%)
FluA	5	0/5 (0%)	n/a	3/5 (60%)
PIV	3	1/3 (33%)	1/1 (100%)	2/3 (66%)
FluB	2	1/2 (50%)	1/1 (100%)	1/2 (50%)
RSV	2	0/2 (0%)	n/a	2/2 (100%)
hMPV	1	0/1 (0%)	n/a	0/1 (0%)
AdV	1	0/1 (0%)	n/a	1/1 (100%)
Legionella	1	0/1 (0%)	n/a	1/1 (100%)
Mycoplasma	1	0/1 (0%)	n/a	1/1 (100%)
CoV+hMPV	1	1/1 (100%)	1 (100%)	0/1 (0%)
hRV/EV+PIV	3	0/3 (0%)	n/a	1/3 (33%)
hRV/EV+CoV	1	0/1 (0%)	n/a	0/1 (0%)
hMPV+FluA+CoV	1	0/1 (0%)	n/a	1/1 (100%)
None Detected	211	79/211 (37%)	76/79 (96.2%)	129/211 (61%)

~80% Missed diagnosis b/c not in differential
…but are they clinically significant?

- 27/48 (56%) specimens with a viral detection were **negative** for bacterial targets

FluA: No SOC orders, uncommon HAI, specific therapy available

Others: <20% SOC orders, no specific therapy, Serious infection in compromised patients, Infection control/cohorting

Impact of FilmArray result on Management

- Time to result and antibiotic adjustments

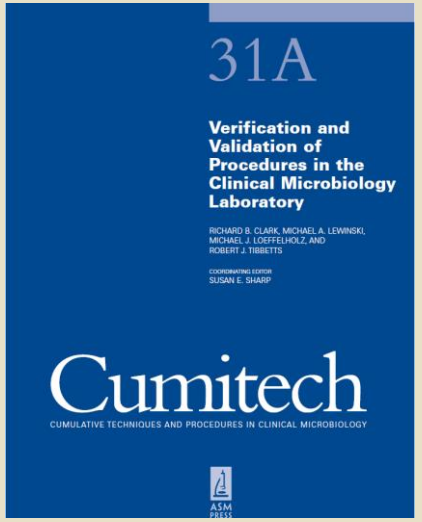
Potential Change, no.	Antimicrobials	Patients	Hours
Appropriate de-escalation/discontinuation	206	122 (48%)	18,284.07
Appropriate escalation/initiation	5	5 (2%)	184.66
Inappropriate de-escalation/discontinuation	6	6 (2%)	-
Inappropriate escalation/continuation	42	42 (17%)	-
No change	-	78 (31%)	-
Unable to assess*	-	16	-

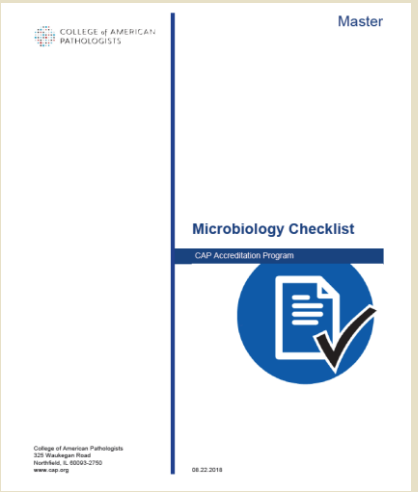
* Date or time not included for antimicrobials, concomitant infection present (cannot determine which antimicrobials are used for LRTI versus other infection), etc.

- Antibiotic adjustment could be made on 165/243 (68%) evaluable patients
  - 10 patients fell into multiple categories
- Multiple antibiotic interventions could be made/patient (avg 1.48/patient)
  - >18,000 antibiotic hours saved (avg. 6.2 d/patient, 3.8 d/abx)

Question #3: Verification

- How do you approach validation of FDA-cleared tests?
  - What components do you assess? e.g. precision, LoD, accuracy?
- What are your specimen criteria?
  - How many total? What proportion clinical vs. spiked vs. retrospective?
- Do all targets need to be evaluated for a multiplex test?
  - Spiked? Reference material? Archived specimens?
  - How do you control cost of validation for expensive tests $$$?
- What is deemed "acceptable" performance to approve validation?
  - What is "gold standard"
  - What sens/spec is required?

