Novel diagnostic technologies for respiratory disease

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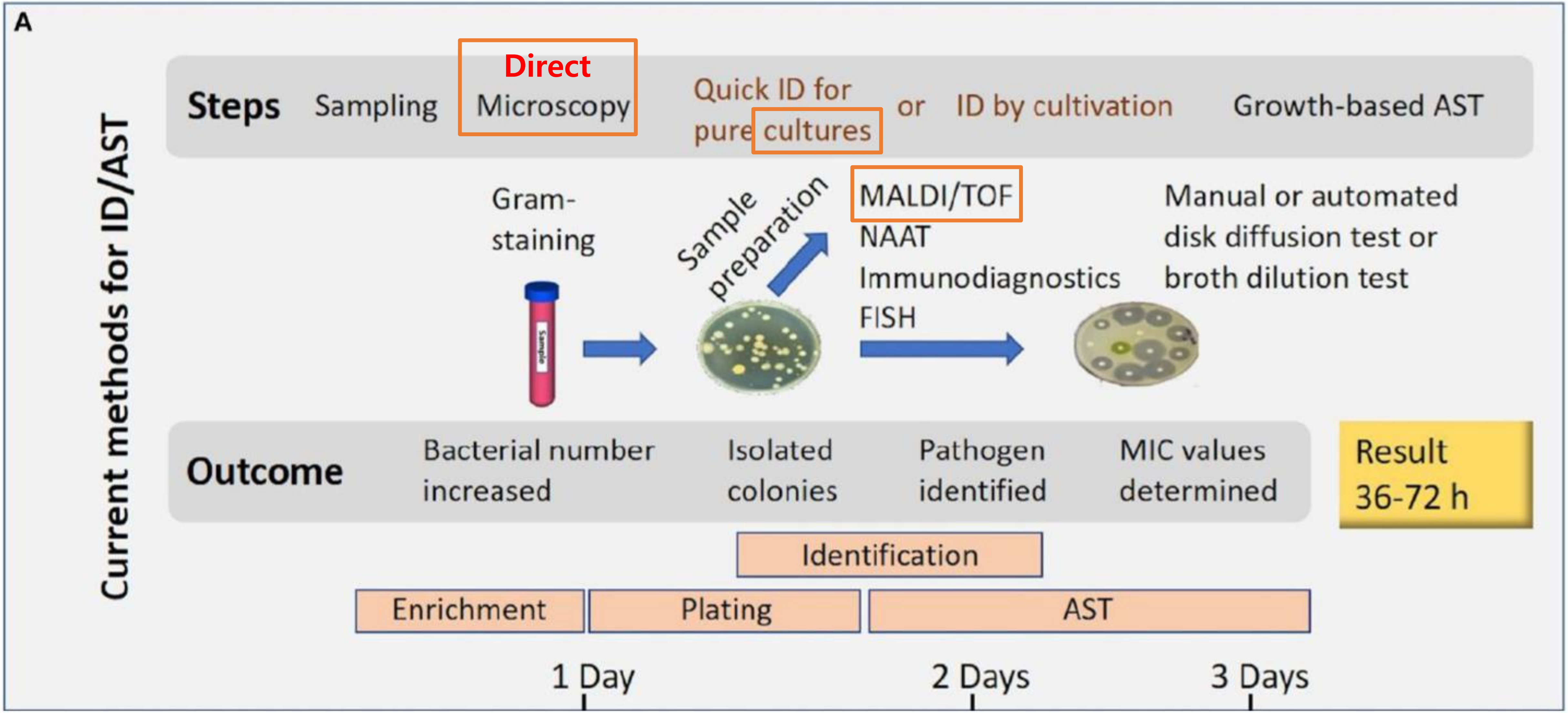
CONVENTIONAL MICROBIOLOGIC TESTS



Front. Cell. Infect. Microbiol., 15 July 2020

NOVEL DIAGNOSTIC TECH. FOR MICROBES

- Microscopy
  - Smear & stain
- Culture/Identification
  - selective media
  - biochemical/immunological
  - MALDI-TOF
  - NAAT
- Antimicrobial susceptibility test
  - Disk diffusion
  - Dilution

Culture based

- Quantitative/Qualitative PCR
  - **Full automated multiplex PCR**
  - **ddPCR**
- Metataxonomic/metagenomic analysis
  - **NGS**
- Antimicrobial susceptibility test
  - **Microfluidics assay**
  - **Molecular assay**

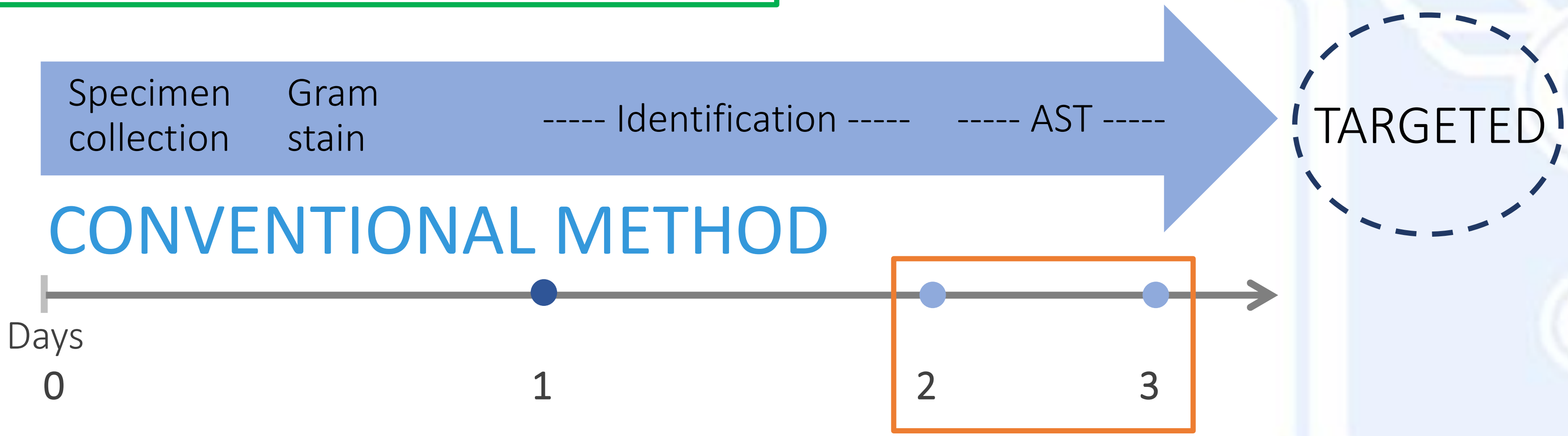
Direct (non-culture based)

ANTIMICROBIAL SUSCEPTIBILITY TEST

Phenotypic AST (Classic methods)

Rapid phenotypic AST (Growth-based assay) ≤8 hr

Rapid Molecular Diagnostics ≤4 hr



2022년 제12회 대한임상미생물학회 심포지엄
Prof 허희재 강의자료

NOVEL TECHNOLOGY FOR AST

Phenotypic AST (Classic methods)

Rapid phenotypic AST (Growth-based assay)

Rapid Molecular Diagnostics

Rapid phenotypic AST

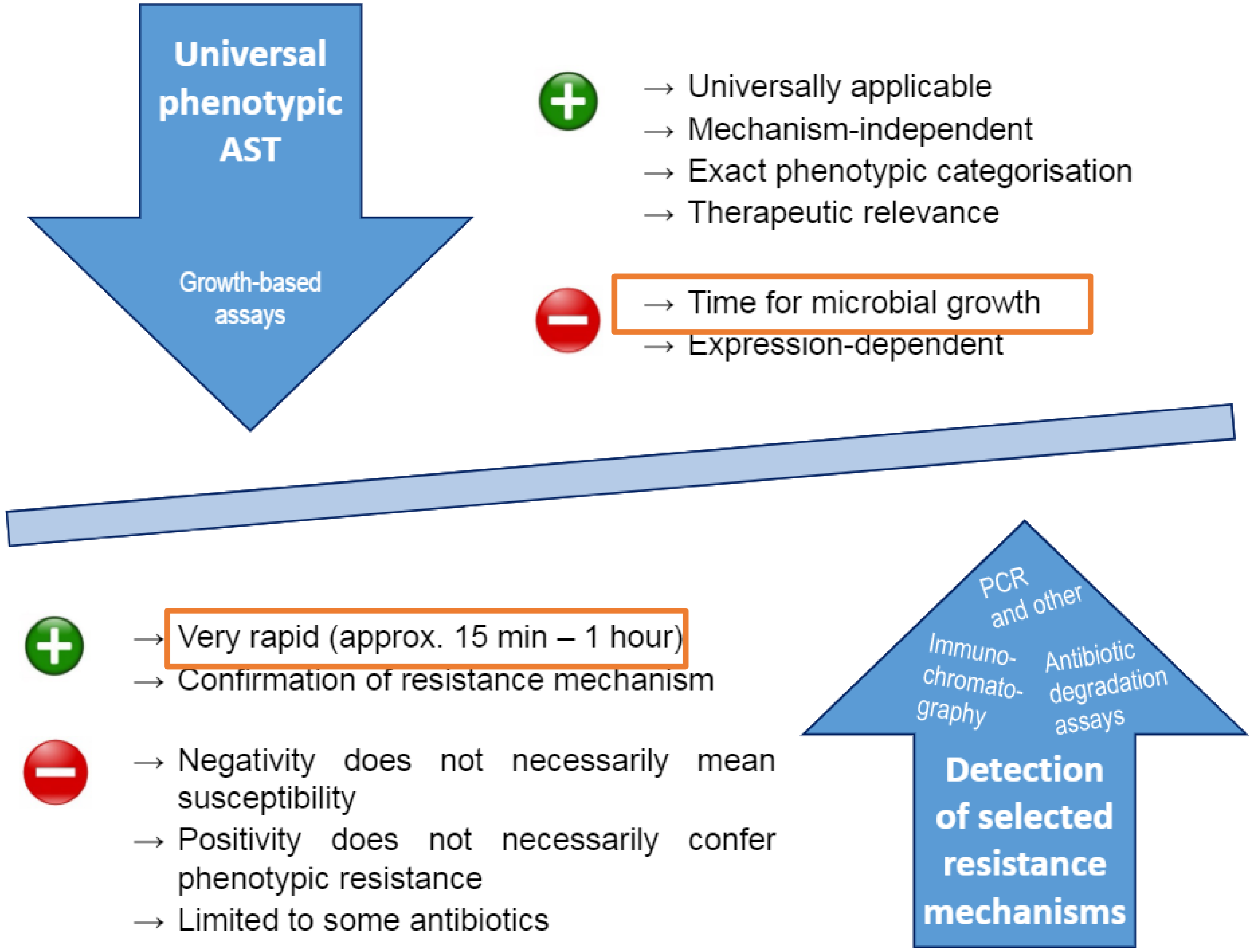
- Growth-based assay
- Mechanism-independent
- e.g. Microfluidic chip technology, morphokinetic cellular analysis

Rapid molecular diagnostics

- Rapid detection of particular resistance mechanisms
- DNA amplification methods
- Targeting resistance genes or sequences

Clin Microbiol Infect. 2019 Nov;25(11):1347-1355.

NOVEL TECHNOLOGY FOR AST



Universal phenotypic AST

Growth-based assays

+
- → Universally applicable
- → Mechanism-independent
- → Exact phenotypic categorisation
- → Therapeutic relevance

−
- → Time for microbial growth
- → Expression-dependent

+
- → Very rapid (approx. 15 min – 1 hour)
- → Confirmation of resistance mechanism

−
- → Negativity does not necessarily mean susceptibility
- → Positivity does not necessarily confer phenotypic resistance
- → Limited to some antibiotics

PCR and other

Immuno-chromato-graphy

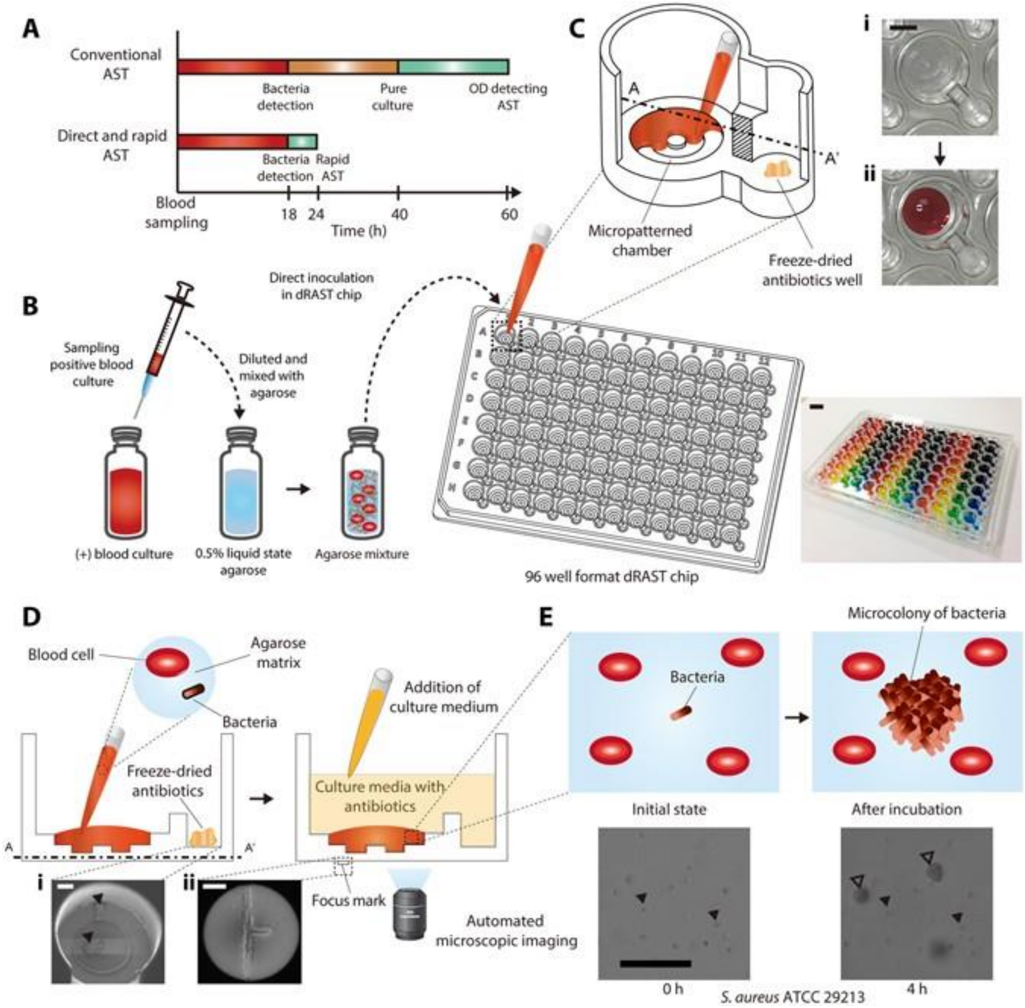
Antibiotic degradation assays

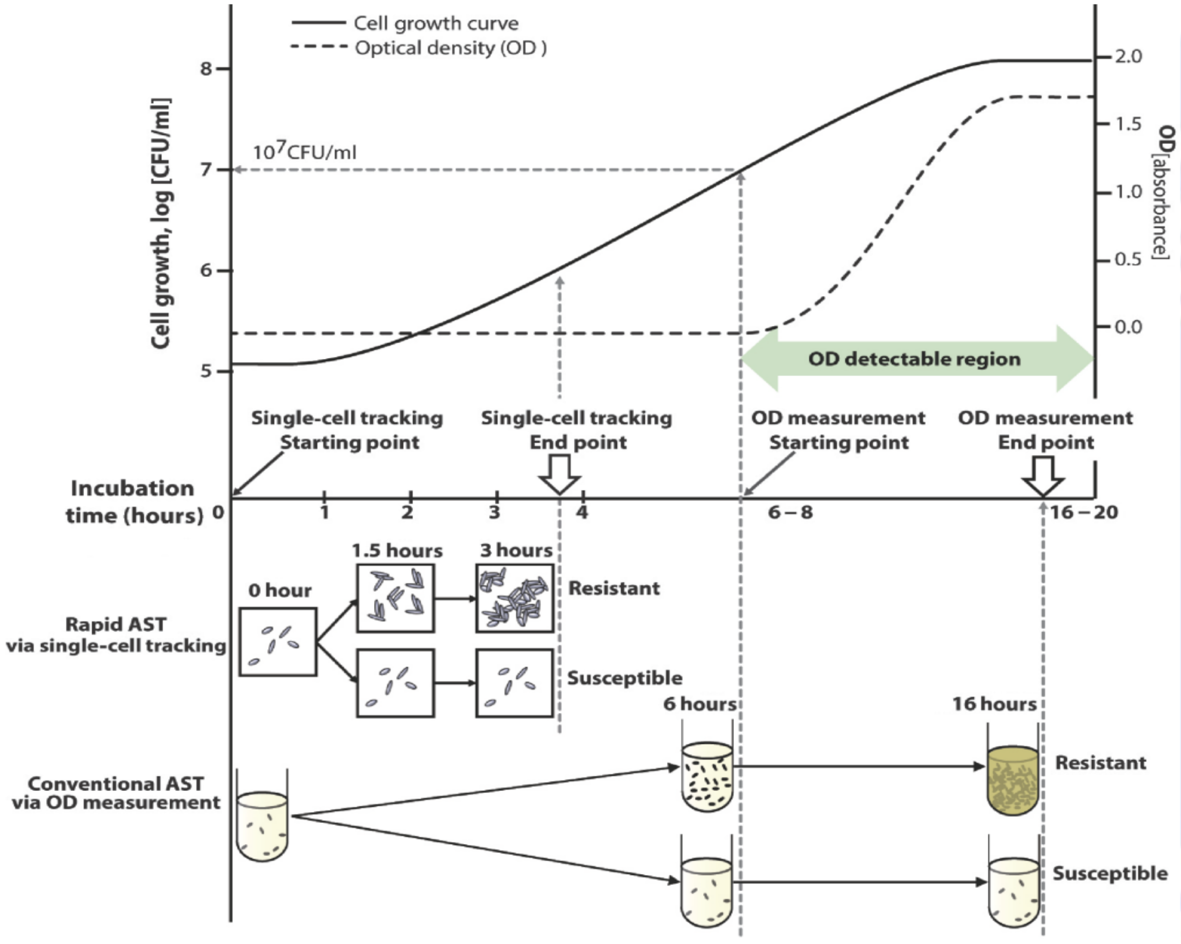
Detection of selected resistance mechanisms

Clin Microbiol Infect. 2019 Nov;25(11):1347-1355.

