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Diagnostic Stewardship Aspects of Rapid Diagnostics

Advancement in Molecular Diagnosis in Infectious Diseases

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Outline of talk

- Diagnostic Stewardship in Microbiology with a focus on molecular microbiology and rapid diagnostics in clinical syndrome
- Example of a Stewardship study on carbapenemase Gram-negative bacteria screening
- Summary

Disclosures

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- I have received in kind support previously for research from BioFire and Rosco Diagnostica

Diagnostic Stewardship

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- Concepts in diagnostic stewardship such as quality, cost-effectiveness, and clinical relevance are not new
- Should not be misconstrued as being primarily to reduce test usage and associated costs although these are important ‘side effects’ of diagnostic stewardship
- World Health Organization’s (WHO) Global Antimicrobial Resistance Surveillance System (GLASS), “coordinated guidance and interventions to improve ***appropriate*** use of microbiological diagnostics to guide therapeutic decisions. It should promote ***appropriate, timely diagnostic testing***, including specimen collection, and pathogen identification and ***accurate, timely reporting*** of results to guide patient treatment”

Performing the right test for the right patient for the right reasons at the right time, with the right interpretation which results in the right treatment, optimizing patient care

Analytic Phases of Testing

* Tools for diagnostic stewardship

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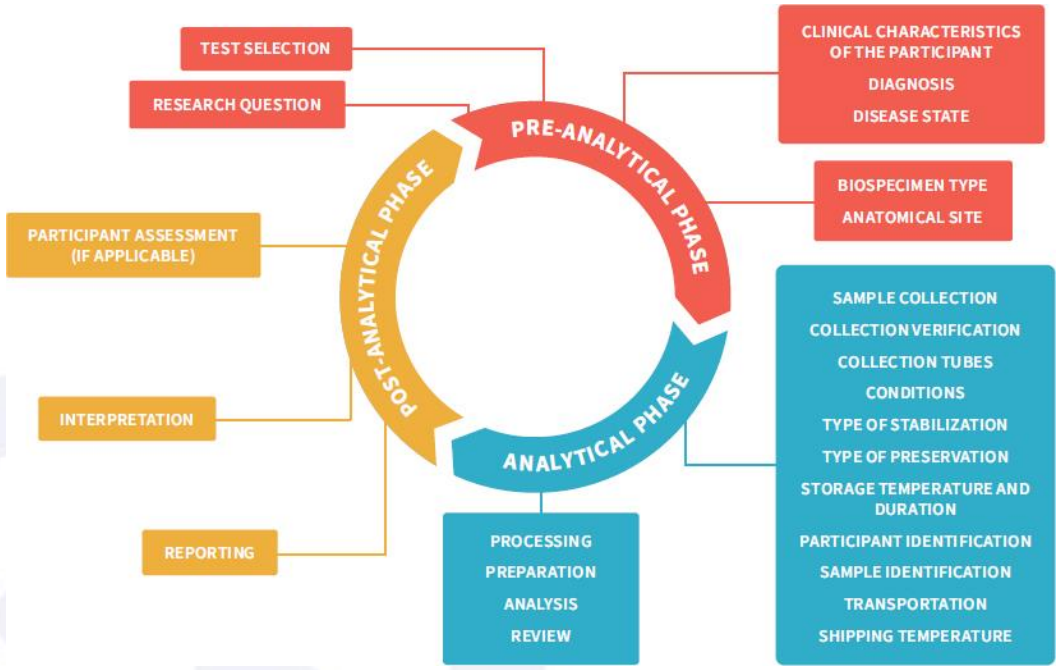
*Post-analytic

•Interpretive comments
•Templated comments (e.g., about test rejection, test performance)
•Expert guidance (e.g., infectious diseases physician and/or laboratory consultation, microbial sequencing boards)
•Provision of utilization report cards
•Utilization of adjunct tests to distinguish colonization from infection
•Laboratory notifications of time-sensitive actionable results and reporting times



*Pre-analytic:

•Removal or introduction of tests
•Clinical decision support system (CDSS), changing order options
•Display costs
•Minimum reordering interval and prevention of duplicate orders
•Development of diagnostic algorithms
•Laboratory formularies with test restriction/requiring prior authorization
•Hold back orders for review prior to authorization
•Education: formal laboratory communications, informal or ad-hoc communications
•Laboratory test utilization committees
•Test reimbursement policies (e.g., insurance reimbursement policies)

*Analytic

• Clear specimen rejection and criterion
• Reflex testing
• Reflective testing
• On-demand vs batch testing
•Cascade testing and reporting (e.g., antimicrobial susceptibility testing)

• Clinical and analytic sensitivity/specificity
Test selection and implementation and factors which influence:
•Predictive values
•Cost
•Test volumes
•Feasibility

Figure: https://nap.nationalacademies.org/read/25094/chapter/5

Minimum Retesting Intervals

'MRIs'

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- Repeated testing may be essential for purposes of patient monitoring and management for certain conditions.
- But testing at excessively frequent intervals may provide no useful information and result in resource wastage and inconvenience to patients
- MRIs aim to optimize testing frequency, which may be affected by
  - Physiological properties and biological half-life of the monitored biomarker;
  - Analytical aspects of the test being performed;
  - Treatment and monitoring requirements of the condition being assessed

The Royal College of Pathologists
Pathology: the science behind the cure

The Association for Clinical Biochemistry & Laboratory Medicine
Better Science, Better Testing, Better Care

IBMS Institute of Biomedical Science

National minimum retesting intervals in pathology

March 2021

Authors: Dr Tim Lang, County Durham and Darlington NHS Foundation Trust
Dr Bernie Croal, Aberdeen Royal Infirmary, NHS Grampian