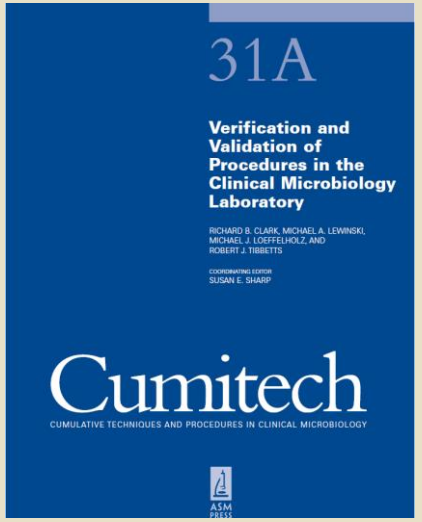


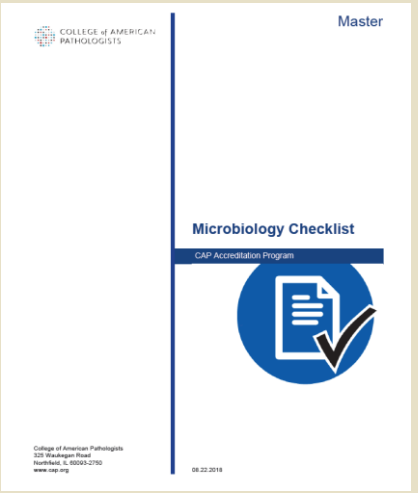


Verification

- FDA-cleared tests
  - Determine if manufacturer claimed performance can be achieved in your lab
  - Precision, accuracy, reportable range, reference range (PARR)

- Precision
  - Within run, between run, between operator
    - Qualitative
      - Include both positive and negative samples, preferably near LoD
      - Replicate samples in at least duplicate (same run and different run)…3x5 day? 20 day?
      - Multiplex → analytes not included can serve as "negative" specimens…reduce cost
    - Quantitative
      - No specific guidance for "acceptable precision"
      - Dependent on assay analytical precision, clinical decision points





Verification

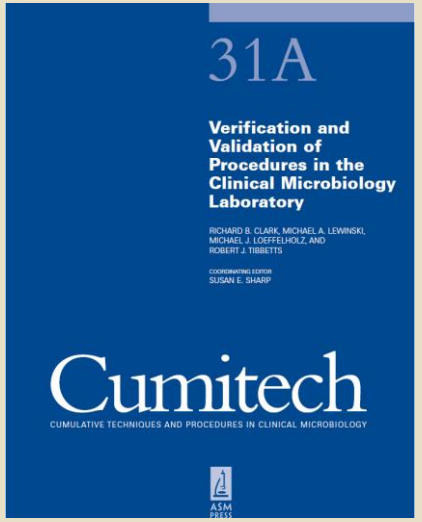
- Precision – *Sample plan*
  - Contrive a specimen containing **three** bacterial analytes that **span AMR**
    - e.g. 10^4 CFU/ml *E.coli*, 10^5 CFU/mL *P. aeruginosa*, $>10^7$ CFU/mL S. *pneumoniae*
    - Spike into neg matrix or flu(+) matrix
  - Test **same** contrived specimens in **duplicate** on 3 days
    - Confirms that MolDx test calls correct targets (and relative abundance if applicable)
    - Also confirms 72 h stability at 4C