NOVEL TECHNOLOGY FOR AST

QuantaMatrix dRAST





Scientific Reports | 7: 1148 | DOI:10.1038/s41598-017-01278-2

Sci Transl Med. 2014 Dec 17;6(267):267

NOVEL TECHNOLOGY FOR AST

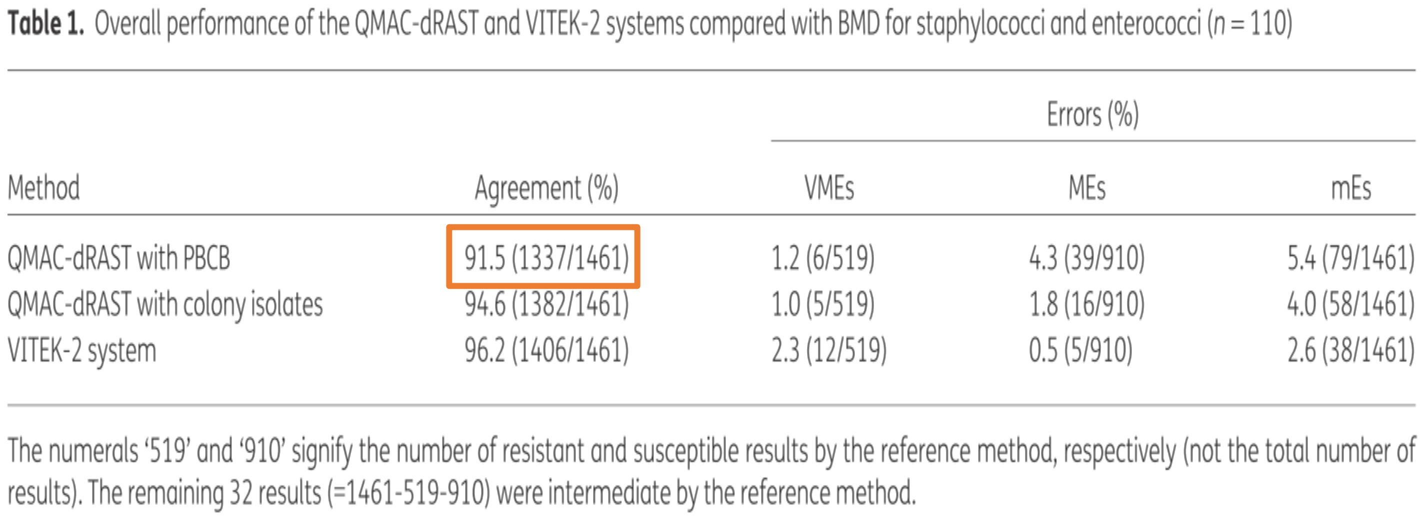
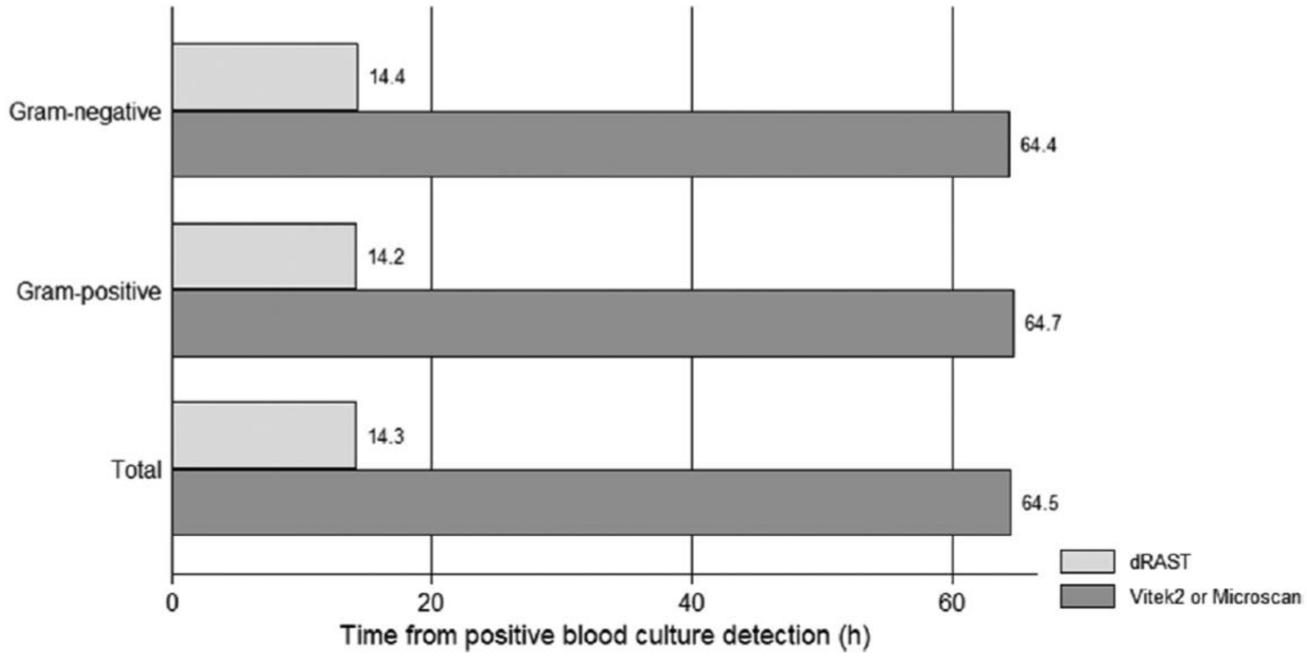


Table 1. Overall performance of the QMAC-dRAST and VITEK-2 systems compared with BMD for staphylococci and enterococci (*n* = 110)

		Errors (%)		
Method	Agreement (%)	VMEs	MEs	mEs
QMAC-dRAST with PBCB	91.5 (1337/1461)	1.2 (6/519)	4.3 (39/910)	5.4 (79/1461)
QMAC-dRAST with colony isolates	94.6 (1382/1461)	1.0 (5/519)	1.8 (16/910)	4.0 (58/1461)
VITEK-2 system	96.2 (1406/1461)	2.3 (12/519)	0.5 (5/910)	2.6 (38/1461)

The numerals '519' and '910' signify the number of resistant and susceptible results by the reference method, respectively (not the total number of results). The remaining 32 results (=1461-519-910) were intermediate by the reference method.

J Antimicrob Chemother. 2018 May 1;73(5):1267-1271.

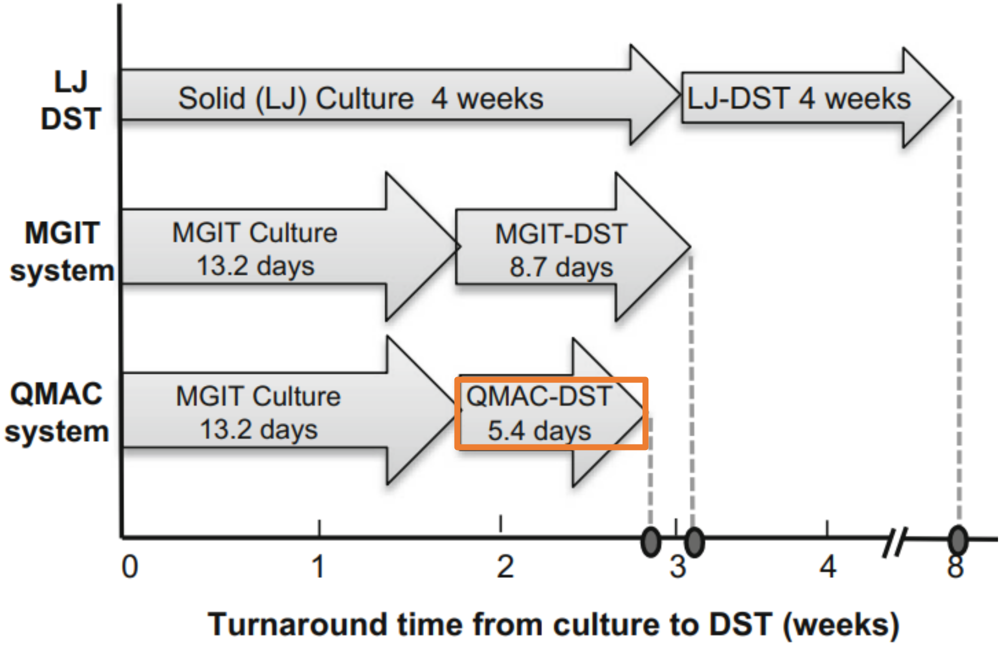


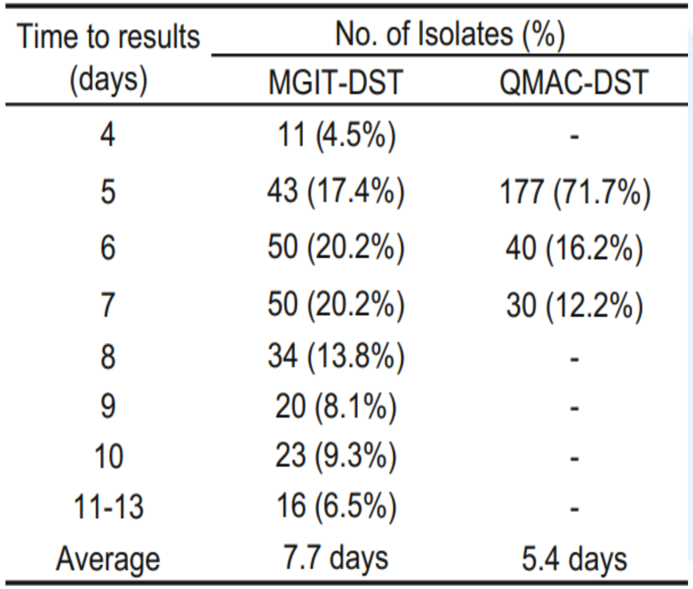
J Med Microbiol . 2018 Mar;67(3):325-331

ANTIMICROBIAL SUSCEPTIBILITY TEST

QuantaMatrix QDST

- first and second line agents
- susceptibility results in 7 days

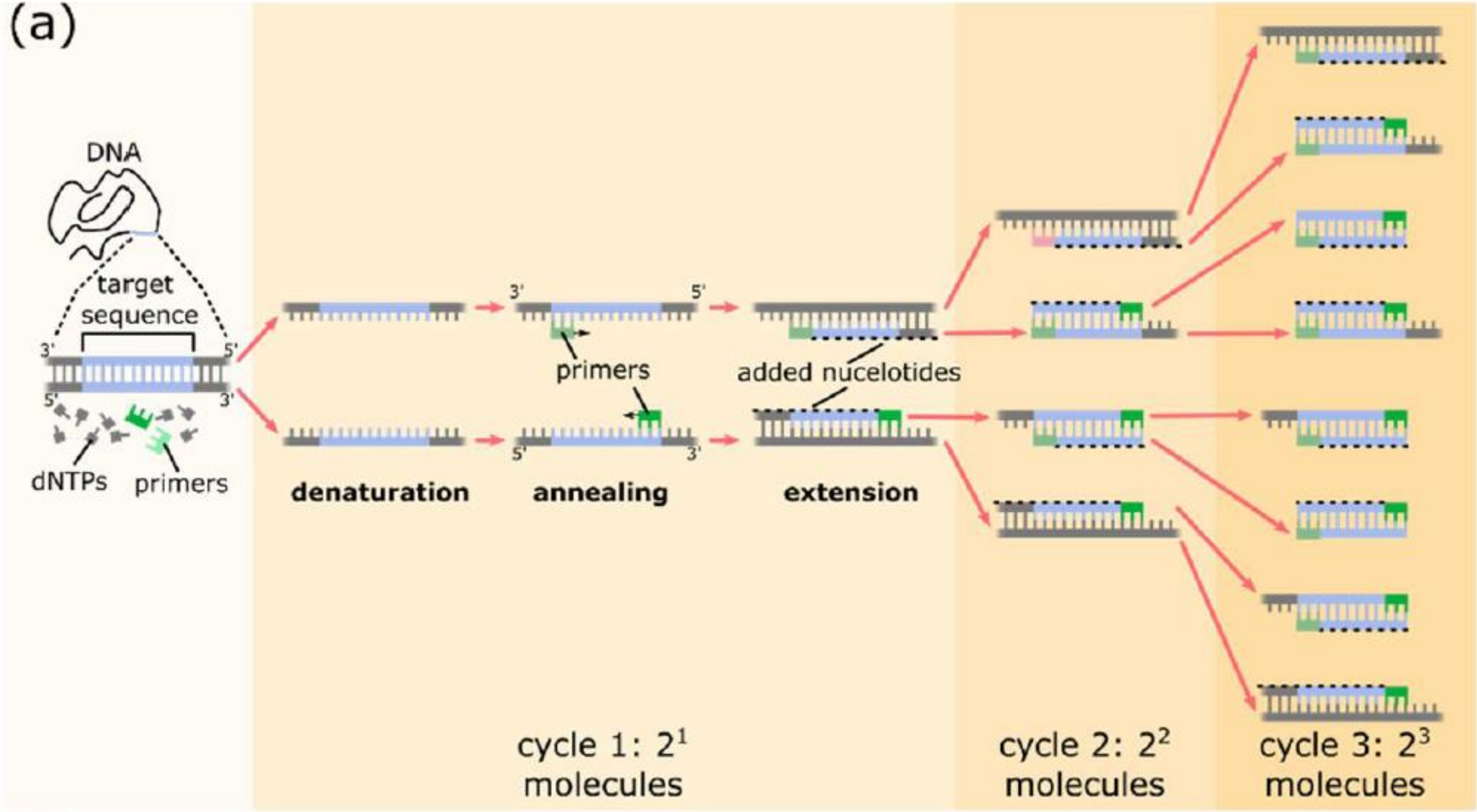




Time to results (days)	No. of Isolates (%)	
	MGIT-DST	QMAC-DST
4	11 (4.5%)	-
5	43 (17.4%)	177 (71.7%)
6	50 (20.2%)	40 (16.2%)
7	50 (20.2%)	30 (12.2%)
8	34 (13.8%)	-
9	20 (8.1%)	-
10	23 (9.3%)	-
11-13	16 (6.5%)	-
Average	7.7 days	5.4 days