Unique document number	G147

DE GRUYTER Clin Chem Lab Med 2021; 59(1): 39–50

Mini Review

Tim Lang*

Minimum retesting intervals in practice: 10 years experience

- **Relative lack of evidence based MRIs in microbiology; Many based on Good Practice Points (i.e., clinical experience)**
- The main tests for which evidence based MRIs are available:

Test	MRI (RCPath unless otherwise stated)	Notes
Galactomannan	Twice weekly	patients with hematological malignancies or who are post-allogenic stem cell transplant Single negative may exclude IA, two consecutive positives (or same sample retested) good PPV, reduction in OD in 1st 2 weeks predicts response
Beta-D-Glucan	Twice weekly	Single negative can exclude most IFI (except Mucorales, cryptococcus, some dimorphs and rare fungi)
Clostridioides difficile	7 days [neg] (IDSA) 28 days [positive] 24 hours [neg]	Do not test for cure
Blood borne virus exposures (HIV, HCV, HBV)	e.g. infant born to HCV pos mother HCV RNA 2-3 months, then anti HCV 18 months	Maternal anti-HCV antibody may persist up to 18 months, about 95% cleared by 12 months. HCV RNA positivity in infancy may not predict chronic infection because of possibility of clearance Early HCV testing is associated with engagement with care

Diagnostic Stewardship in Bacteremia, Endovascular Infections, and Sepsis

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- Blood cultures should be limited to patients that have intermediate (>10-50%; e.g., acute pyelonephritis) or high (>50%; e.g., severe sepsis, endocarditis, line infection, meningitis) probability of bacteremia,
- They are unnecessary for low-probability conditions (<10%; e.g., cystitis, non-severe pneumonias, post-operative fevers in first 48 hours)

Fabre Clin Infect Dis 71:1339–1347

- Rapid detection of pathogens
  - MALDI-TOF MS on early growth
  - Multiplex molecular and probe-based assays
- Rapid detection of resistance
  - MALDI-TOF MS on early growth
  - Multiplex molecular and probe-based assays

RCTs / Studies with these technologies have found decreased time to pathogen/resistance detection.

But need to be coupled to robust antimicrobial stewardship programme to benefit time to optimal therapy.

No clear significant differences in other clinical outcome measures (mortality, LOS, adverse events) but neeed studies in other contexts (e.g. LMICs)

TABLE 2
Pathogen and Resistance Gene Identification Libraries for FDA-approved Rapid Blood Culture Diagnostic Assays

Diagnostic Assay	Gram-Positive Bacteria	Gram-Negative Bacteria	Yeast/Candida spp.	Resistance Genes
Verigene BC-GP	*Staphylococcus spp.* *Staphylococcus aureus* *Staphylococcus lugdunensis* *Staphylococcus epidermidis* *Streptococcus anginosus* *Streptococcus agalactiae* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Enterococcus faecalis* *Enterococcus faecium* *Streptococcus spp.* *Listeria* *Micrococcus spp.*[1]	—	—	*mecA* *vanA/B*
Verigene BC-GN	—	*Escherichia coli* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Pseudomonas aeruginosa* *Serratia marcescens*[2] *Acinetobacter spp.* *Citrobacter spp.* *Enterobacter spp.* *Proteus spp.*	—	CTX-M (ESBL) IMP (CRE) KPC (CRE) NDM (CRE) OXA (CRE) VIM (CRE)
BioFire FilmArray BCID Version 1	*Enterococcus spp.* *Listeria monocytogenes* *Staphylococcus spp.* *Staphylococcus aureus* *Streptococcus spp.* *Streptococcus agalactiae* *Streptococcus pneumoniae* *Streptococcus pyogenes*	*Acinetobacter baumannii* *Haemophilus influenzae* *Neisseria meningitidis* *Pseudomonas aeruginosa* *Enterobacteriaceae* *Enterobacter cloacae complex* *Escherichia coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Proteus spp.* *Serratia marcescens*	*C. albicans* *C. glabrata* *C. krusei* *C. parapsilosis* *C. tropicalis*	*mecA* *vanA/B* KPC (CRE)
T2Biosystem T2Candida	—	—	*C. albicans* *C. glabrata* *C. krusei* *C. parapsilosis* *C. tropicalis*	—
T2Biosystem T2Bacteria	*Staphylococcus aureus* *Enterococcus faecium*	*Pseudomonas aeruginosa* *Escherichia coli* *Klebsiella pneumoniae*	—	—
Accelerate Pheno	Coagulase-negative *Staphylococcus spp.* *Staphylococcus aureus* *Staphylococcus lugdunensis* *Streptococcus spp.*[3] *Enterococcus faecalis* *Enterococcus faecium*	*Acinetobacter baumannii* *Citrobacter spp.* *Enterobacter spp.* *Escherichia coli* *Klebsiella spp.* *Pseudomonas aeruginosa* *Proteus spp.* *Serratia marcescens*	*C. albicans*[4] *C. glabrata*[4]	—

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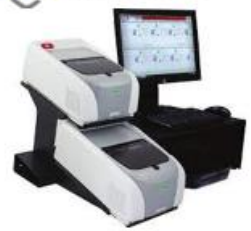
Verigene BC



BioFire BCID

T2

Accelerate Pheno

https://www.medlabmag.com/article/
https://www.biofiredx.com/
https://acceleratediagnostics.com/
https://www.luminexcorp.com/verigene-nanogrid-technology/
https://www.t2biosystems.com/

BCID1 and BCID2

BioFire FilmArray Blood Culture Identification Panel

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BCID 1

Enterococcus
Listeria monocytogenes

Staphylococcus
Staphylococcus aureus

Streptococcus
Streptococcus agalactiae
Streptococcus pyogenes
Streptococcus pneumoniae