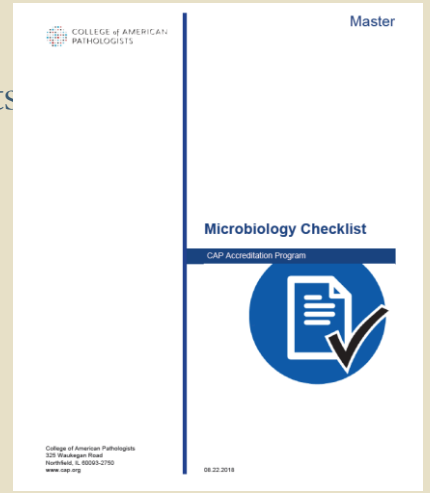
Total of **6 tests** used for Precision study but evaluated multiple targets, quantitation, and stability

After implementation, record daily QC results for rotating controls to support verification/IQCP

Verification

- Accuracy
  - Number of specimens
    - Cumitech – **"Minimum of 20 specimens"**, positive and negative, high and low concentration
      - May include reference material or archived clinical specimens
    - CAP – **"A sufficient number"**, may include reference material in matrix
  - Breadth of targets
    - Cumitech – **"Clinically relevant organisms for the institution"**, i.e. ok to exclude rare targets
    - CAP - Must include specimens representing **each strain** tested (may include spiked)
  - Acceptable performance
    - Cumitech – **"At least 90% accuracy compared to reference method"**, e.g. existing test
    - CAP – **"Performs in accordance with mfr. claims"**
      - Document investigation of discordant results





Verification

- What does the package insert say?
  - Qualitative agreement vs. SOC Cx

Table 16. Bacterial Detections (as compared to SOC) in BAL Specimens for the FilmArray Pneumonia Panel Clinical Evaluation

BAL				
FilmArray Pneumonia Panel Result (n=846)	Positive SOC Culture	Negative SOC Culture		SOC Culture Not Performed
		No Growth	NOF Reported	
Detected (n=322)	195/322 (60.6%)	43/322 (13.4%)	79/322 (24.5%)	5/322 (1.6%)
Not Detected (n=524)	11/524 (2.1%)	268/524 (51.1%)	242/524 (46.2%)	3/524 (0.6%)

Sensitivity: ~98%

- 51% "No growth"
- 46% "NOF"
  - Truly negative or Below $10^{3.5}$ (clinically insignificant)

Verification

- What does the package insert say?
  - Qualitative agreement vs. SOC Cx

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Specificity: ~61%

- 35% of "False positive" were "No growth"
  - Antibiotic effect?

Verification

- What does the package insert say?
  - Qualitative agreement vs. SOC Cx

Table 16. Bacterial Detections (as compared to SOC) in BAL Specimens for the FilmArray Pneumonia Panel Clinical Evaluation

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Not Detected (n=524)	11/524 (2.1%)	268/524 (51.1%)	242/524 (46.2%)	3/524 (0.6%)

Specificity: ~61%

- 65% of "False positive" were "NOF"
  - Antibiotic effect?
  - Below Cx LoD
  - Below lab rubric for reporting?

Clinical significance?

Big or little misses?

Verification

- What does the package insert say?
  - Quantitative agreement vs. quantitiative REF Cx

Table 41. FilmArray Pneumonia Panel Overall bin performance for BAL Specimens (qRefCx)

BAL							
qRefCx Range of Values [CFU/mL] (Predicted FilmArray Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
FilmArray Bin (copies/mL)	ND	12202	1	2	0	0	0
	10^4	116	1	3	0	0	0
	10^5	90	10	11	0	1	0
	10^6	61	10	17	2	1	0
	≥10^7	61	10	36	32	11	12
% concordant		**12202/12530 (97.4%)**	**1/32 (3.1%)**	**14/69 (20.3%)**	**2/34 (5.9%)**	**12/13 (92.3%)**	**12/12 (100%)**
					41/160 (25.6%)		

25% agreement in "on-scale" binned values (96% for >10^6 bins)

Verification

- What does the package insert say?
  - Quantitative agreement vs. quantitiative REF Cx