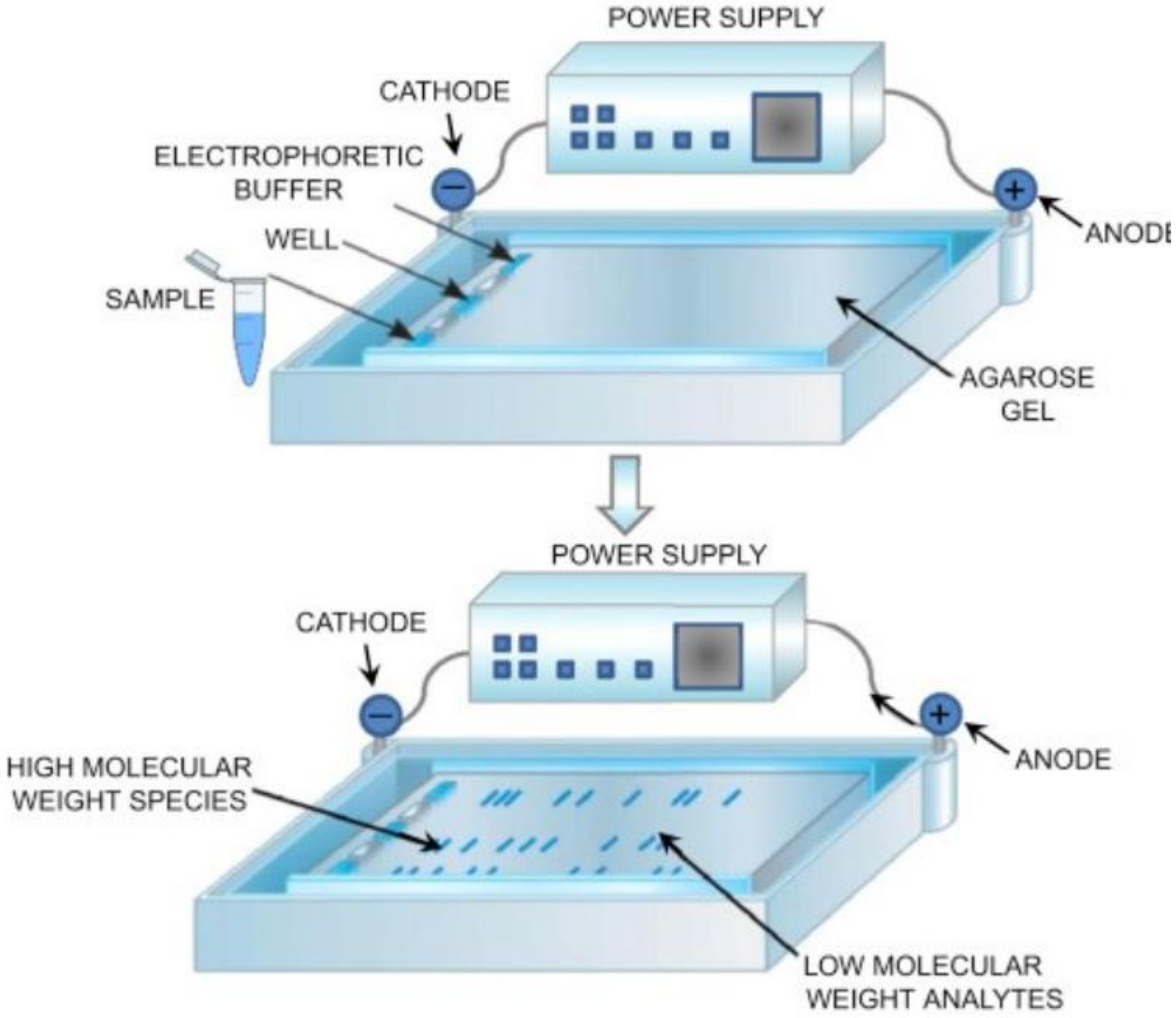
Appl Microbiol Biotechnol. 2021 Aug;105(14-15):6059-6072.

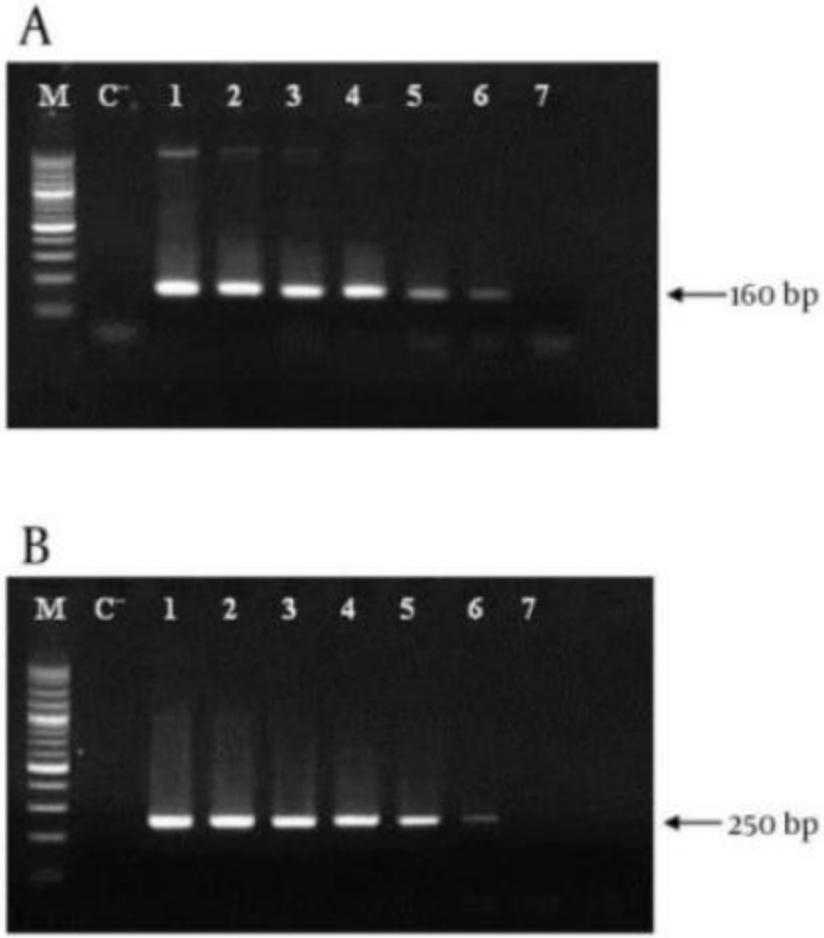
PCR



Sensors (Basel). 2018 Apr 20;18(4):1271

PCR (CONVENTIONAL PCR)

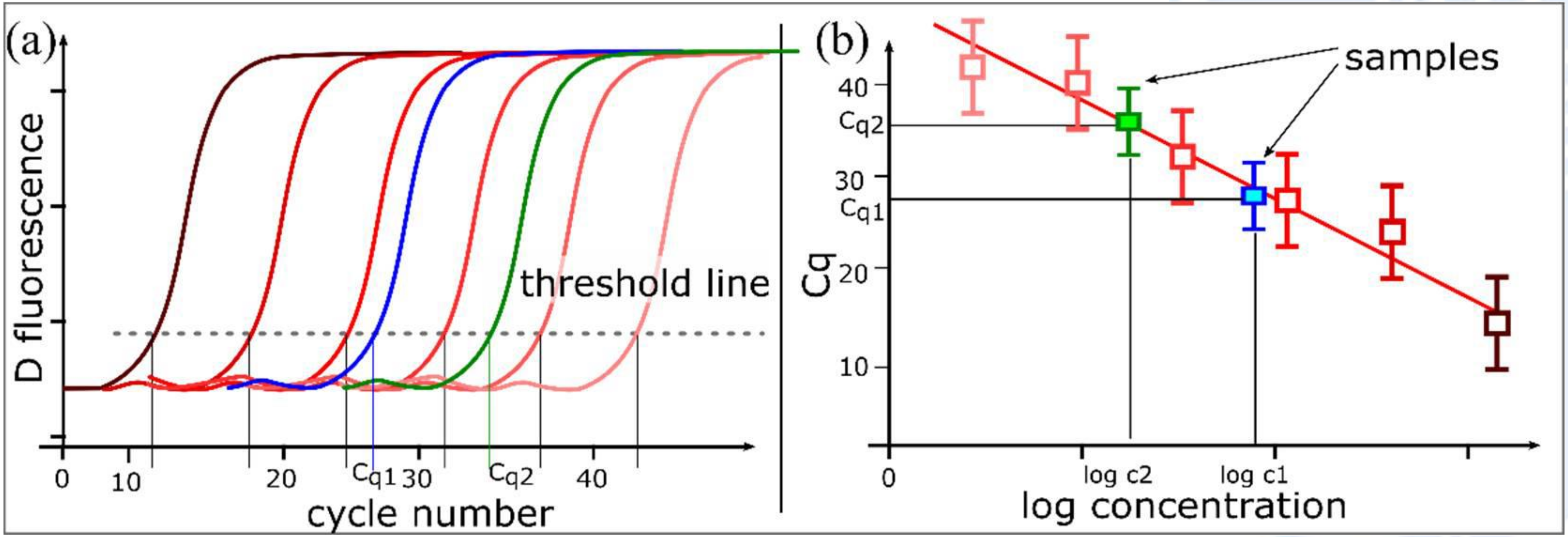




Sensors (Basel). 2018 Apr 20;18(4):1271

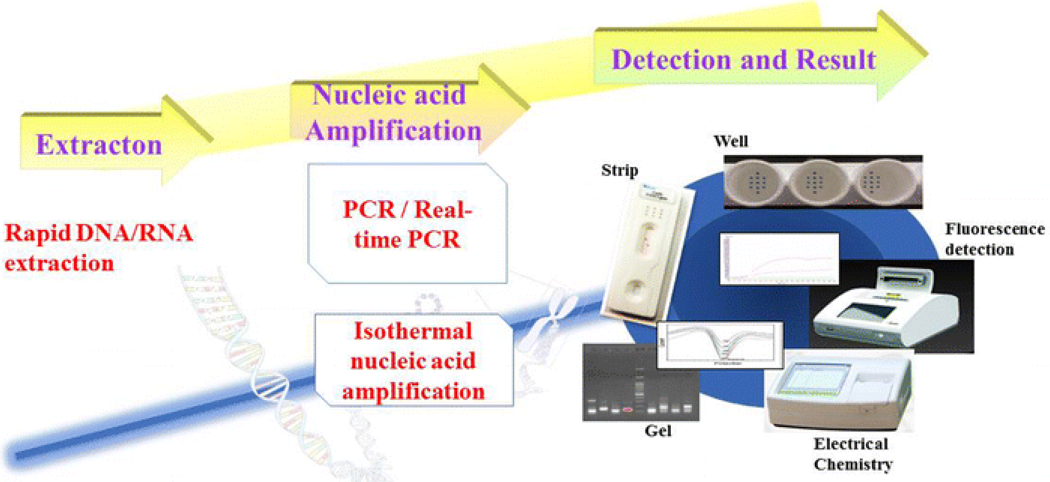
QUANTITATIVE PCR

Realtime PCR



Sensors (Basel). 2018 Apr 20;18(4):1271

FULLY AUTOMATED REALTIME PCR



Extracton

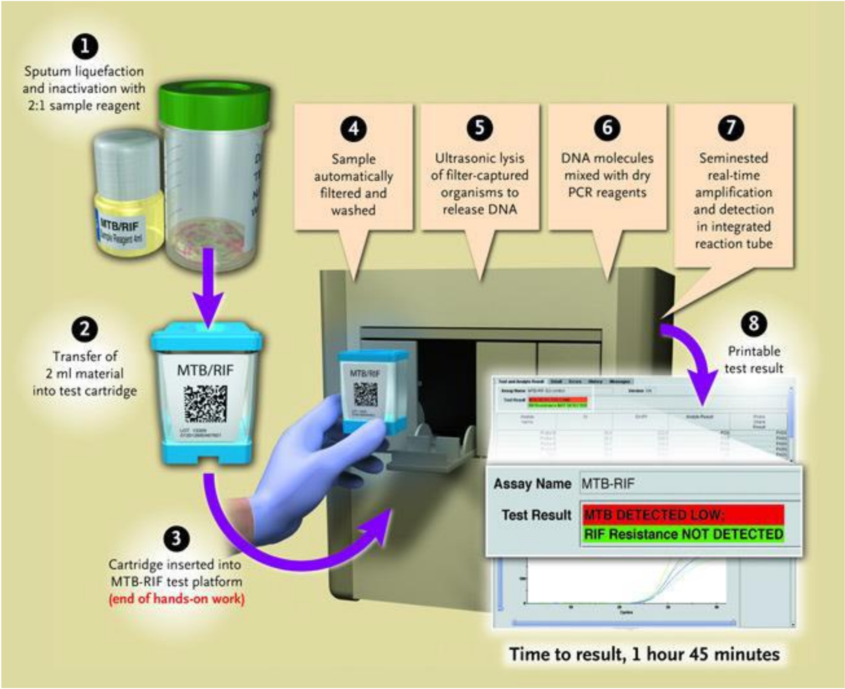
Nucleic acid Amplification

Detection and Result

Rapid DNA/RNA extraction

PCR / Real-time PCR

Isothermal nucleic acid amplification



① Sputum liquefaction and inactivation with 2:1 sample reagent

② Transfer of 2 ml material into test cartridge

③ Cartridge inserted into MTB-RIF test platform (end of hands-on work)

④ Sample automatically filtered and washed

⑤ Ultrasonic lysis of filter-captured organisms to release DNA

⑥ DNA molecules mixed with dry PCR reagents

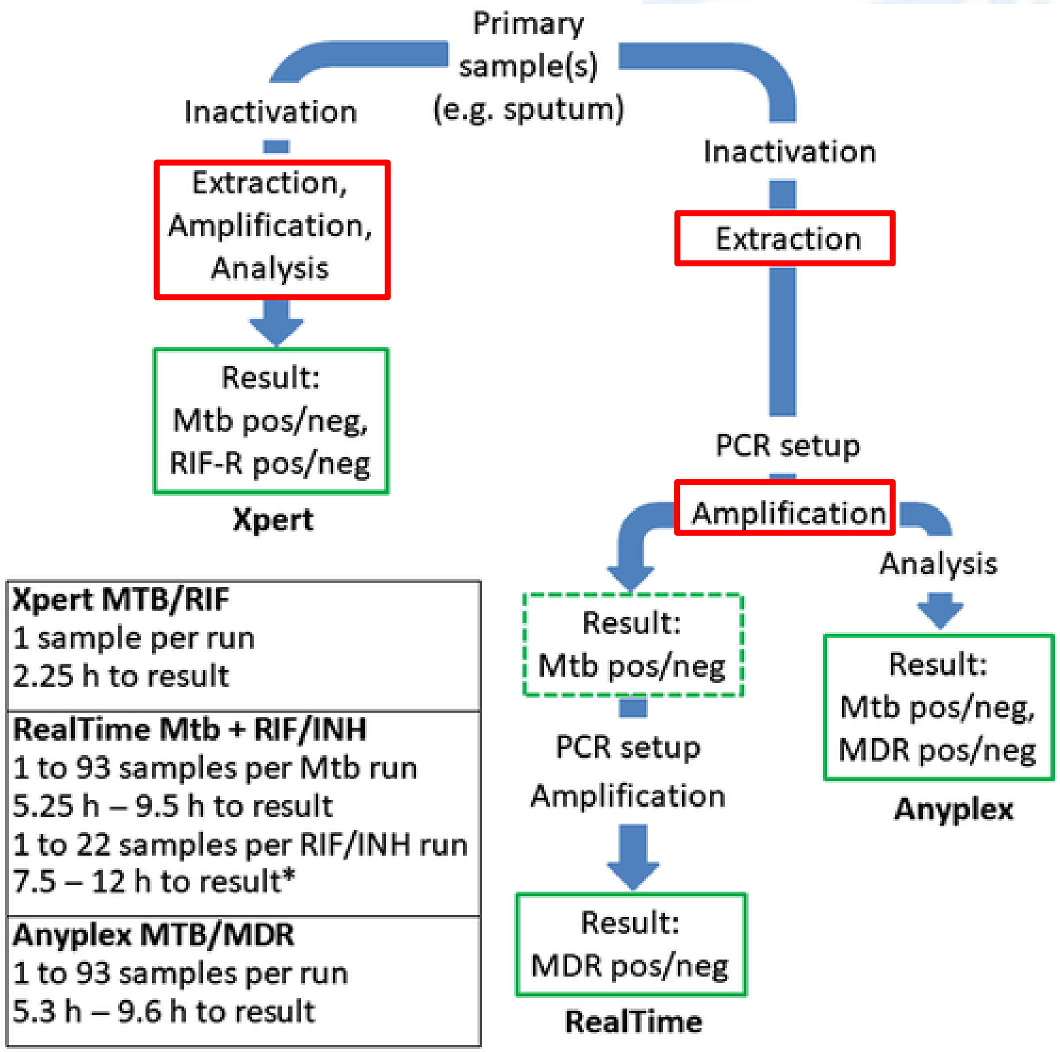
⑦ Seminested real-time amplification and detection in integrated reaction tube

⑧ Printable test result

Assay Name MTB-RIF

Test Result MTB DETECTED LOW; RIF Resistance NOT DETECTED

Time to result, 1 hour 45 minutes



Primary sample(s) (e.g. sputum)