Acinetobacter baumannii
Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa

Enterobacteriaceae
Enterobacter cloacae complex
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumoniae
Proteus
Serratia marcescens

Candida albicans
Candida glabrata
Candida krusei
Candida parapsilosis
Candida tropicalis

mecA - methicillin resistant
vanA/B - vancomycin resistant
KPC - carbapenem resistant

Multiplex PCR in pouch, with multiple wells within a enclosed pouch

BCID 2

BioFire FilmArray Blood Culture Identification 2 (BCID2) Panel

Gram-negative Bacteria
Acinetobacter calcoaceticus-baumannii complex
Bacteroides fragilis
Enteric Bacteria
Enterobacter cloacae complex
Escherichia coli
Klebsiella aerogenes
Klebsiella oxytoca
Klebsiella pneumoniae group
Proteus spp.
Salmonella spp.
Serratia marcescens
Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa
Stenotrophomonas maltophilia

Yeast
Candida albicans
Candida auris
Candida glabrata
Candida krusei
Candida parapsilosis
Candida tropicalis
Cryptococcus neoformans/gattii

Gram-positive Bacteria
Enterococcus faecalis
Enterococcus faecium
Listeria monocytogenes
Staphylococcus spp.
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus lugdunensis
Streptococcus spp.
Streptococcus agalactiae (Group B)
Streptococcus pneumoniae
Streptococcus pyogenes (Group A)

Antimicrobial Resistance Genes
bla_{CTX-M}
bla_{IMP}
bla_{KPC}
mcr-1
mecA/C and MREJ
bla_{NDM}
$bla_{OXA-48-like}$
bla_{VIM}
vanA/B

https://www.biofiredx.com/

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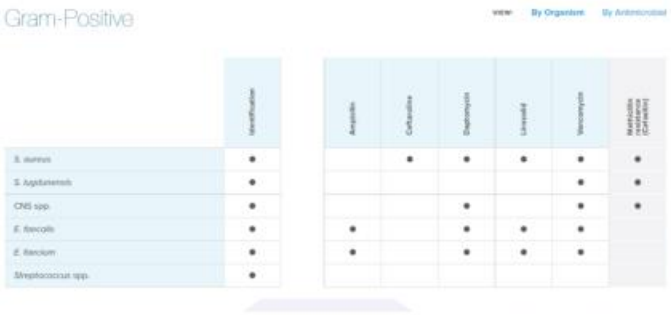

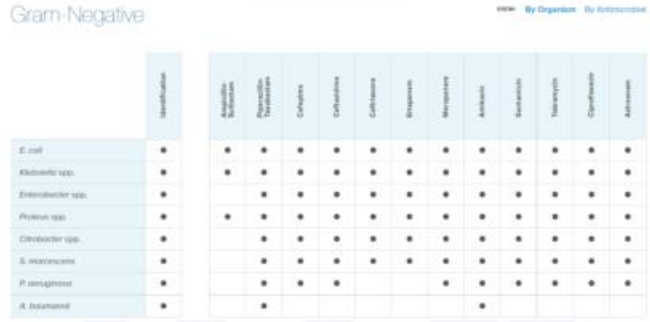

Accelerate ID/Pheno : automated fluorescence in situ hybridization technology with morphokinetic cellular analysis to provide rapid species identification (ID) and antimicrobial susceptibility testing (AST) results

https://acceleratediagnostics.com/

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ePlex BCID-GP Panel
Gram-Positive Organisms
Bacillus cereus group
Bacillus subtilis group
Corynebacterium
Cutibacterium acnes
(Propionibacterium acnes)
Enterococcus
Enterococcus faecalis
Enterococcus faecium
Lactobacillus
Listeria
Listeria monocytogenes
Micrococcus
Staphylococcus
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus lugdunensis
Streptococcus
Streptococcus agalactiae (GBS)
Streptococcus anginosus group
Streptococcus pneumoniae
Streptococcus pyogenes (GAS)
Resistance Genes
mecA
mecC
vanA
vanB

Pan Targets
Pan Gram-Negative
Pan Candida

ePlex BCID-GN Panel
Gram-Negative Organisms
Acinetobacter baumannii
Bacteroides fragilis
Citrobacter
Cronobacter sakazakii
Enterobacter (non-cloacae complex)
Enterobacter cloacae complex
Escherichia coli
Fusobacterium nucleatum
Fusobacterium necrophorum
Haemophilus influenzae
Klebsiella oxytoca
Klebsiella pneumoniae group
Morganella morganii
Neisseria meningitidis
Proteus
Proteus mirabilis
Pseudomonas aeruginosa
Salmonella
Serratia
Serratia marcescens
Stenotrophomonas maltophilia
Resistance Genes
CTX-M
IMP
KPC
NDM
OXA (OXA-23 and OXA-48)
VIM

Pan Targets
Pan Gram-Positive
Pan Candida

ePlex BCID-FP Panel
Fungal Organisms
Candida albicans
Candida auris
Candida dubliniensis
Candida famata
Candida glabrata
Candida guilliermondii
Candida kefyr
Candida krusei
Candida lusitaniae
Candida parapsilosis
Candida tropicalis
Cryptococcus gattii
Cryptococcus neoformans
Fusarium
Rhodotorula

ePLEX: multiplex nucleic acid amplification assay based on competitive DNA hybridization and electrochemical detection using eSensor technology

https://genmarkdx.com/systems/eplex-system/

Comparison of Pathogen ID and AST for EDA-approved Rapid blood culture diagnostic assays