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qRefCx Range of Values [CFU/mL] (Predicted FilmArray Bin)		**ND to <10^3.5** (ND)	**10^3.5 to <10^4.0** (10^4)	**10^4.0 to <10^5.0** (10^4 or 10^5)	**10^5.0 to <10^6.0** (10^5 or 10^6)	**10^6.0 to <10^7.0** (10^6 or 10^7)	**≥10^7.0** (≥10^7)
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	10^4	116	1	3	0	0	0
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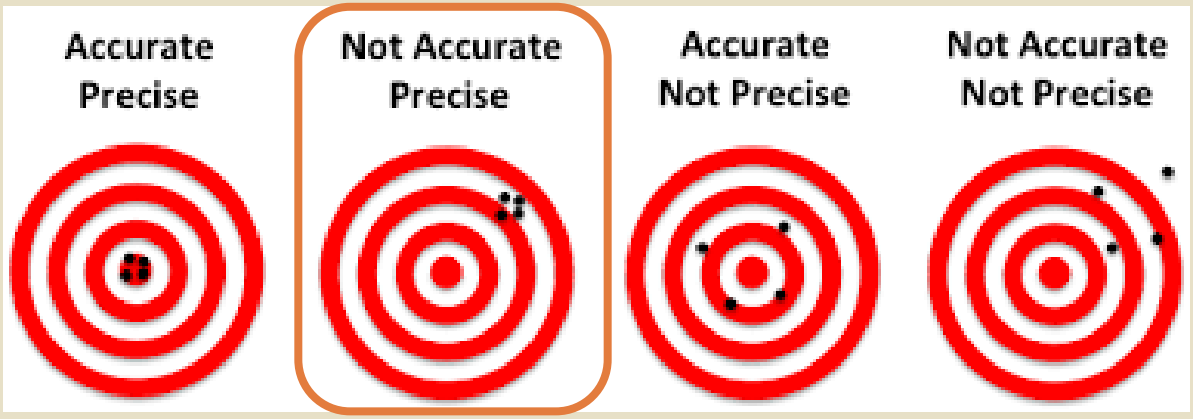
			41/160 (25.6%)				

"Lower left shift" bias in molecular quantification → over quantification vs. culture

Verification

- What does the package insert say?
  - Disclaimers/Limitations



NOTE: Detection of bacterial nucleic acid may be indicative of colonizing or normal respiratory flora and may not indicate the causative agent of pneumonia. Semi-quantitative Bin (copies/mL) results generated by the FilmArray Pneumonia Panel are not equivalent to CFU/mL and do not consistently correlate with the quantity of bacterial analytes compared to CFU/mL. For specimens with multiple bacteria detected, the relative abundance of nucleic acids (copies/mL) may not correlate with the relative abundance of bacteria as determined by culture (CFU/mL). Clinical correlation is advised to determine significance of semi-quantitative Bin (copies/mL) for clinical management.

Verification

- Accuracy – *Sample plan*
  - Goal: Clinical specimens (n=20)
    - Qualitative detection → 98% sensitivity, 60% specificity
      - Discordant analysis - Chart review to determine potential antibiotic effect, NOF
    - Quantitative detection → 25% concordance within bin, e.g. "exact agreement"
      - Consider assessment of "essential agreement" and/or "categorical agreement"
  - Goal: Contrived specimens (n=10-20)
    - Spike negative matrix with 2-4 targets at varying concentration (including $<10^3$/mL)
    - Add same target at different conc. in different specimen (think "checkerboard")
      - Expect better "exact agreement" → fresh bugs, no abx exposure
        - <u>Should build confidence that MolcDx is the "true" result, Cx is flawed</u>

Implementation and Result reporting

- Engage your client!!!!!!
  - Share data, interpretation, limitations
    - Report actual molecular quantitative number? or commute to relative +1, +2, +3, +4?
      - ~ 1 log bias toward higher Mol quantitation → impact to clinical interpretation/management
        - Do your providers adhere to ATS guidelines?
    - Commute to relative +1, +2, +3, +4?
      - Reporting relative values may relieve some of the confusion when Q-culture results do not match
        - Recall only 25% "exact agreement" with bind values but 50-70% agreement with relative abundance
    - Ask how these results will be utilized
      - Abx stewardship → Current practice to stop or continue Abx on negative Cx or continue?
      - Will "10^4 CFU/mL" result in over prescription?