Inactivation

Extraction, Amplification, Analysis

Result: Mtb pos/neg, RIF-R pos/neg

Xpert

Inactivation

Extraction

PCR setup

Amplification

Analysis

Result: Mtb pos/neg

PCR setup

Amplification

Result: MDR pos/neg

RealTime

Result: Mtb pos/neg, MDR pos/neg

Anyplex

Xpert MTB/RIF
1 sample per run
2.25 h to result

RealTime Mtb + RIF/INH
1 to 93 samples per Mtb run
5.25 h – 9.5 h to result
1 to 22 samples per RIF/INH run
7.5 – 12 h to result*

Anyplex MTB/MDR
1 to 93 samples per run
5.3 h – 9.6 h to result

APMIS Volume 127, Issue 6 p. 462-467

FULLY AUTOMATED REALTIME PCR

THE BIOFIRE RESPIRATORY 2.1 PANEL MENU

Overall 97.1% sensitivity and 99.3% specificity (prospective specimens) [1]

SARS-CoV-2 98.4% PPA and 98.9% NPA[2]

Sample Type: Nasopharyngeal swab in transport media or saline

VIRUSES:

- Adenovirus
- Coronavirus HKU1
- Coronavirus NL63
- Coronavirus 229E
- Coronavirus OC43
- **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)**
- Human Metapneumovirus
- Human Rhinovirus/Enterovirus
- Influenza A
- Influenza A/H1
- Influenza A/H3
- Influenza A/H1-2009
- Influenza B
- Parainfluenza Virus 1
- Parainfluenza Virus 2
- Parainfluenza Virus 3
- Parainfluenza Virus 4
- Respiratory Syncytial Virus

BACTERIA:

- *Bordetella parapertussis*
- *Bordetella pertussis*
- *Chlamydia pneumoniae*
- *Mycoplasma pneumoniae*

THE BIOFIRE PNEUMONIA PANEL MENU

SAMPLE TYPE:
BAL: (including mini-BAL)
Sputum: (including endotracheal aspirate)

PERFORMANCE:
BAL: 96.2% Sensitivity and 98.3% Specificity[1]
Sputum: 96.3% Sensitivity and 97.2% Specificity[1]

PART NUMBER:
BioFire Pneumonia 30 Pouch Kit: RFIT-ASY-0144

VIRUSES:

- Adenovirus
- Coronavirus
- Human Metapneumovirus
- Human Rhinovirus/Enterovirus
- Influenza A
- Influenza B
- Parainfluenza Virus
- Respiratory Syncytial Virus

ANTIMICROBIAL RESISTANCE GENES:

Methicillin resistance:
- *mecA*/C and MREJ

Carbapenemases:
- KPC
- NDM
- Oxa-48-like
- VIM
- IMP

ESBL:
- CTX-M

BACTERIA:
Semi-Quantitative Bacteria

- *Acinetobacter calcoaceticus-baumannii* complex
- *Enterobacter cloacae* complex
- *Escherichia coli*
- *Haemophilus influenzae*
- *Klebsiella aerogenes*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae* group
- *Moraxella catarrhalis*
- *Proteus* spp.
- *Pseudomonas aeruginosa*
- *Serratia marcescens*
- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*

ATYPICAL BACTERIA:
Qualitative Bacteria

- *Chlamydia pneumoniae*
- *Legionella pneumophila*
- *Mycoplasma pneumoniae*

Fully automated realtime PCR

Pathogen detection

Pathogen	C(+) PP(+)	C(+) PP(-)	C(-) PP(+)	C(-) PP(-)	Performance (%, 95% confidence interval)	
					Sensitivity	Specificity
Gram-positive bacteria						
Staphylococcus aureus	16	0	14	69	100 (75.9 – 100)	83.3 (73.0 – 90.1)
Streptococcus agalactiae	1	0	4	94	100 (5.5 – 100)	95.9 (89.3 – 98.7)
Streptococcus pneumoniae	1	0	3	95	100 (5.5 – 100)	96.9 (90.1 – 99.2)
Gram-negative bacteria						
Enterobacter cloacae complex	2	0	7	90	100 (19.8 – 100)	92.8 (85.2 – 96.8)
Escherichia coli	3	0	8	88	100 (31.0 – 100)	91.7 (83.8 – 96.1)
Klebsiella aerogenes	1	1	3	94	50.0 (2.7 – 97.3)	96.9 (90.6 – 99.2)
Klebsiella oxytoca	0	0	1	98	-	99.0 (93.7 – 99.9)
Klebsiella pneumonia group	5	0	12	82	100 (46.3 – 100)	87.2 (78.4 – 92.9)
Moraxella catarrhalis	0	0	1	98	-	99.0 (93.7 – 99.9)
Proteus spp.	0	0	1	98	-	99.0 (93.7 – 99.9)
Serratia marcescens	0	0	3	96	-	97.0 (90.8 – 99.2)
A. baumannii complex	21	0	9	69	100 (80.8 – 100)	88.5 (78.7 – 94.3)
Pseudomonas aeruginosa	19	0	9	71	100 (79.1 – 100)	88.8 (79.2 – 94.4)
Haemophilus influenzae	0	0	5	94	-	94.9 (88.1 – 98.1)
Total	**64**	**1**	**8**	**26**	**98.5 (90.6 – 99.9)**	**76.5 (58.4 – 88.6)**

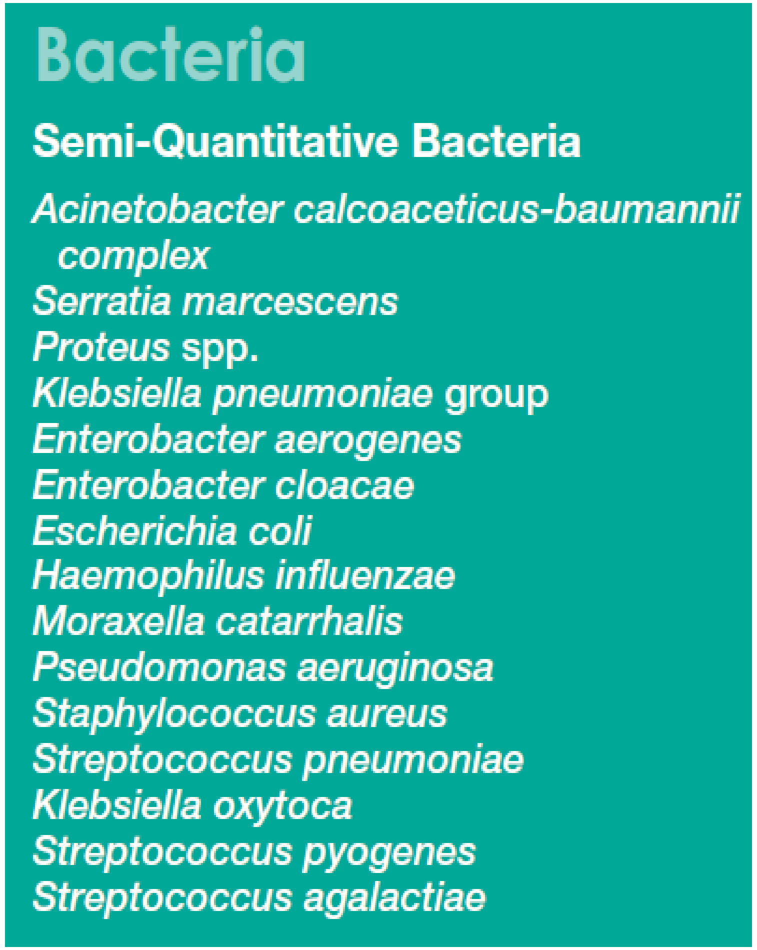
Yoo IY et al. Int J Infect Dis. 2020;95:326-331.

FULLY AUTOMATED REALTIME PCR

Carbapenem : *KPC, NDM, OXA-48, VIM, IMP*

3^{rd} **generation Cephalosporin** : *CTX-M*

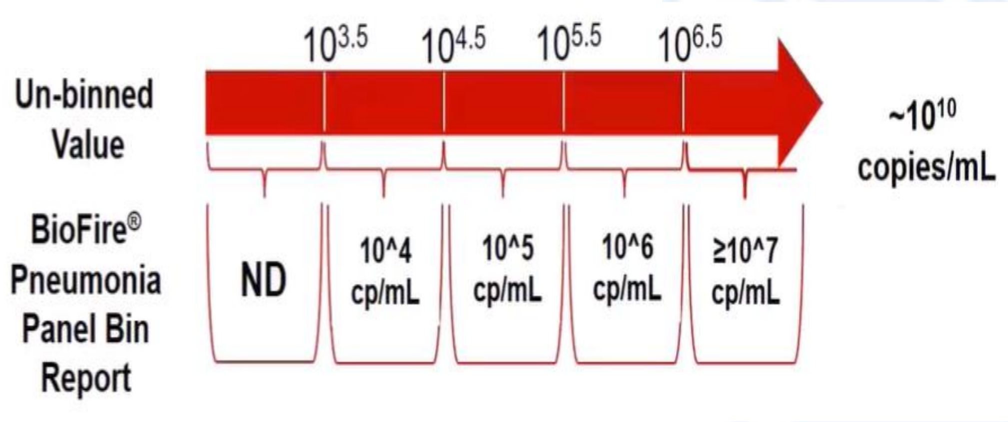
Oxacillin: *mecA/mecC* and *MREJ*



Bacteria

Semi-Quantitative Bacteria

Acinetobacter calcoaceticus-baumannii complex
Serratia marcescens
Proteus spp.
Klebsiella pneumoniae group
Enterobacter aerogenes
Enterobacter cloacae
Escherichia coli
Haemophilus influenzae
Moraxella catarrhalis
Pseudomonas aeruginosa
Staphylococcus aureus
Streptococcus pneumoniae
Klebsiella oxytoca
Streptococcus pyogenes
Streptococcus agalactiae



FULLY AUTOMATED REALTIME PCR

/QUALITATIVE PCR

Organism group Gene(s)	AST (R)/ PP (+)	AST (R)/ PP (-)	AST (S)/PP (+)	
			No.	No. of positive results in sequencing
Methicillin-resistant Staphylococcus aureus				
mecA/C and MREJ	10	1	11	9
ESBL-producing Enterobacteriaceae				
CTX-M	4	0	12	10
Carbapenemase-producing organisms				
KPC	1	0	0	0
NDM	1	0	0	0
IMP	0	0	1	1
VIM	1	0	1	0

Yoo IY et al. Int J Infect Dis. 2020;95:326-331.

FULLY AUTOMATED REALTIME PCR

Culture	FilmArray Pneumonia Panel plus, Bin (DNA copies/mL)					Total
Amount (semi-quantitative)	Not detected	10^4	10^5	10^6	$\geq 10^7$	
Significant amount						
Moderate[a]	0	0	1	**8**	**15**	24
Many[b]	1	0	0	**4**	**40**	45
No significant amount	26	3	3	4	5	41
Total	27	3	4	16	60	110

[a] Growth up to the second quadrant of the plate.

[b] Growth up to the third or fourth quadrant of the plate

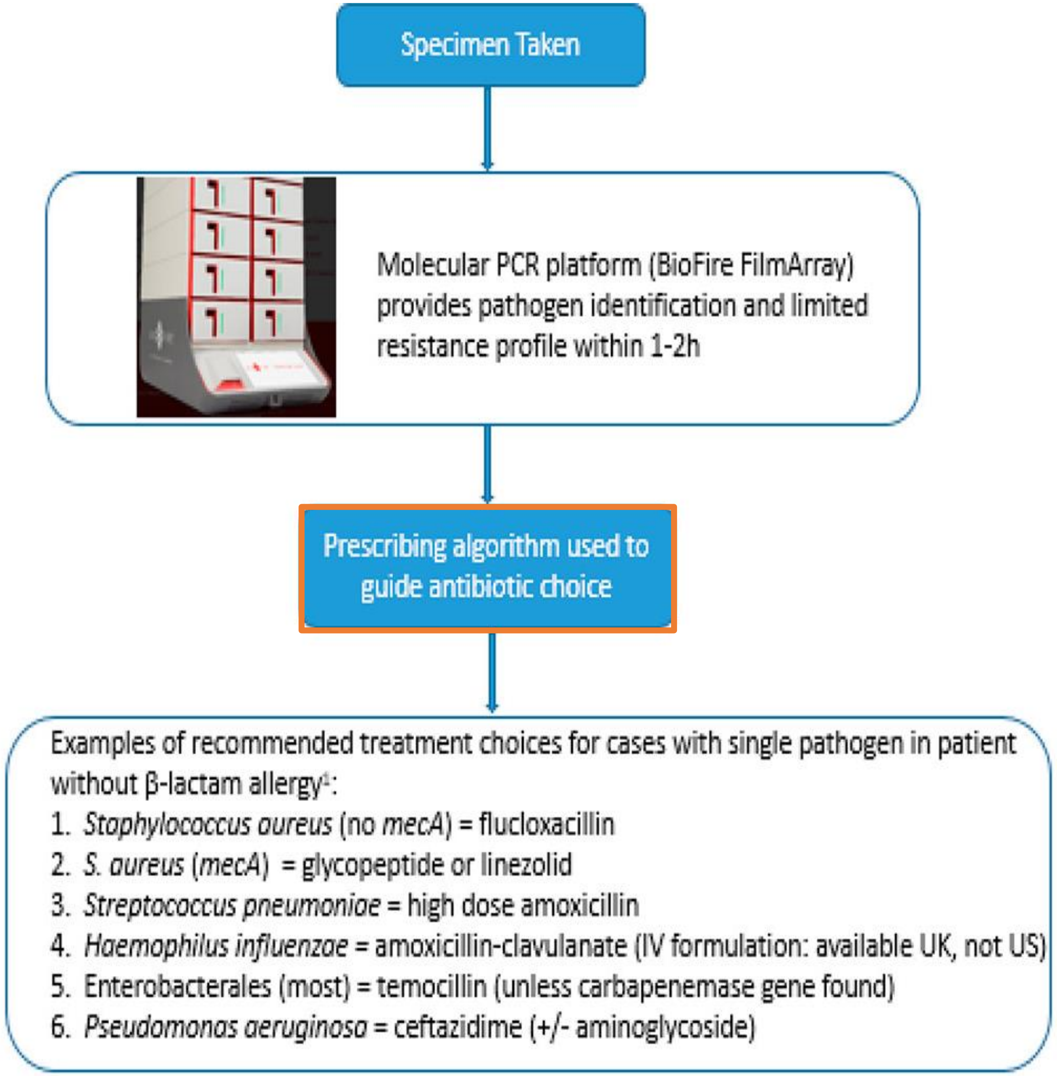
Yoo IY et al. Int J Infect Dis. 2020;95:326-331.

FULLY AUTOMATED REALTIME PCR

Treatment modification	Cases [no. (%)]
No modification	23 (50.0%)
Treatment changed	23 (50.0%)
1. Antibiotic escalation	13 (56.5%)
Vancomycin	3
Carbapenem	2
Agents covering CRGNB	8
2. Antibiotic de-escalation	10 (43.5%)
Vancomycin	3
Meropenem	3
Piperacillin/tazobactam	2
Vancomycin and meropenem	1
Vancomycin and amikacin	1

Yoo IY et al. Int J Infect Dis. 2020;95:326-331.

FULLY AUTOMATED REALTIME PCR



Specimen Taken

Molecular PCR platform (BioFire FilmArray) provides pathogen identification and limited resistance profile within 1-2h

Prescribing algorithm used to guide antibiotic choice

Examples of recommended treatment choices for cases with single pathogen in patient without β-lactam allergy[1]:

1. *Staphylococcus aureus* (no *mecA*) = flucloxacillin
2. *S. aureus* (*mecA*) = glycopeptide or linezolid
3. *Streptococcus pneumoniae* = high dose amoxicillin
4. *Haemophilus influenzae* = amoxicillin-clavulanate (IV formulation: available UK, not US)
5. Enterobacterales (most) = temocillin (unless carbapenemase gene found)
6. *Pseudomonas aeruginosa* = ceftazidime (+/- aminoglycoside)

Dhesi et al. ACS Pharmacol Transl Sci. 2020. 12;3(3):401-417

FULLY AUTOMATED REALTIME PCR

Translating quicker tests into antibiotic savings
: is antibiotic (de)-escalation safe?