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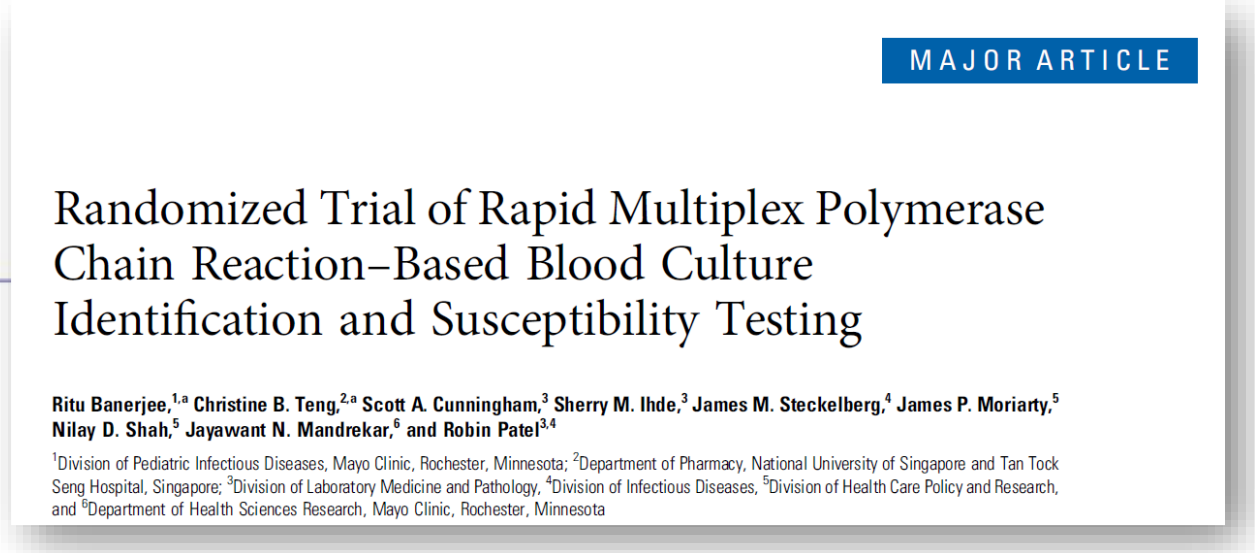
Assay	Bacterial ID	Resistance Genes	AST	Fungal ID	TAT (est)	Comments
Biofire Film Array BCID (27 targets)	√**(16 spp, 3 genera/family**	√ **(3)**		√ **(5)**	1 hr	From positive blood cultures
Biofire Film Array BCID2 (43 targets)	√ **(23 spp, 3 genera/family)**	√ **(10)**		√ **(7)**	1 hr	From positive blood cultures
Verigene BC-GP / GN	√ **(GP: 8 spp, 1 grp, 4 genera) (GN – 5 spp, 4 genera)**	√ **(6)**		-	<2-2.5 hr	From positive blood cultures
Accelerate Pheno	√ **(GP -6, GN- 8, 2 yeast)**	-	√	√	ID 2 hr AST 7 hr	From positive blood cultures
GenMark ePLEX	√ **(GP 12 spp/grp, 6 genera) (GN 16 spp/grp, 5 genera), Pan GP/GN**	√	-	√ **(13 spp, 2 genera), Pan Candida**		From positive blood cultures
T2Candida	-	-	-	√ **(5 spp.)**	3-7 hr	Direct from blood
T2Bacteria	√ **(6 spp.)**	-	-	-	3-7 hr	Direct from blood



MAJOR ARTICLE

Randomized Trial of Rapid Multiplex Polymerase Chain Reaction–Based Blood Culture Identification and Susceptibility Testing

Ritu Banerjee,[1,a] Christine B. Teng,[2,a] Scott A. Cunningham,[3] Sherry M. Ihde,[3] James M. Steckelberg,[4] James P. Moriarty,[5] Nilay D. Shah,[5] Jayawant N. Mandrekar,[6] and Robin Patel[3,4]

[1]Division of Pediatric Infectious Diseases, Mayo Clinic, Rochester, Minnesota; [2]Department of Pharmacy, National University of Singapore and Tan Tock Seng Hospital, Singapore; [3]Division of Laboratory Medicine and Pathology, [4]Division of Infectious Diseases, [5]Division of Health Care Policy and Research, and [6]Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota

- 617 patients in 3 arms
- Standard – control arm,
- 2 intervention arms
  - BCID+templated comments
  - BCID+templated comments+ASP

Clin Infect Dis. 2015 Oct 1;61(7):1071-80.

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Results

- Microorganism identification: BCID 1.3 hrs vs control 22.3 hrs

- Decreased piperacillin-tazobactam use
- Decreased treatment of contaminants
- Increased narrow spectrum for Gram positives
- No worsening of clinical outcomes
- Faster escalation (both intervention arms)
- Fastest de-escalation (BCID+ASP)
- Groups did not differ in mortality, LOS, or cost.

Randomized Trial of Rapid Multiplex Polymerase Chain Reaction–Based Blood Culture Identification and Susceptibility Testing