Goal is added value…not added cost!

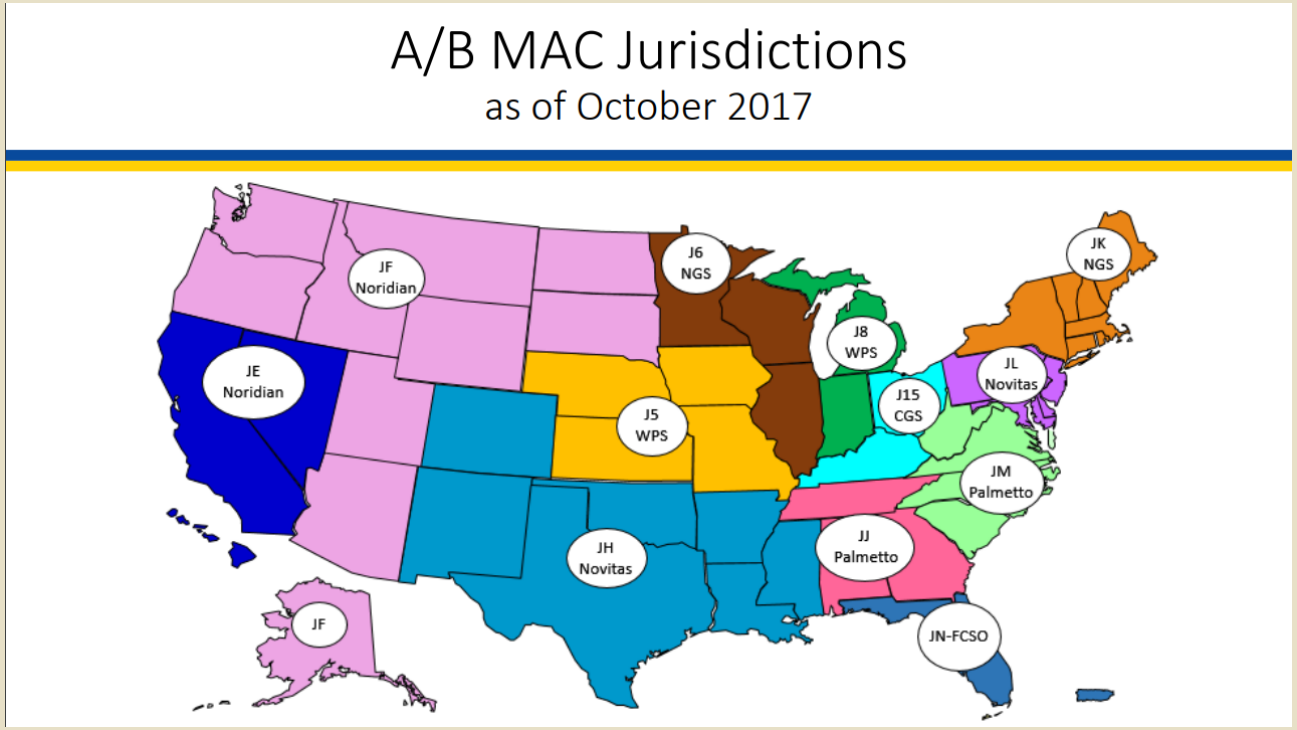
Question #5: Reimbursement

- How does your lab get paid?
  - Charges to outpatient (CPT?) vs inpatient (DRG/RVU?)
- Current status of reimbursement for multiplex panels?
  - Any difference in getting reimbursed for outpatient vs. impatient?
  - Any idea how CPT equates to RVU charges?
  - Is your lab/your hospital getting reimbursed the entire amount charged?
- What is the DRG for HAP/VAP?
  - HAIs not covered: CAUTI, CLABSI, SSI, bed sores, falls, retained foreign objects (post-surgical)