Detection of colonizing flora

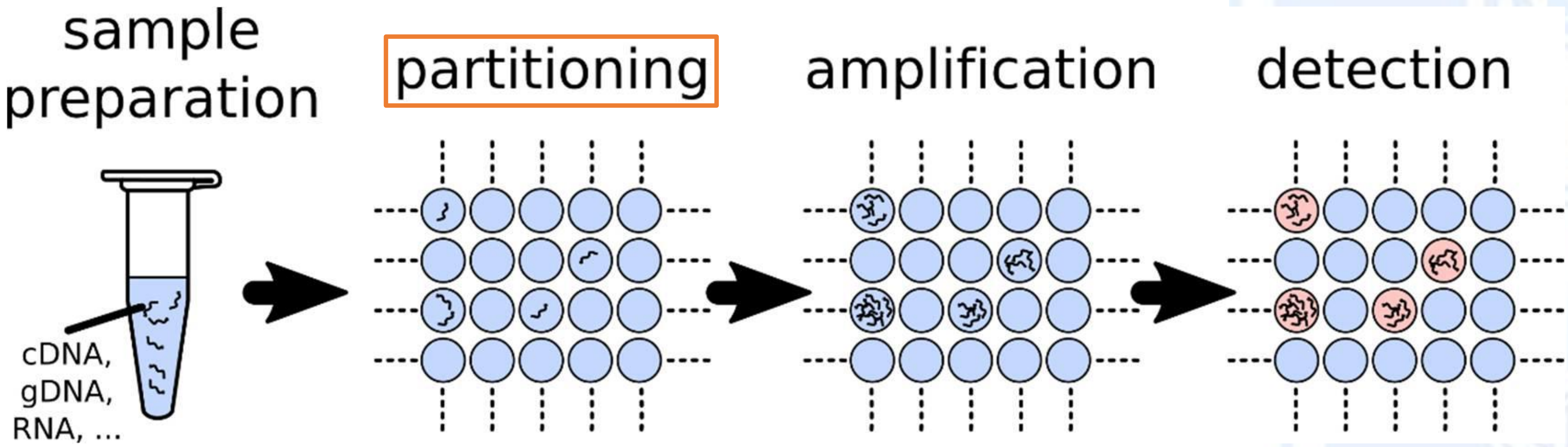
Interpreting genotypic resistance results

Practical issues: costs

Poole et al. J Infect. 2020. 80(1):1-7.

DIGITAL PCR

Digital PCR

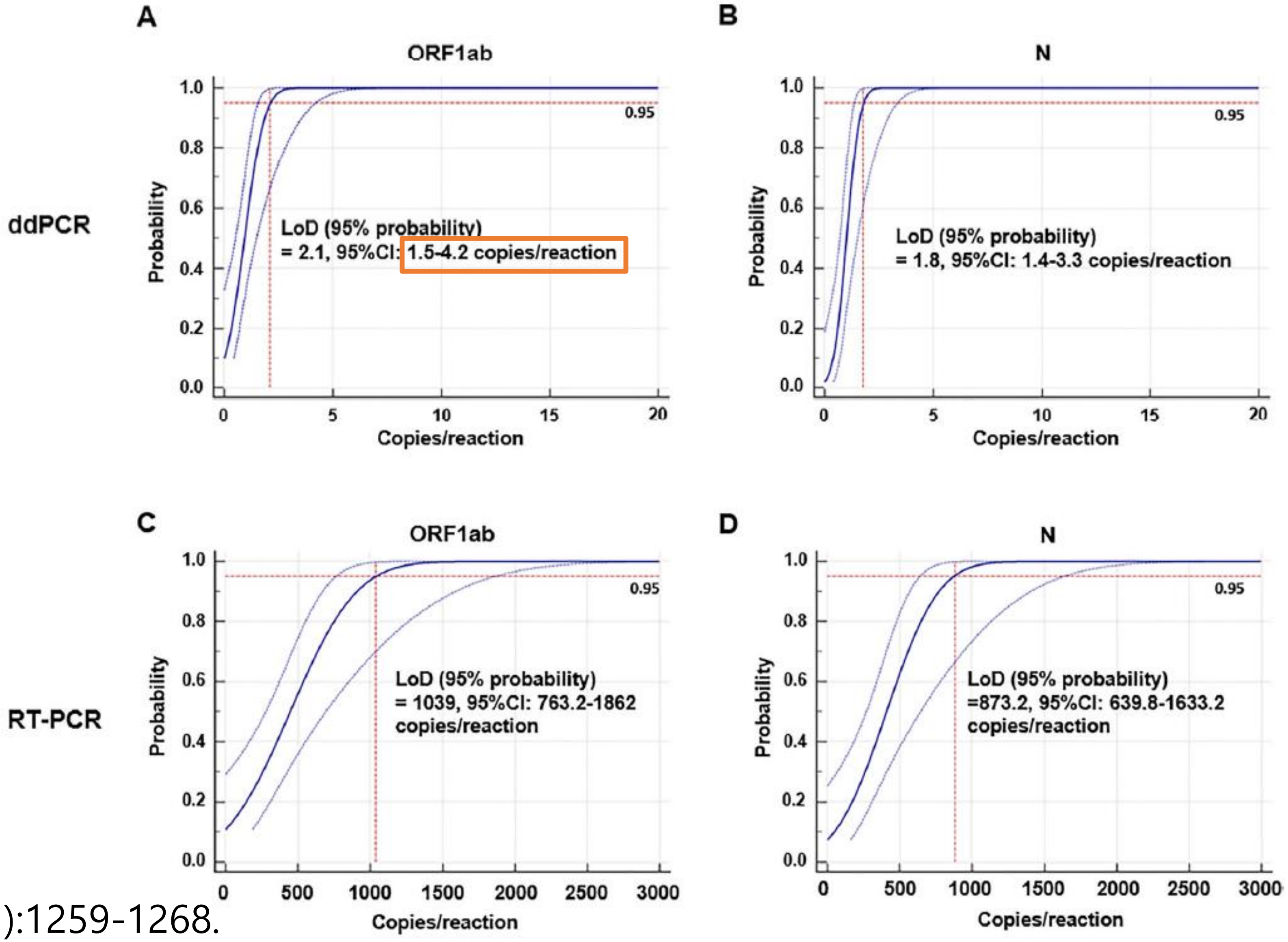


Poisson distribution -> absolute quantification w/o standard curve
Higher analytical sensitivity
Droplet, microwells, chamber ……

Sensors (Basel). 2018 Apr 20;18(4):1271

DIGITAL PCR

Digital PCR for SARS-CoV-2



Emerg Microbes Infect. 2020 Dec;9(1):1259-1268.

Digital PCR for Tuberculosis

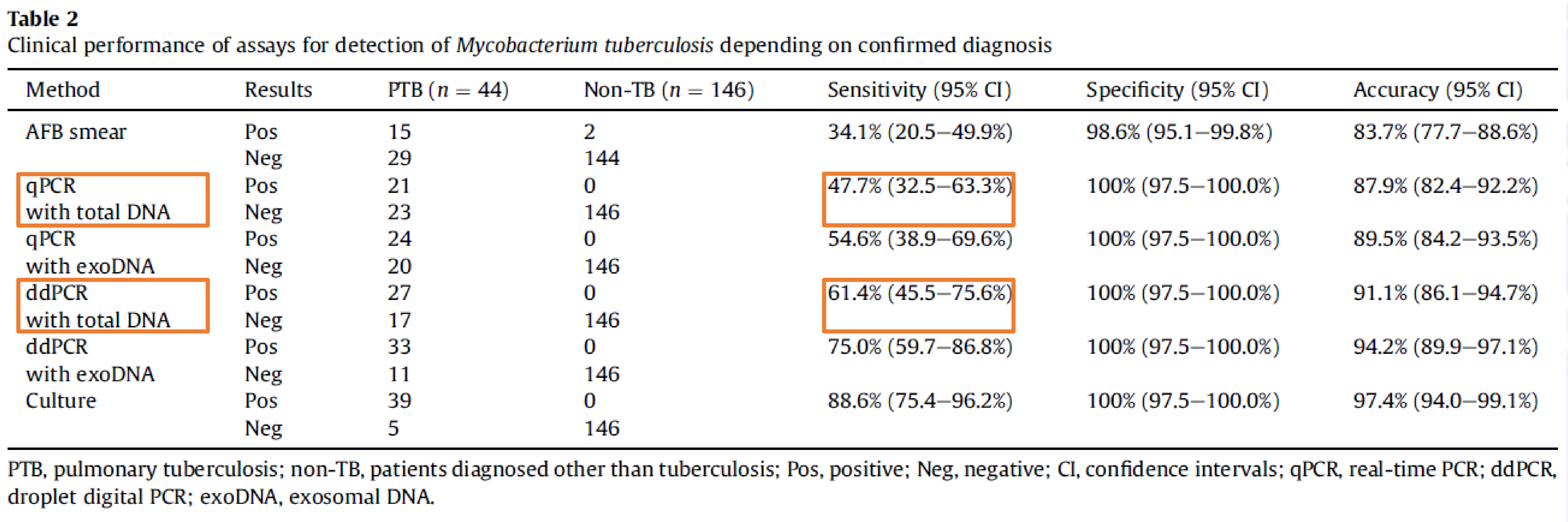


Table 2
Clinical performance of assays for detection of *Mycobacterium tuberculosis* depending on confirmed diagnosis

Method	Results	PTB ($n = 44$)	Non-TB ($n = 146$)	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
AFB smear	Pos	15	2	34.1% (20.5–49.9%)	98.6% (95.1–99.8%)	83.7% (77.7–88.6%)
	Neg	29	144			
qPCR with total DNA	Pos	21	0	47.7% (32.5–63.3%)	100% (97.5–100.0%)	87.9% (82.4–92.2%)
	Neg	23	146			
qPCR with exoDNA	Pos	24	0	54.6% (38.9–69.6%)	100% (97.5–100.0%)	89.5% (84.2–93.5%)
	Neg	20	146			
ddPCR with total DNA	Pos	27	0	61.4% (45.5–75.6%)	100% (97.5–100.0%)	91.1% (86.1–94.7%)
	Neg	17	146			
ddPCR with exoDNA	Pos	33	0	75.0% (59.7–86.8%)	100% (97.5–100.0%)	94.2% (89.9–97.1%)
	Neg	11	146			
Culture	Pos	39	0	88.6% (75.4–96.2%)	100% (97.5–100.0%)	97.4% (94.0–99.1%)
	Neg	5	146			

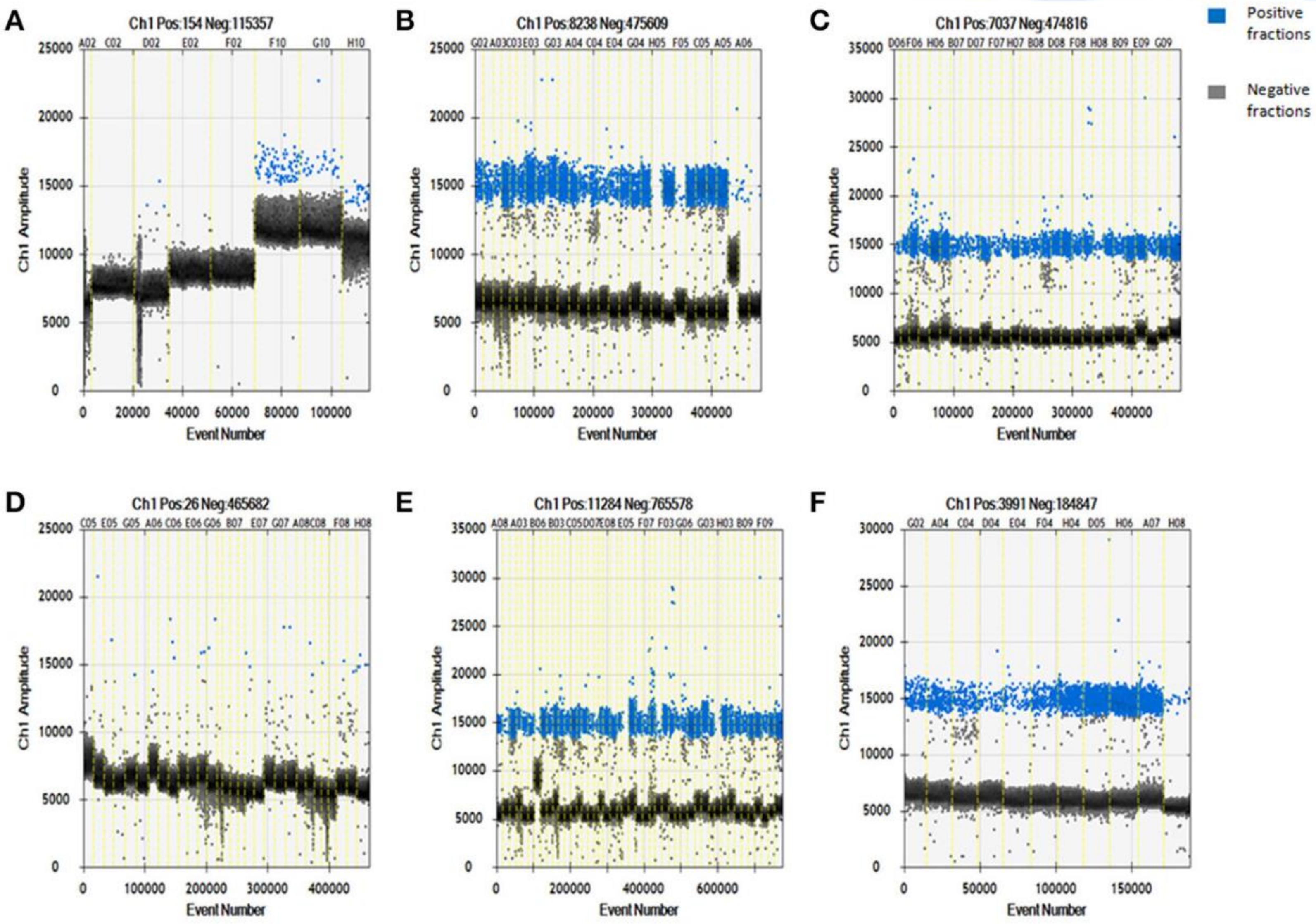
PTB, pulmonary tuberculosis; non-TB, patients diagnosed other than tuberculosis; Pos, positive; Neg, negative; CI, confidence intervals; qPCR, real-time PCR; ddPCR, droplet digital PCR; exoDNA, exosomal DNA.

Clinical Microbiology and Infection 26 (2020) 942.e1e942.e5

DIGITAL PCR

Digital PCR for Tuberculosis

(Circulating TB DNA)



A: control
B: pul. TB
C: extrapul. TB
D: healthy

Front Cell Infect Microbiol. 2017 Aug 11;7:369.

DIGITAL PCR

Digital PCR for *Pneumocystis jirovecii*

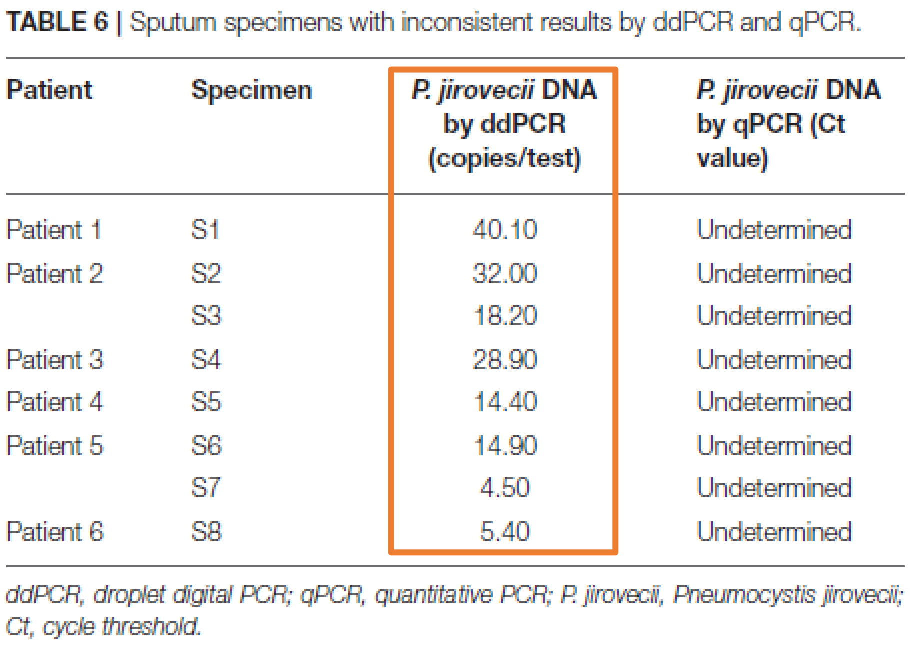


TABLE 6 | Sputum specimens with inconsistent results by ddPCR and qPCR.

Patient	Specimen	*P. jirovecii* DNA by ddPCR (copies/test)	*P. jirovecii* DNA by qPCR (Ct value)
Patient 1	S1	40.10	Undetermined
Patient 2	S2	32.00	Undetermined
	S3	18.20	Undetermined
Patient 3	S4	28.90	Undetermined
Patient 4	S5	14.40	Undetermined
Patient 5	S6	14.90	Undetermined
	S7	4.50	Undetermined
Patient 6	S8	5.40	Undetermined

ddPCR, droplet digital PCR; qPCR, quantitative PCR; P. jirovecii, Pneumocystis jirovecii; Ct, cycle threshold.