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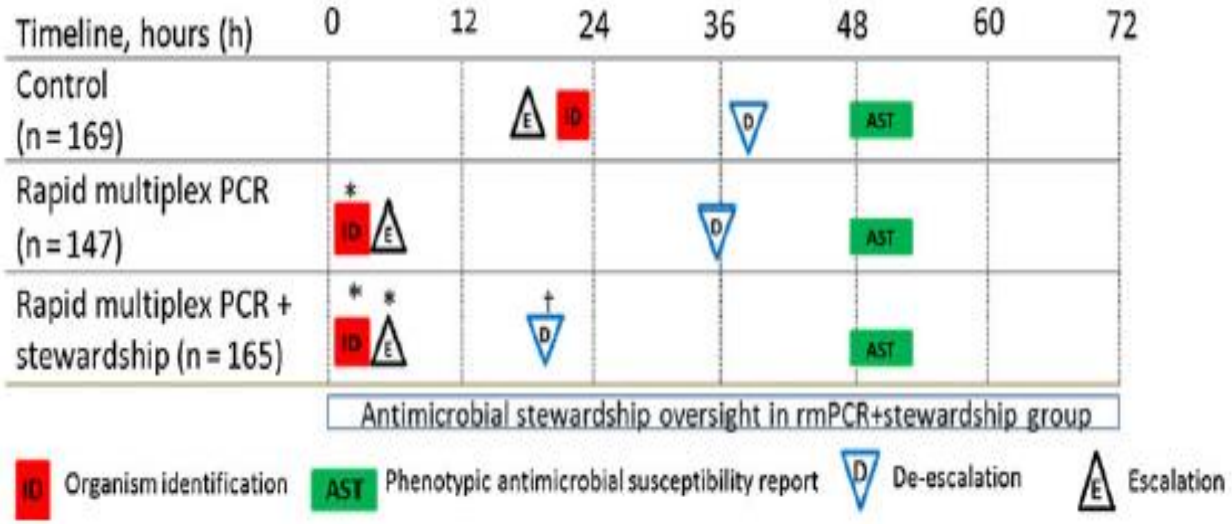
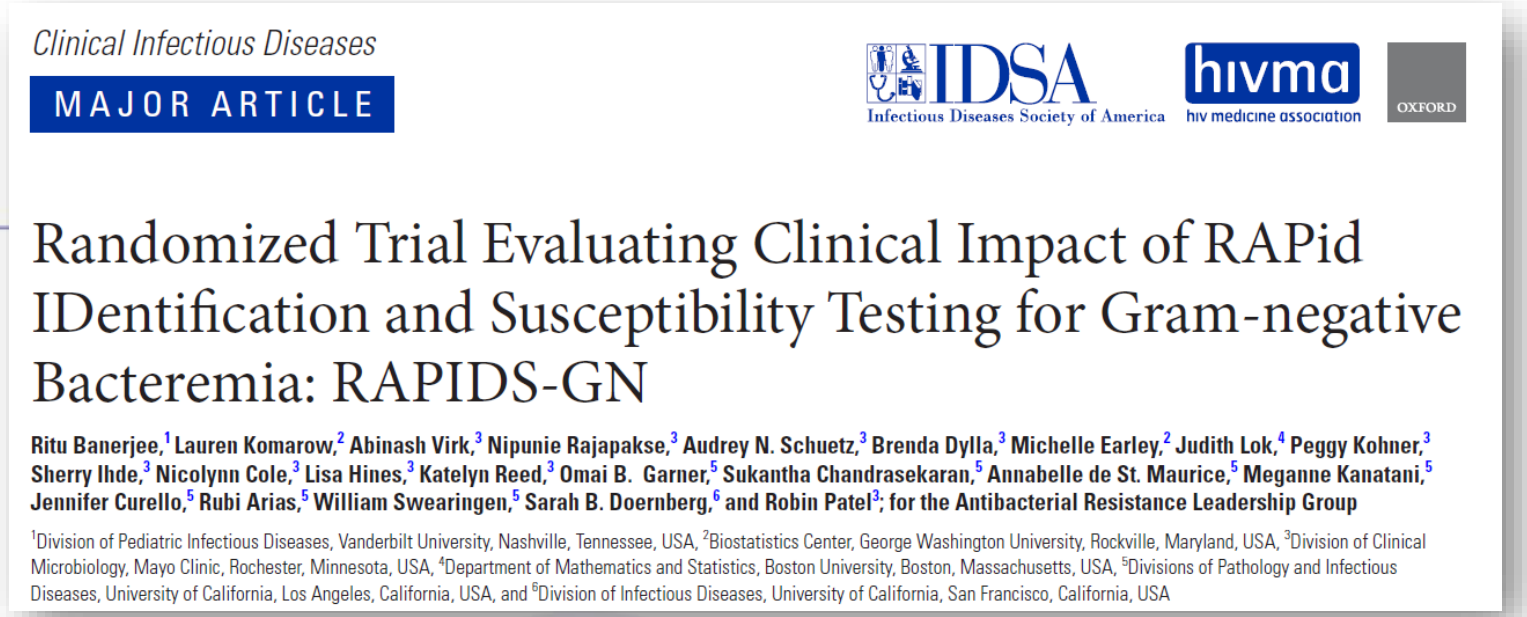


Figure 2. Comparison of time to organism identification, availability of phenotypic antimicrobial susceptibility results, and first appropriate modification of antimicrobial therapy for the subset of study subjects with organisms represented on the rapid multiplex polymerase chain reaction (rmPCR) panel (n = 481). Time 0 is when the positive Gram stain result was reported. Median time in hours (interquartile range [IQR]) to organism identification: control 22.3 (17–28), both rmPCR and rmPCR + stewardship 1.3 (0.9–1.6); de-escalation: control 39 (19–56), rmPCR 36 (22–61), rmPCR + stewardship 20 (6–36); escalation: control 18 (2–63), rmPCR 4 (1.5–24), rmPCR + stewardship 4 (1.8–9). *$P < .05$ vs control; †$P < .05$ vs control and rmPCR groups.

Banerjee and Teng et al. Clin Infect Dis. 2015 Oct 1;61(7):1071-80.