https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/Medicare-Provider-Charge-Data/Inpatient2016.html
https://www.cms.gov/medicare/medicare-fee-for-service-payment/hospitalacqcond/hospital-acquired_conditions.html

Reimbursement

- Location matters
  - Medicare Administrative Contractor (MAC)
    - Advise **Medicare** on reimbursement for fee for service charges (e.g. **outpatient CPT billing**)



Palmetto MolDx
- Limited coverage of resp and GI panels
- "Medical necessity" and outcome data

NGS
- No limited coverage ruling…..
- Panels typically covered

Private payors (BCBS, UHC, etc.)
- Independent, sometimes follow MACs

https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/Medicare-Provider-Charge-Data/Inpatient2016.html

Reimbursement

- Location matters…or does it?
  - DRG "Simple pneumonia"

Location	Charge	Avg Payment	Avg Medicare
Froedtert	$17,494	$6,774	$4,810
Albuquerque, NM	$8,146	$6,703	$5,594
Corpus Christi, TX	$59,216	$5,468	$4,199
New Brunswick, NJ	$120,730	$5,728	$3,802

  - DRG "Resp Dx with ventilator support"

Location	Charge	Avg Payment	Avg Medicare
Froedtert	$71,234	$22,212	$16,697
New Brunswick, NJ	$159,050	$21,440	$19,414

https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/Medicare-Provider-Charge-Data/Inpatient2016.html

Reimbursement

- Outpatient CPT vs Inpatient RVU (different by institution)

Target	CPT Code	CPT charge	RVU*	RVU charge
Bacteria (quantitative)	87798 (x15)	$38.99 ($584.85)	6.6 (x15)	$764.28
L. pneumophila	87541	$38.99	6.6	$50.95
M. pneumoniae	87581	$38.99	6.6	$50.95
C. pneumoniae	87486	$38.99	6.6	$50.95
Viruses (5-11)	87632	$237.14	16	$132.52
Resistance markers	87150 (x?)	$38.99 (?)	6.6 (?)	$50.95 (?)
Total		**$938.96 + R**		**$1,049.65 + R**

1 RVU = $7.72

https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/Medicare-Provider-Charge-Data/Inpatient2016.html

Reimbursement

- Simple math for inpatients

"Simple pneumonia"

DRG:	$17,494
Avg payment:	$6,774
Lab charge:	**$1,049.65**

"Pneumonia with ventilation"

DRG:	$71,234
Avg payment:	$22,212
Lab charge:	**$1,049.65**

Does the result reduce cost of care by >$1,049.65?

Reduction in length of stay → ICU $3,300 - $28,000/day
Reduction in mechanical ventilation → $1,500/day
Reduction in antibiotics → $50-$450/day
Reduction in HAIs →$1,000s - $10,000s

https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/Medicare-Provider-Charge-Data/Inpatient2016.html

Conclusions

- MolDx are capable of detecting bacterial pathogens in clinical specimens with **high sensitivity**
  - Detect potential pathogens in 60-70% more specimens, not subject to NOF, fastidious growth, Abx
  - Excel at complex specimens with multiple pathogens or those with higher viscosity
- **Semi-quantitative** results may aid interpretation, esp. for complex/polymicrobial specimens
  - FA-Pneumo results in relative agreement with qCulture results
- Results are **clinically actionable**
  - Abx adjustment in >60% of patients ~ 3-4 days sooner than culture
  - 50% of patients could have therapy narrowed
- **Potential** to reduce the overall cost of care
  - Studies needed!!!!!

END