Targeted

Microbiome

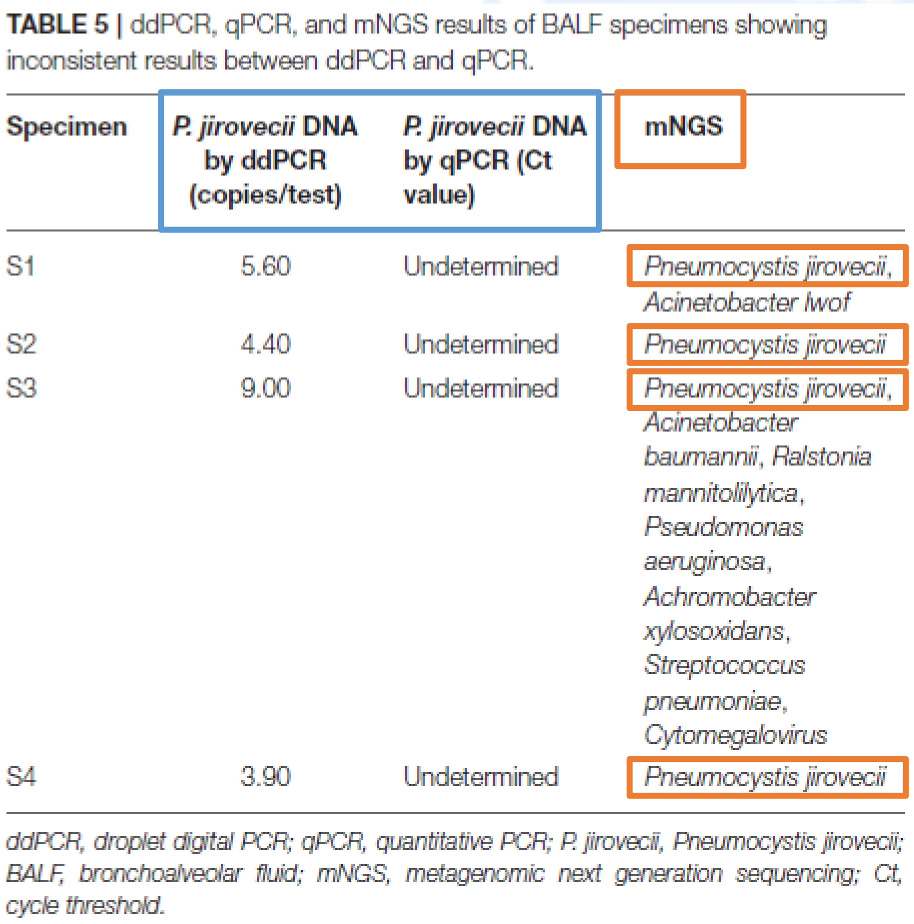


TABLE 5 | ddPCR, qPCR, and mNGS results of BALF specimens showing inconsistent results between ddPCR and qPCR.

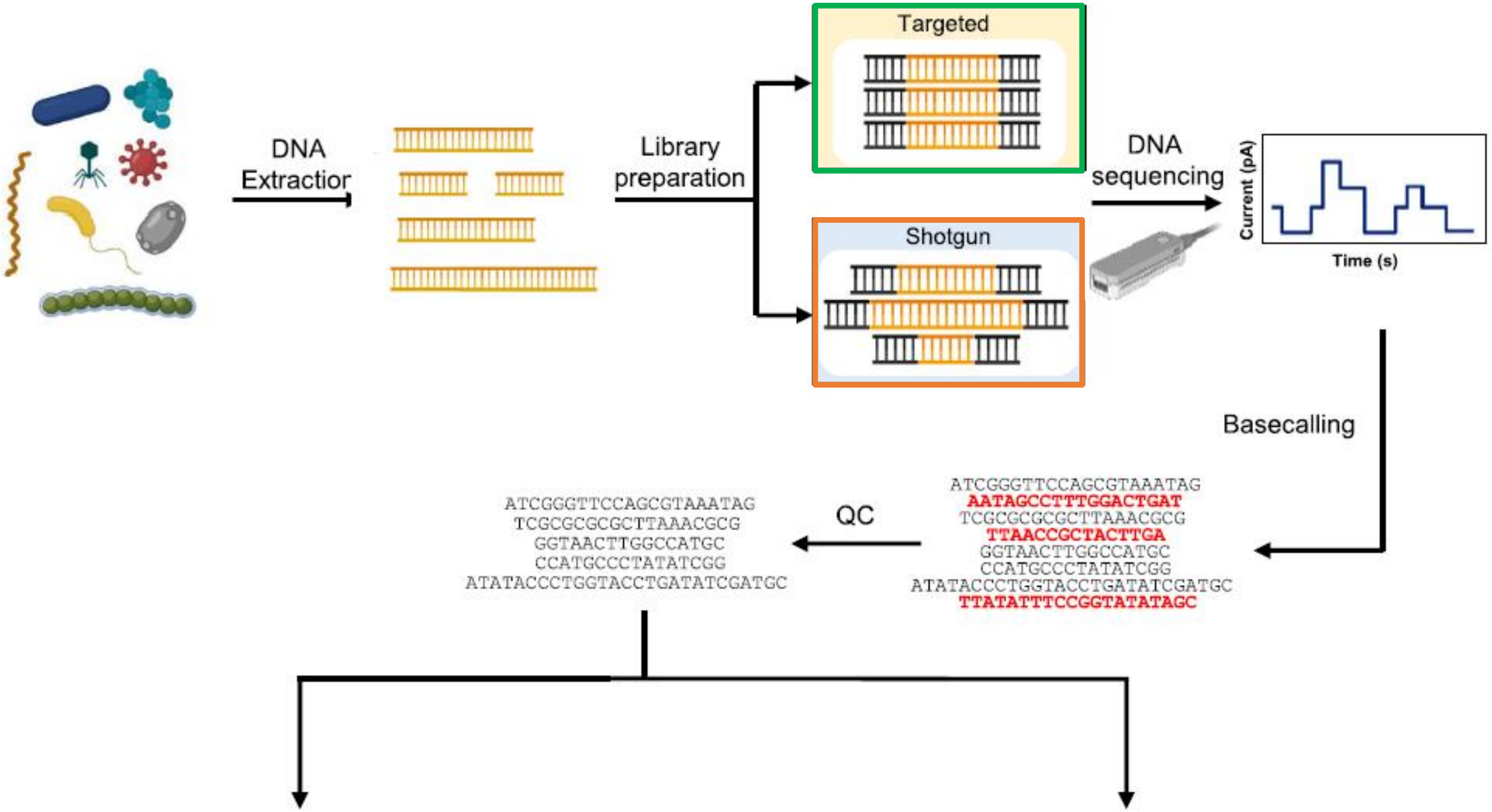
Specimen	*P. jirovecii* DNA by ddPCR (copies/test)	*P. jirovecii* DNA by qPCR (Ct value)	mNGS
S1	5.60	Undetermined	*Pneumocystis jirovecii, Acinetobacter lwof*
S2	4.40	Undetermined	*Pneumocystis jirovecii*
S3	9.00	Undetermined	*Pneumocystis jirovecii, Acinetobacter baumannii, Ralstonia mannitolilytica, Pseudomonas aeruginosa, Achromobacter xylosoxidans, Streptococcus pneumoniae, Cytomegalovirus*
S4	3.90	Undetermined	*Pneumocystis jirovecii*

ddPCR, droplet digital PCR; qPCR, quantitative PCR; P. jirovecii, Pneumocystis jirovecii; BALF, bronchoalveolar fluid; mNGS, metagenomic next generation sequencing; Ct, cycle threshold.

Front Med (Lausanne). 2021 Dec 22;8:761788.

MICROBIOME ANALYSIS

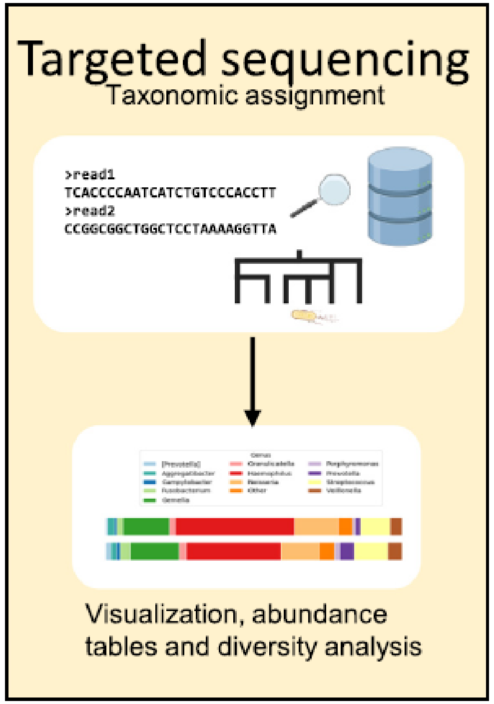
NGS_Microbiome



Targeted sequencing

Shotgun metagenomics

L. Ciuffreda et al. Computational and Structural Biotechnology Journal 19 (2021) 1497–1511



Targeted sequencing

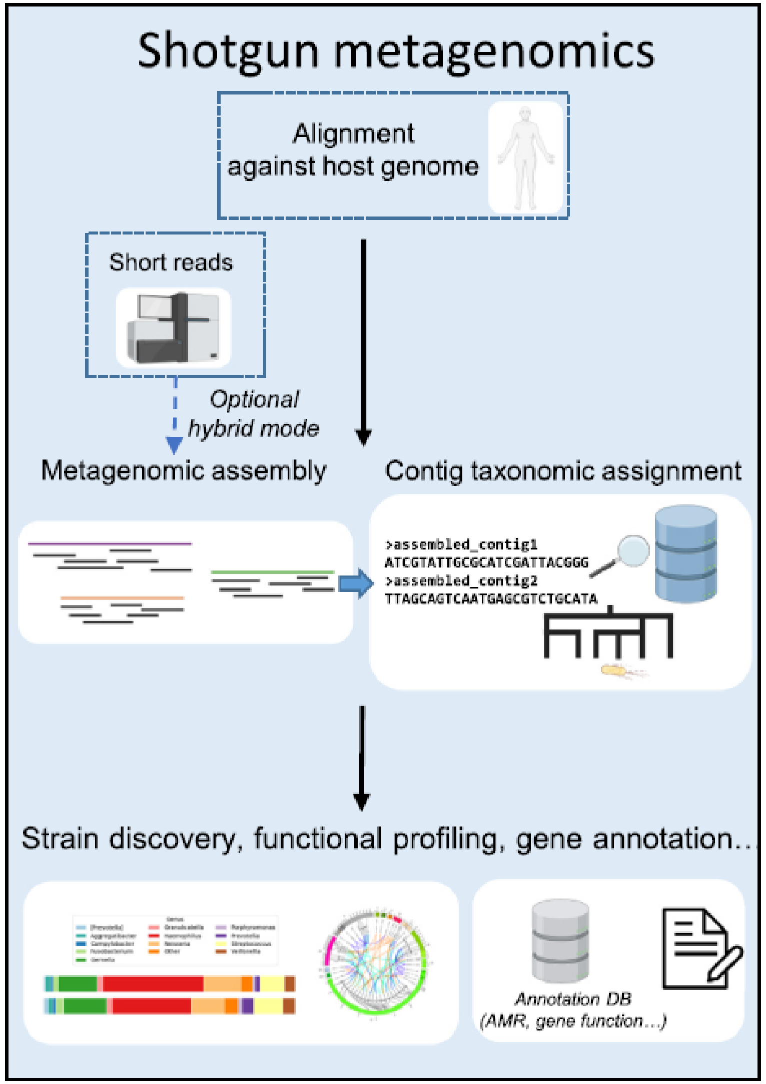
Taxonomic assignment

```
>read1
TCACCCCAATCATCTGTCCCACCTT
>read2
CCGGCGGCTGGCTCCTAAAAGGTTA
```

Visualization, abundance tables and diversity analysis

Metataxonomic

Bacteria – 16s rRNA
Fungus – ITS region



Shotgun metagenomics

Alignment against host genome

Short reads

Optional hybrid mode

Metagenomic assembly

Contig taxonomic assignment

```
>assembled_contig1
ATCGTATTGCGCATCGATTACGGG
>assembled_contig2
TTAGCAGTCAATGAGCGTCTGCATA
```

Strain discovery, functional profiling, gene annotation…

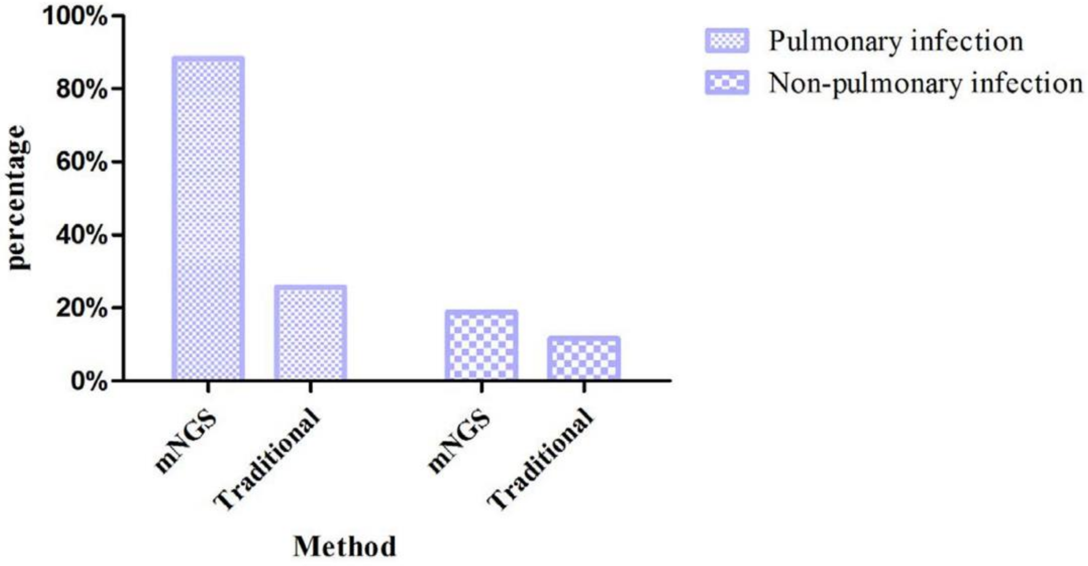
Annotation DB (AMR, gene function…)

Metagenomic

Non-targeted

MICROBIOME ANALYSIS

NGS_pathogen detection



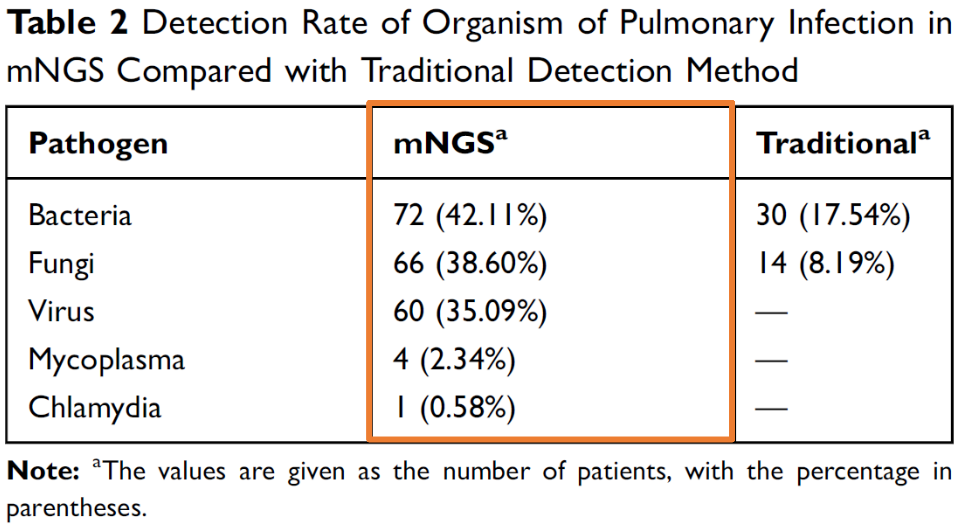


Table 2 Detection Rate of Organism of Pulmonary Infection in mNGS Compared with Traditional Detection Method

Pathogen	mNGS[a]	Traditional[a]
Bacteria	72 (42.11%)	30 (17.54%)
Fungi	66 (38.60%)	14 (8.19%)
Virus	60 (35.09%)	—
Mycoplasma	4 (2.34%)	—
Chlamydia	1 (0.58%)	—

Note: [a]The values are given as the number of patients, with the percentage in parentheses.