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MAJOR ARTICLE

IDSA Infectious Diseases Society of America · hivma hiv medicine association · OXFORD

Randomized Trial Evaluating Clinical Impact of RAPid IDentification and Susceptibility Testing for Gram-negative Bacteremia: RAPIDS-GN

Ritu Banerjee,[1] Lauren Komarow,[2] Abinash Virk,[3] Nipunie Rajapakse,[3] Audrey N. Schuetz,[3] Brenda Dylla,[3] Michelle Earley,[2] Judith Lok,[4] Peggy Kohner,[3] Sherry Ihde,[3] Nicolynn Cole,[3] Lisa Hines,[3] Katelyn Reed,[3] Omai B. Garner,[5] Sukantha Chandrasekaran,[5] Annabelle de St. Maurice,[5] Meganne Kanatani,[5] Jennifer Curello,[5] Rubi Arias,[5] William Swearingen,[5] Sarah B. Doernberg,[6] and Robin Patel[3]; for the Antibacterial Resistance Leadership Group

[1]Division of Pediatric Infectious Diseases, Vanderbilt University, Nashville, Tennessee, USA, [2]Biostatistics Center, George Washington University, Rockville, Maryland, USA, [3]Division of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota, USA, [4]Department of Mathematics and Statistics, Boston University, Boston, Massachusetts, USA, [5]Divisions of Pathology and Infectious Diseases, University of California, Los Angeles, California, USA, and [6]Division of Infectious Diseases, University of California, San Francisco, California, USA

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- 500 patients with GNB BSI, two US centers
- 226 control (SOC+ASP), 222 RAPID (Accelerate Pheno System) + ASP
- Time to results RAPID vs SOC : 2.7 vs 11.7 hrs (P <0.01)
- Time to AST 13.5 vs 44.9 hrs (P <0.01)
- Time to (RAPID vs SOC):
  - first overall antibiotic modification – 8.6 vs 14.9 hrs (P = 0.02)
  - Gram negative antibiotics modification – 17.3 vs 42.1 hrs
  - Antibiotic escalation : 18.4 hrs vs 61.7 hrs
- Arms did not differ in clinical outcomes including mortality, time to death, and length of stay

Banerjee, CID 2020

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BRIEF REPORT

Accuracy of a Rapid Multiplex Polymerase Chain Reaction Plus a Chromogenic Phenotypic Test Algorithm for Detection of Extended-Spectrum β-Lactamase and Carbapenemase-Producing Gram-Negative Bacilli in Positive Blood Culture Bottles

Sean Wei Xiang Ong,[1,2] Pei Yun Hon,[1] Sharon Syn Hui Wee,[3] Jonathan Wei Zhong Chia,[4] Shehara Mendis,[4] Ezlyn Izharuddin,[1] Ray Junhao Lin,[1,2] Po Ying Chia,[1,2,5] Rees Chin Swee Sim,[4] Mark I-Cheng Chen,[1,6] Angela Chow,[5,6,7] Joanne Yoong,[6] David Chien Lye,[1,2,5,8] Christine B. Teng,[9] Paul Anantharajah Tambyah,[8,10] Ritu Banerjee,[11] Robin Patel,[12] Partha Pratim De,[4] and Shawn Vasoo[1,2,5]

[1]National Centre for Infectious Diseases, Singapore; [2]Department of Infectious Diseases, Tan Tock Seng Hospital, Singapore; [3]Clinical Research and Innovation Office, Tan Tock Seng Hospital, Singapore; [4]Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore; [5]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore; [6]Saw Swee Hock School of Public Health, National University of Singapore, Singapore; [7]Department of Clinical Epidemiology, Office of Clinical Epidemiology, Analytics, and Knowledge (OCEAN), Tan Tock Seng Hospital, Singapore; [8]Infectious Diseases Translational Research Program, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [9]Department of Pharmacy, National University of Singapore, Singapore; [10]Division of Infectious Disease, National University Hospital, Singapore; [11]Division of Pediatric Infectious Diseases, Vanderbilt University, Nashville, Tennessee, USA; and [12]Divisions of Clinical Microbiology and Infectious Diseases, Mayo Clinic, Rochester, Minnesota, USA

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- Initial analysis of GN strategy for Rabbit Trial at NCID /TTSH
- Verification phase 97 pos blood cultures (54 clinical, 43 spiked)
- Prospective validation phase 123 blood cultures positive for GNB by Gram stain part of interim safety analysis of a randomized controlled trial, Impact of Rapid Pathogen Identification From Blood Cultures (RABbIT) (ClinicalTrials.gov identifier NCT02743585).
- BCID assay + Rosco ESBL/Carbapenemase kits

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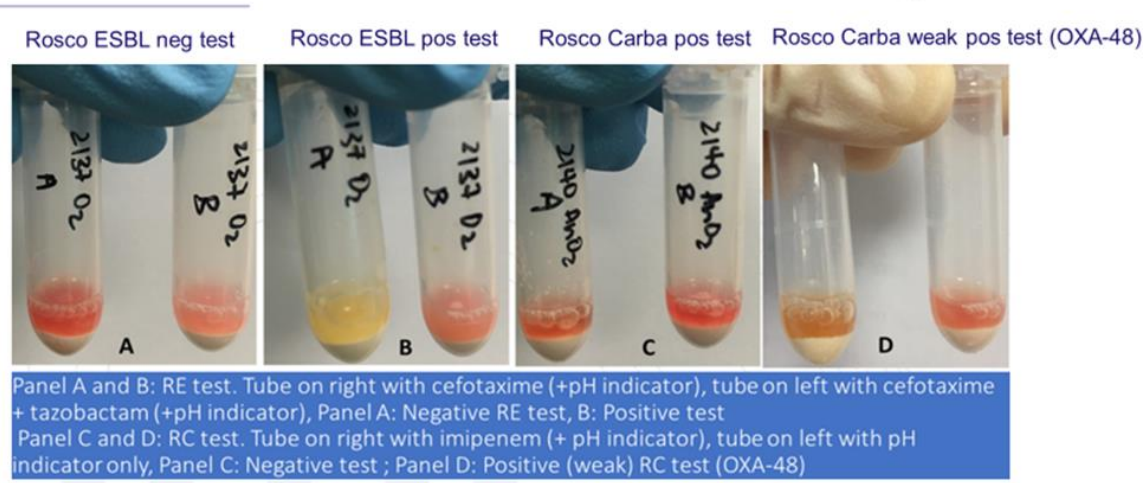