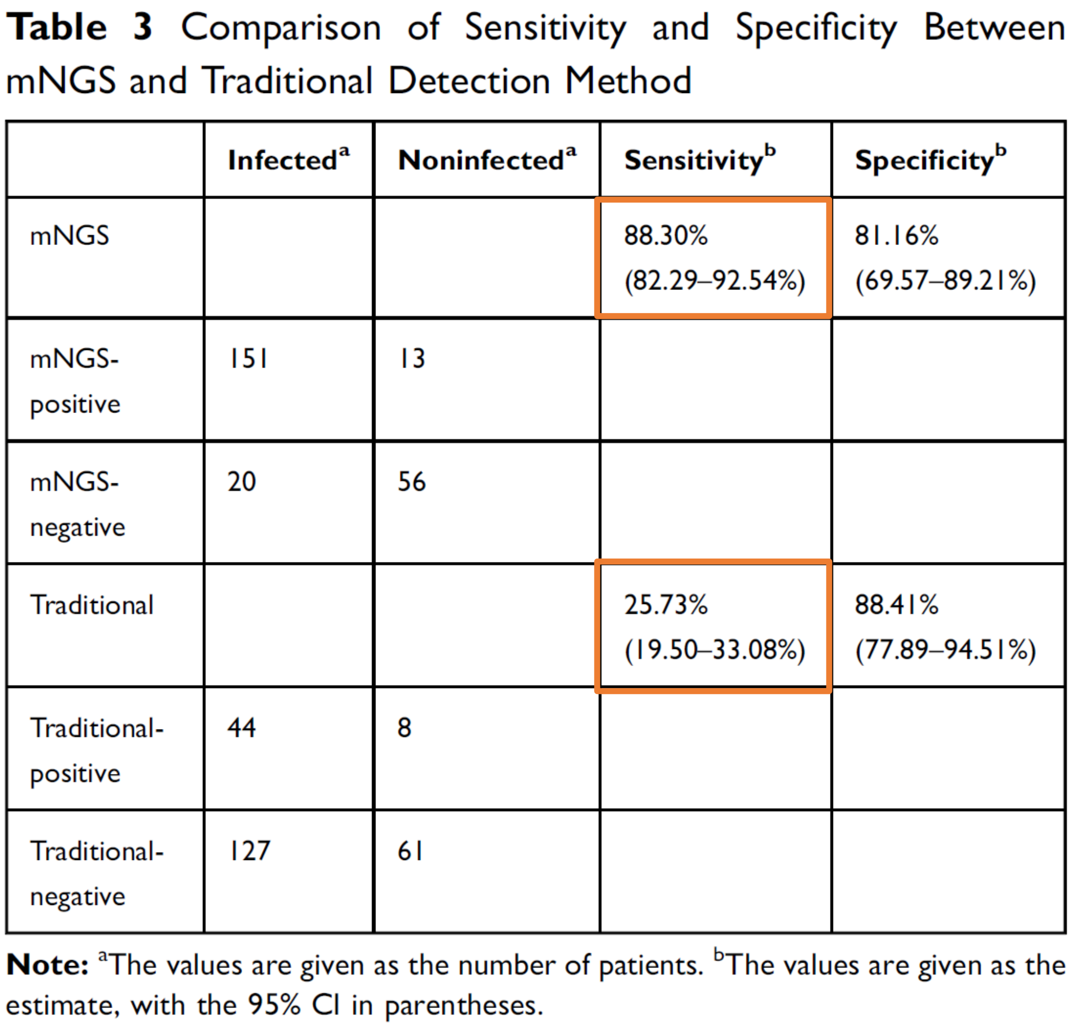


Table 3 Comparison of Sensitivity and Specificity Between mNGS and Traditional Detection Method

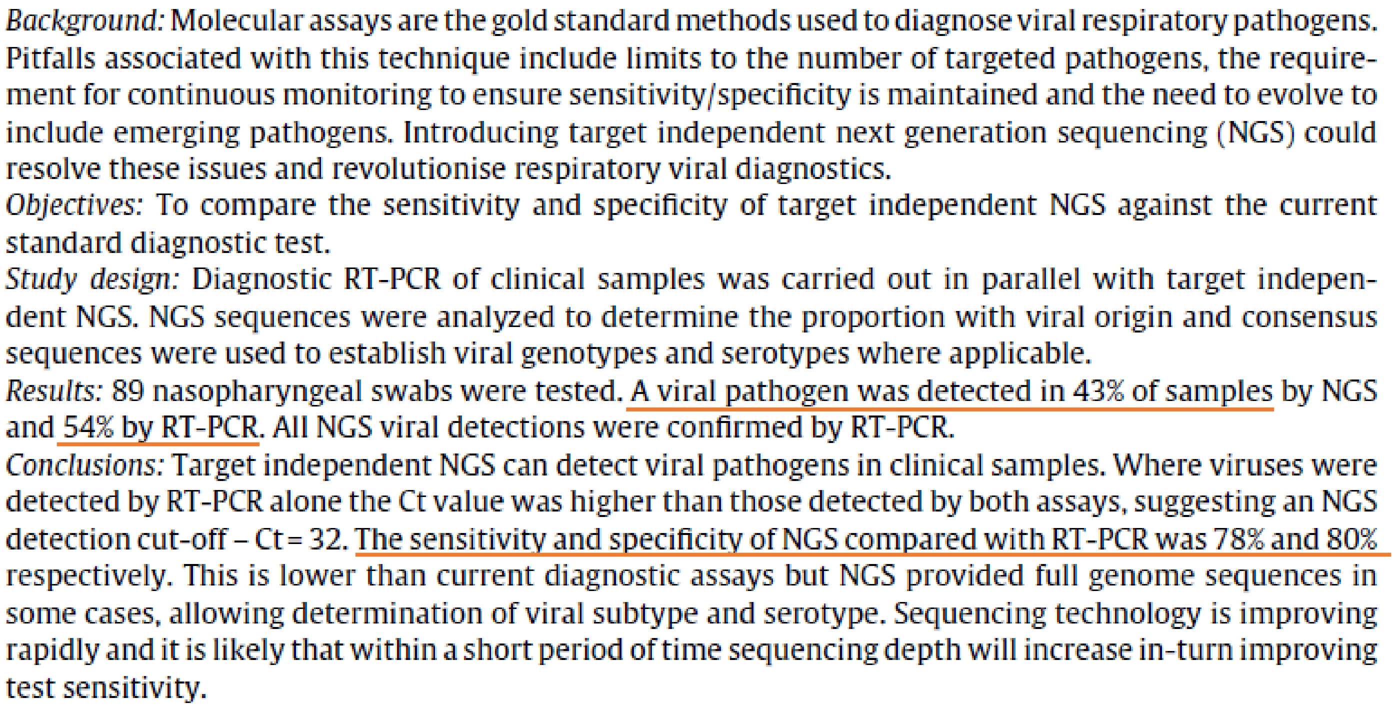
	Infected[a]	Noninfected[a]	Sensitivity[b]	Specificity[b]
mNGS			88.30% (82.29–92.54%)	81.16% (69.57–89.21%)
mNGS-positive	151	13		
mNGS-negative	20	56		
Traditional			25.73% (19.50–33.08%)	88.41% (77.89–94.51%)
Traditional-positive	44	8		
Traditional-negative	127	61		

Note: [a]The values are given as the number of patients. [b]The values are given as the estimate, with the 95% CI in parentheses.

Infect Drug Resist. 2020 Feb 19;13:567-576.

MICROBIOME ANALYSIS

NGS



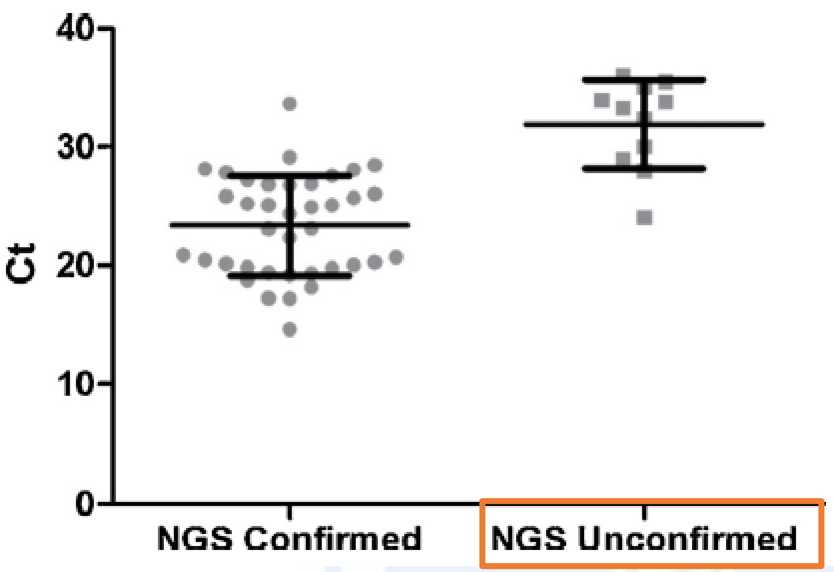
Background: Molecular assays are the gold standard methods used to diagnose viral respiratory pathogens. Pitfalls associated with this technique include limits to the number of targeted pathogens, the requirement for continuous monitoring to ensure sensitivity/specificity is maintained and the need to evolve to include emerging pathogens. Introducing target independent next generation sequencing (NGS) could resolve these issues and revolutionise respiratory viral diagnostics.

Objectives: To compare the sensitivity and specificity of target independent NGS against the current standard diagnostic test.

Study design: Diagnostic RT-PCR of clinical samples was carried out in parallel with target independent NGS. NGS sequences were analyzed to determine the proportion with viral origin and consensus sequences were used to establish viral genotypes and serotypes where applicable.

Results: 89 nasopharyngeal swabs were tested. A viral pathogen was detected in 43% of samples by NGS and 54% by RT-PCR. All NGS viral detections were confirmed by RT-PCR.

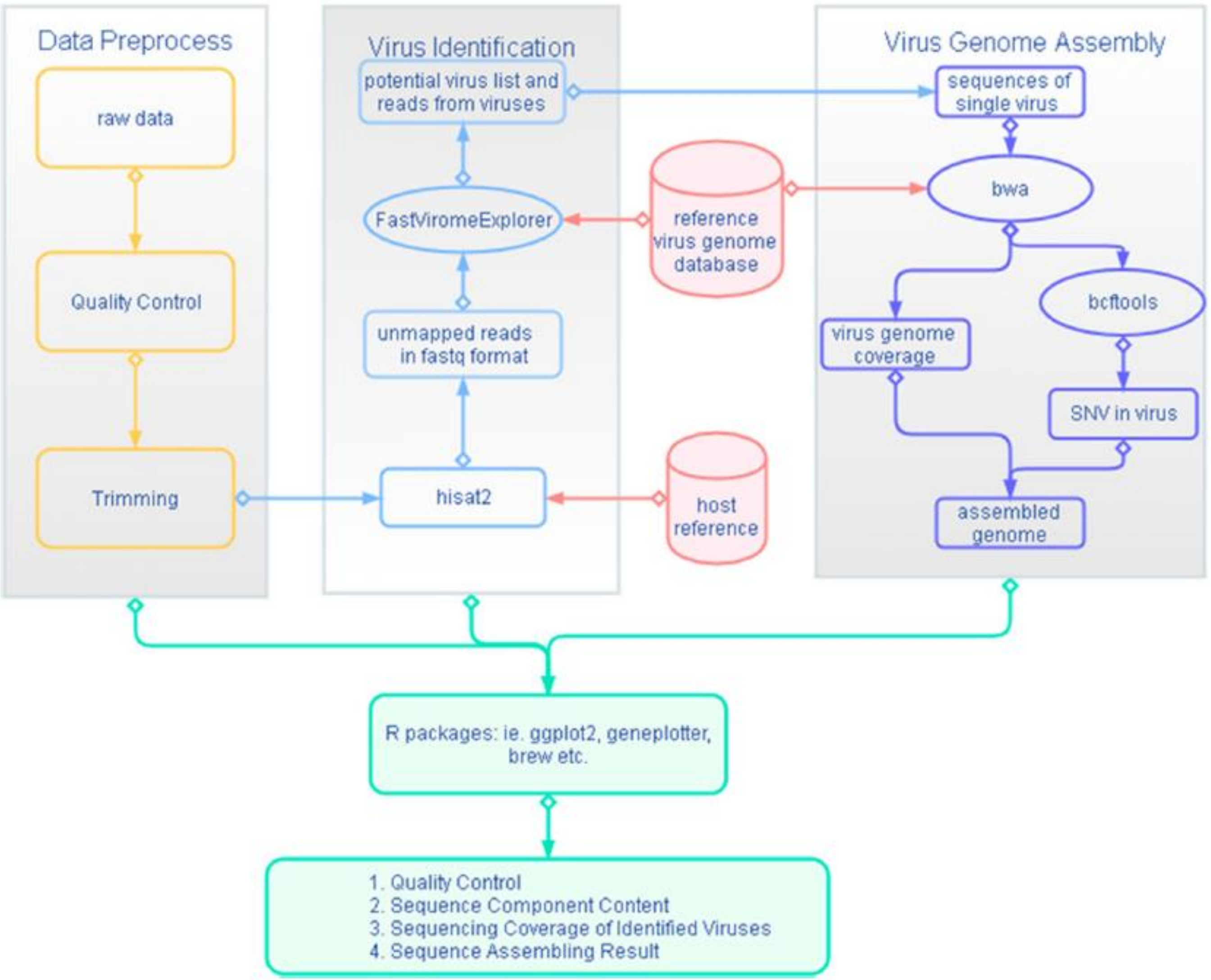
Conclusions: Target independent NGS can detect viral pathogens in clinical samples. Where viruses were detected by RT-PCR alone the Ct value was higher than those detected by both assays, suggesting an NGS detection cut-off – Ct = 32. The sensitivity and specificity of NGS compared with RT-PCR was 78% and 80% respectively. This is lower than current diagnostic assays but NGS provided full genome sequences in some cases, allowing determination of viral subtype and serotype. Sequencing technology is improving rapidly and it is likely that within a short period of time sequencing depth will increase in-turn improving test sensitivity.



Journal of Clinical Virology 69 (2015) 96–100

METATAXONOMIC/METAGENOMIC ANALYSIS

NGS



Data Preprocess
raw data
Quality Control
Trimming

Virus Identification
potential virus list and reads from viruses
FastViromeExplorer
unmapped reads in fastq format
hisat2

reference virus genome database
host reference

Virus Genome Assembly
sequences of single virus
bwa
bcftools
virus genome coverage
SNV in virus
assembled genome

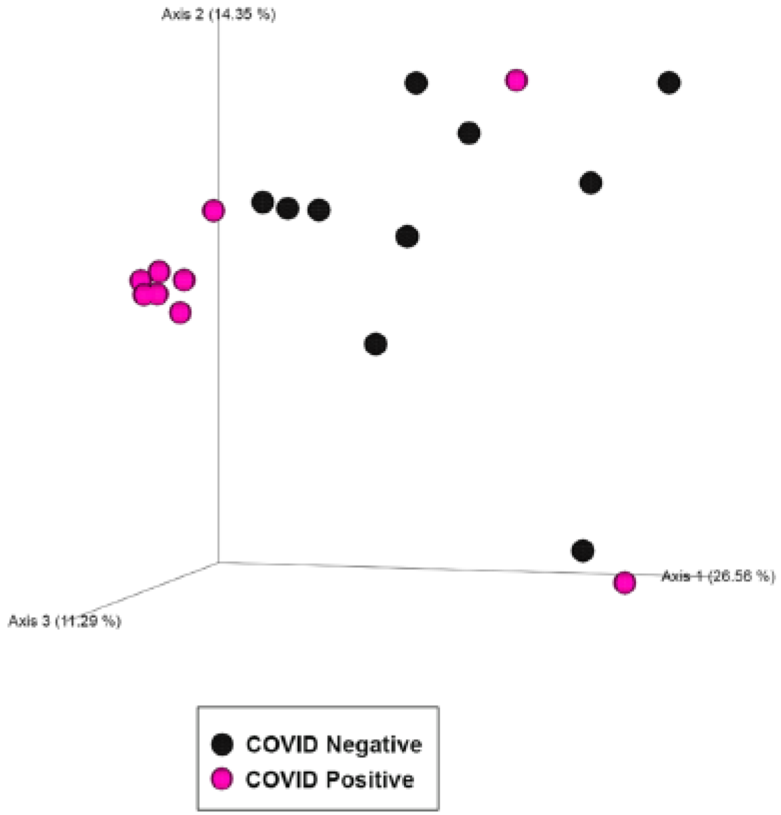
R packages: ie. ggplot2, geneplotter, brew etc.