Panel A and B: RE test. Tube on right with cefotaxime (+pH indicator), tube on left with cefotaxime + tazobactam (+pH indicator), Panel A: Negative RE test, B: Positive test
Panel C and D: RC test. Tube on right with imipenem (+ pH indicator), tube on left with pH indicator only, Panel C: Negative test ; Panel D: Positive (weak) RC test (OXA-48)

Clin Infect Dis. 2021 Sep 23:ciab848. doi: 10.1093/cid/ciab848. Epub ahead of print. PMID: 34554228.

Table 1. Results of Extended-Spectrum β-Lactamase and Carbapenemase Testing in Clinical and Spiked Blood Culture Isolates (n = 97)

Resistance Phenotype (No.)	Resistance Genotype (No.)	Species (No.)	BCID Panel Match, No. of On-Panel Organisms (%)	RE Kit Positive, No. (%)	RC Kit Positive, No. (%)
	Verification phase				
	Spiked samples				
ESBL (18)	CTX-M-15 (11)	*Escherichia coli* (11)	…	11 (100)	0 (0)
	CTX-M-9 (4)	*E. coli* (4), *Enterobacter cloacae* complex (1)	…	4 (100)	0 (0)
	SHV-3 (1)	*E. coli* (1)	…	1 (100)	0 (0)
	SHV-18 (1)	*Klebsiella pneumoniae* (1)	…	1 (100)	0 (0)
	TEM-10 (1)	*E. coli* (1)	…	1 (100)	0 (0)
	Total	…	…	18 (100)	0 (0)
AmpC (2)	Chromosomal AmpC, high-level producer (1)	*E. cloacae* (1); ATCC BAA-1143	…	0 (0)	0 (0)
	Chromosomal AmpC, low-level producer (1)	*K. pneumoniae* (1); ATCC BAA-1144	…	0 (0)	0 (0)
	Total	…	…	0 (0)	0 (0)
Carbapenemase (21)	IMI (3)	*E. cloacae* complex (3)	…	1 (33)	0 (0)
	IMP (4)	*E. coli* (1), *E. cloacae* complex (2), *Serratia marcescens* (1)	…	3 (75)[a]	4 (100)
	KPC (4)	*E. coli* (2), *K. pneumoniae* (2)	…	4 (100)	4 (100)
	NDM (2)	*E. coli* (1), *K. pneumoniae* (1)	…	2 (100)	2 (100)
	OXA-23 with 51-like (1)	*Acinetobacter baumannii* (1)	…	0 (0)	0 (0)
	OXA-232 (3)	*K. pneumoniae* (3)	…	3 (100)	0 (0)
	OXA-48 (1)	*E. coli* (1)	…	1 (100)	0 (0)
	SME-1 (1)	*S. marcescens* (1)	…	0 (0)	0 (0)
	VIM-1 (2)	*K. pneumoniae* (1), *Pseudomonas aeruginosa* (1)	…	0 (0)	0 (0)
	Total	…	…	14 (67)	10 (48)
None (2)	…	*E. coli* (2)	…	0 (0)[b]	0 (0)
	Clinical samples				
Narrow-spectrum β-lactamase (1)	TEM, unspecified (1)	*E. coli* (1)	…	0 (0)	0 (0)
ESBL (7)	CTX-M (1)	*Citrobacter koseri* (1)	…	1 (100)	0 (0)
	CTX-M, OXA (1)	*E. coli* (1)	…	1 (100)	0 (0)
	CTX-M, SHV (1)	*K. pneumoniae* (1)	…	1 (100)	0 (0)
	CTX-M, SHV, OXA (1)	*K. pneumoniae* (1)	…	1 (100)	0 (0)
	CTX-M, TEM (1)	*E. coli* (1)	…	1 (100)	0 (0)
	CTX-M, SHV, OXA, TEM (1)	*K. pneumoniae* (1)	…	1 (100)	0 (0)
	CTX-M, TEM, OXA (1)	*Klebsiella oxytoca* (with susceptible *C. koseri*)[c] (1)	…	1 (100)	0 (0)
	Total	…	…	7 (100)	0 (0)
AmpC (1)	DHA	*Morganella morganii* (with susceptible *K. pneumoniae*, and *Proteus mirabilis*)[c] (1)	…	0 (0)	0 (0)
Carbapenemase (1)	KPC	*E. coli* (1)	…	1 (100)	1 (100)
None (44)	…	*A. baumannii* (1), *Aeromonas hydrophila* (1), anaerobes, species not determined (2), *Burkholderia multivorans* (1), *Burkholderia pseudomallei* (1), *Chryseobacterium gleum* (1), *E. coli* (18)	…	0 (0)	0 (0)
		Klebsiella aerogenes (2), *K. pneumoniae* (9), *P. mirabilis* (3), *P. aeruginosa* (1), *Salmonella enteritidis* (2), *S. marcescens* (1), *K. oxytoca* and *E. coli*[c] (1)			
	Total	…	…	0 (0)	0 (0)

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Verification phase (n=97; 54 clinical, 43 spiked)

RE Kit
PPA 100% (95% CI 83.4%–100%)
NPA 100% (90.0%–100%)

RC kit detected 11 of 22 carbapenemase-producing isolates,
PPA of 50% (95% CI, 28.8%–71.2%)
NPA of 100% (90.0%–100%).