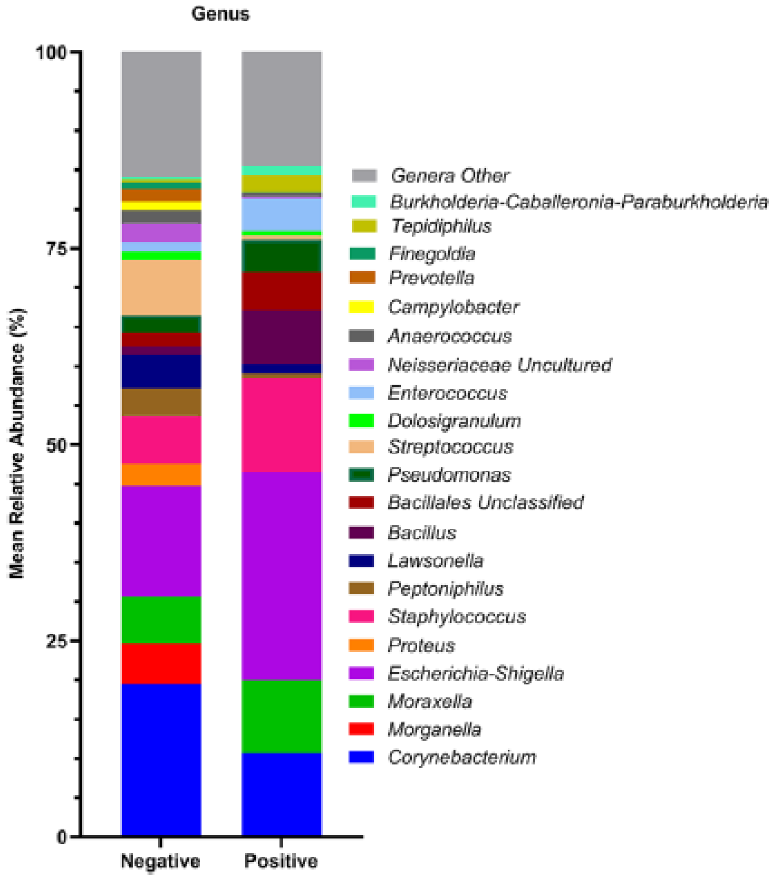
1. Quality Control
2. Sequence Component Content
3. Sequencing Coverage of Identified Viruses
4. Sequence Assembling Result

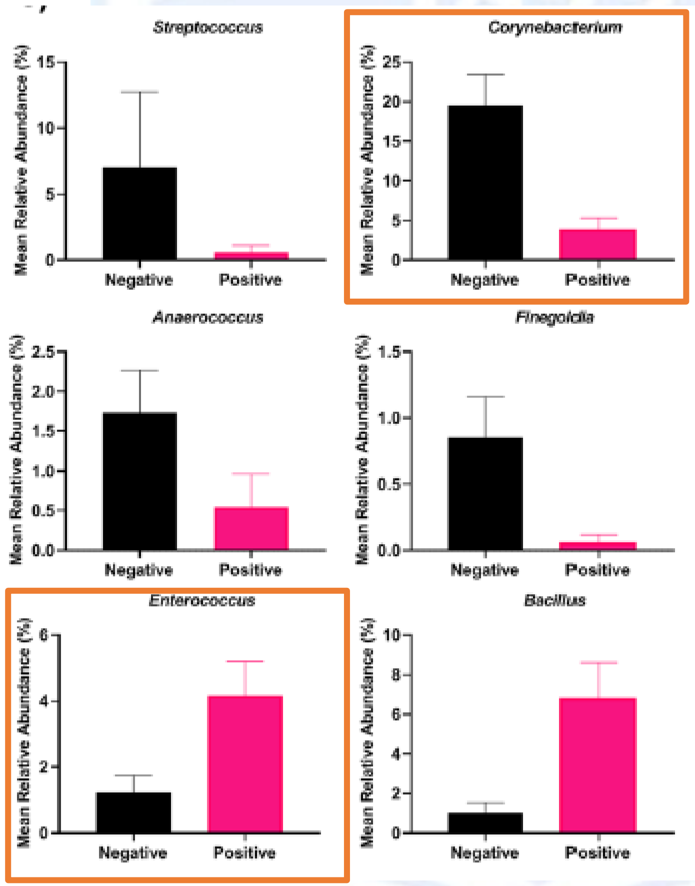
Song et al. BMC Medical Genomics 2021, 14(Suppl 6):289

MICROBIOME ANALYSIS

NGS_microbiome of colonization



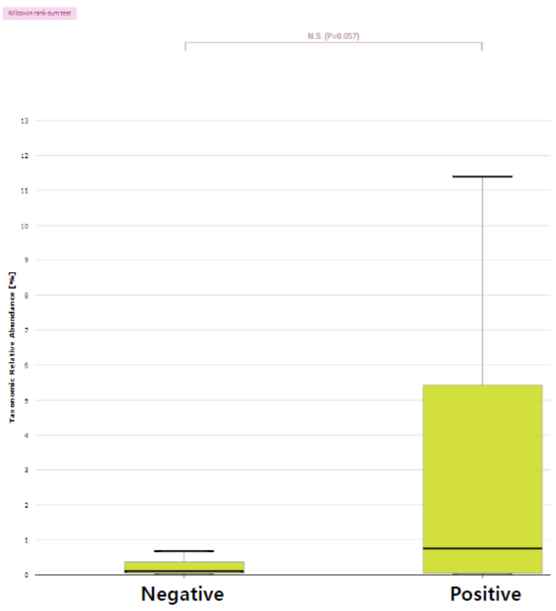




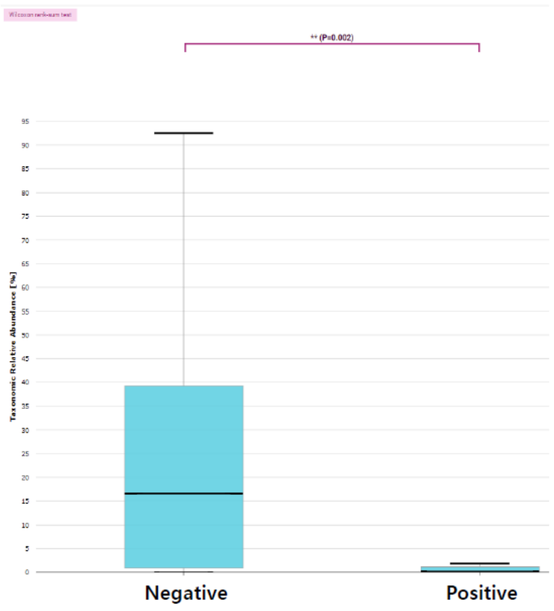
Engen et al. Biol Proced Online (2021) 23:10

MICROBIOME ANALYSIS

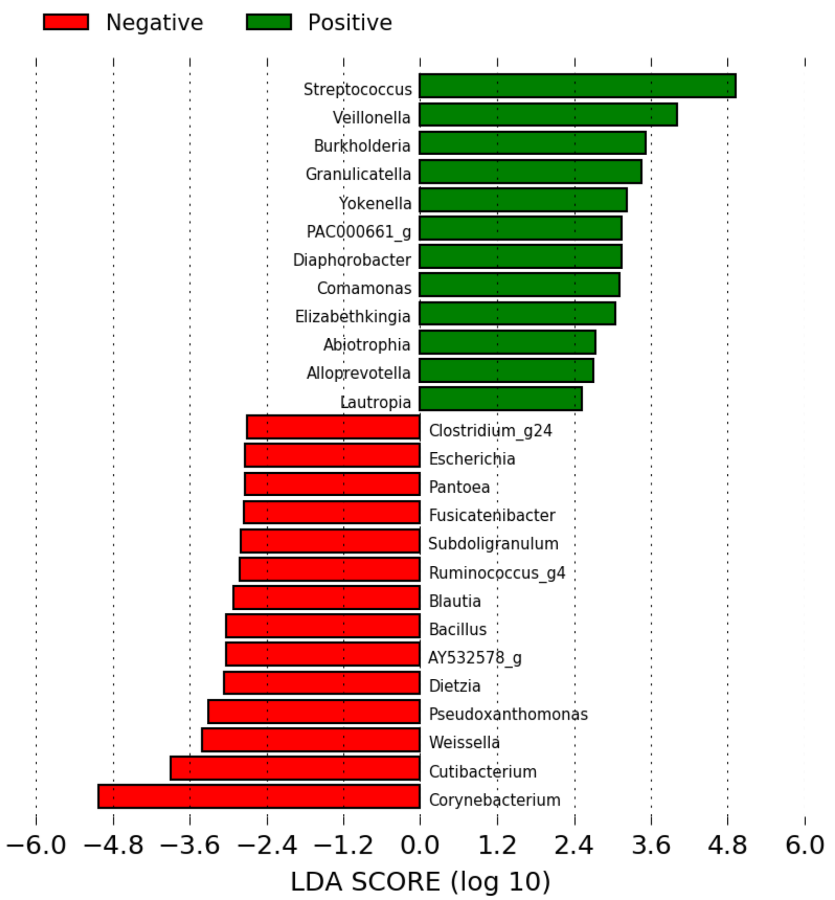
NGS



Streptococcus pneumoniae group

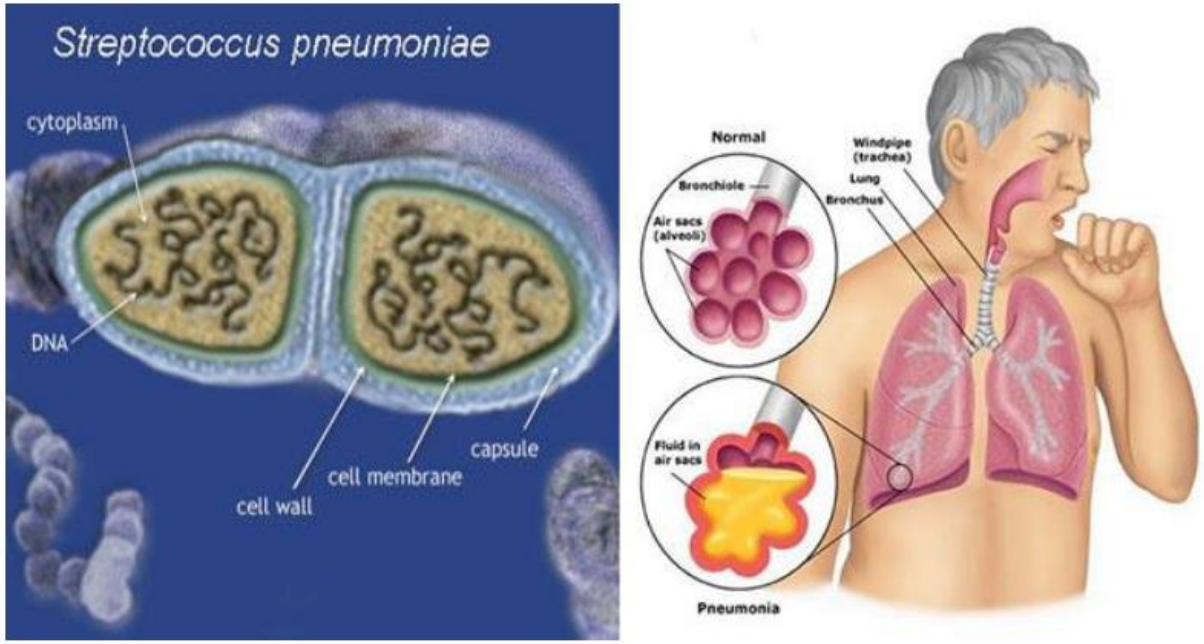


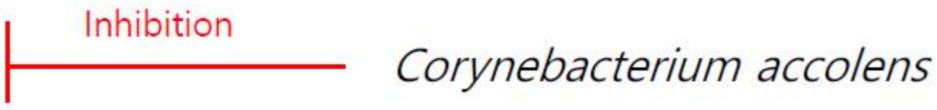
Corynebacterium accolens group



Not published

MICROBIOME ANALYSIS





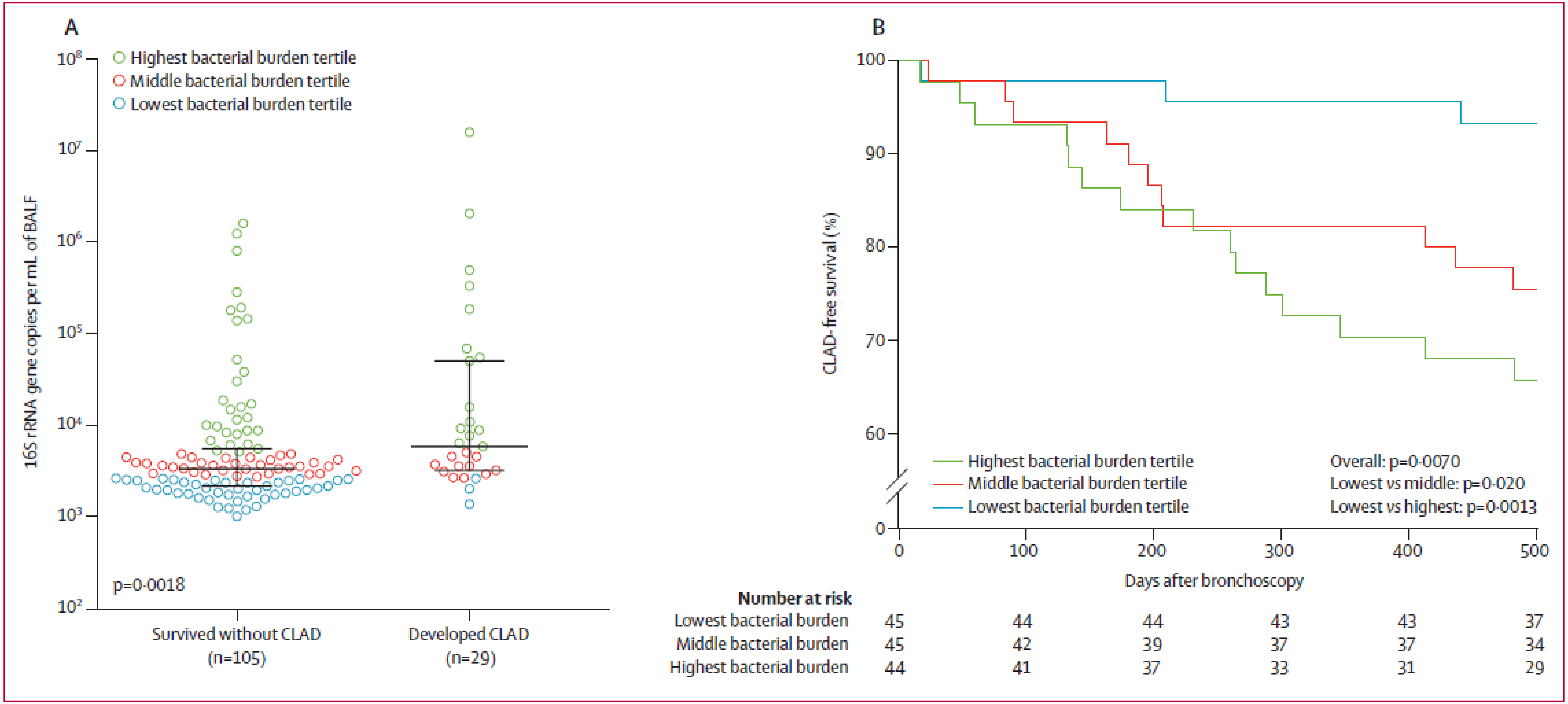
Inhibition

Corynebacterium accolens

- Upper and Lower Respiratory Tract Infection by Streptococcus pneumoniae Is Affected by Pneumolysin Deficiency and Differences in Capsule Type (Kadioglu et al., 2002)
- ***Corynebacterium accolens*** releases Antipneumococcal Free Fatty Acids from Human Nostril and Skin Surface Triacylglycerols (Bomar et al., 2016)

MICROBIOME ANALYSIS

NGS



Number at risk

	0	100	200	300	400	500
Lowest bacterial burden	45	44	44	43	43	37
Middle bacterial burden	45	42	39	37	37	34
Highest bacterial burden	44	41	37	33	31	29

Lancet Respir Med. 2021 Jun;9(6):601-612.

TAKE HOME MESSAGE

Culture -> Direct

1. Novel technology for **A**ntimicrobial **s**usceptibility **t**est
    1. **Phenotypic** vs **genotypic**
    2. Interpretation genotype based AST
    3. Unavailable antibiotics / unavailable pathogen
2. **Full automated Quantitative/Qualitative PCR** for respiratory infection (pneumonia / respiratory viral infection)
    1. **Easy** & **fast** & **safe from contamination**
    2. **Colonizer vs Pathogen**
    3. Cost
3. **Digital PCR**
    1. High analytical sensitivity
    2. Cost & **targeted PCR**
4. **Metataxonomic/metagenomic analysis (microbiome)**
    1. Higher analytical sensitivity -> higher cost

경청해 주셔서 감사합니다.