Resistance Phenotype (No.)	Resistance Genotype (No.)	Species (No.)	BCID Panel Match, No. of On-Panel Organisms (%)	RE Kit Positive, No. (%)	RC Kit Positive, No. (%)
	Clinical phase				
	Monomicrobial specimens				
On BCID panel					
ESBL (25)	CTX-M (3)	*E. coli* (3)	3 (100)	3 (100)	0 (0)
	CTX-M + ACC (2)	*E. coli* (2)	2 (100)	2 (100)	0 (0)
	CTX-M + OXA (2)	*E. coli* (2)	2 (100)	2 (100)	0 (0)
	CTX-M + OXA +SHV (2)	*K. pneumoniae* (2)	2 (100)	2 (100)	0 (0)
	CTX-M + TEM (2)	*E. coli* (2)	2 (100)	2 (100)	0 (0)
	CTX-M + OXA + TEM (2)	*E. coli* (1) *E. cloacae* complex (1)	2 (100)	2 (100)	0 (0)
	CTX-M + TEM +SHV (2)	*K. pneumoniae* (2)	2 (100)	2 (100)	0 (0)
	OXA (1)	*E. coli* (1)	1 (100)[d]	2 (100)	0 (0)
	OXA + SHV (1)	*K. pneumoniae* (1)	1 (100)	1 (100)	0 (0)
	TEM (5)	*P. mirabilis* (1) *E. coli* (4)	5 (100)	1 (20)	0 (0)
	TEM + DHA (1)	*E. coli* (1)	1 (100)	0 (0)	0 (0)
	SHV + CIT (1)	*K. pneumoniae* (1)	1 (100)	0 (0)	0 (0)
	SHV + DHA (1)	*K. pneumoniae* (1)	1 (100)	0 (0)	0 (0)
Carbapenemase (1)	OXA-48-like + SHV + OXA	*K. pneumoniae* (1)	1 (100)	1 (100)	1 (100)
Miscellaneous (2)	Presumptive K1 β-lactamase	*K. oxytoca*(1)	1 (100)	1 (100)	0 (0)
	Extremely drug-resistant (XDR)	*A. baumannii* (1)	1 (100)	1 (100)	0 (0)
None (76)	NA	*A. baumannii* (1), *Acinetobacter pittii* (1), *C. koseri* (1), *E. cloacae* complex (5) *E. coli* (35), *K. pneumoniae* (20), *K. variicola* (1), *P. mirabilis* (5), *P. aeruginosa* (6), *S. marcescens* (1)	76 (100)	0 (0)	0 (0)
	Off BCID panel				
None (8)	NA	*Bacteroides fragilis* (2), *Sphingomonas pseudosanguinis* (1), *Pseudomonas luteola* (1), *Acinetobacter nosocomialis* (2), *Moraxella osloensis* (1), *Stenotrophomonas maltophilia* (1)	NA	0 (0)	0 (0)
	Polymicrobial specimens (all with ≥1 target on BCID panel)				
ESBL (2 specimens, 4 on-panel targets)	CTX-M, TEM, SHV	*K. pneumoniae* (with *A. baumannii*)	2 (100)	1 (100)	0 (0)
	CTX-M, TEM	*E. coli* (ESBL) (with 2nd susceptible *E. coli* strain, *K. pneumoniae*)	2 (100)	1 (100)	0 (0)
Plasmid AmpC (2 specimens, 4 on-panel targets	DHA	*M. morganii* (with *P. mirabilis*, *P. aeruginosa*) (1); *E. coli* and *K. pneumoniae* (1)[d]	4 (80)	0 (0)	0 (0)
None (7 specimens, 16 on-panel targets)	NA	*E. cloacae*, *Enterobacter aerogenes*, *K. pneumoniae* (1); *E. coli* and *Enterococcus faecalis* (1); *E. coli*, *K. pneumoniae*, *Streptococcus* sp. (1); *K. pneumoniae*, *S. marcescens*, *P. aeruginosa* (1); *P. mirabilis*, *E. faecalis* (1); *Moraxella lacunata*, *Staphylococcus warneri* (1); *E. coli* and *P. aeruginosa* (only anaerobic bottle tested) (1)[e]	15 (93.8)	0 (0)	0 (0)

Abbreviations: ATCC, American Type Culture Collection; BCID, blood culture identification; ESBL, extended-spectrum β-lactamase; KPC, *K. pneumoniae* carbapenemase; NA, not applicable; RC, Rosco carbapenemase kit (Neo-Rapid CARB Kit); RE, Rosco ESBL kit (Rapid ESBL Screen Kit); SHV.

[a]One IMP–*S. marcescens* isolate tested negative with the RE kit but was positive with the RC kit.

[b]One *E. coli* isolate tested negative with the RE kit but was found to be reverted to a susceptible phenotype and was negative for CTX-M-9 on retesting.

[c]These 3 clinical samples were polymicrobial.

[d]False-positive *Proteus* species suppressed with manufacturer alert.

[e]Only anaerobic bottle was tested, so *P. aeruginosa* was missed.

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Prospective clinical cohort (n=123)

BCID

on-panel, target-based:
PPA 99.2 (95% CI, 95%-100%)
Sample based: PPA 99.1 (95% CI 94.5%-100%) NPA 100% (59.8%-100%)

RE Kit (27 ESBLs)

All ESBLs PPA 74% (95% CI 53.4%–88.1%)
NPA 100% (95.0%–100%)

CTX-M ESBLs PPA 100% (74.7-100%), NPA 100% (95.1-100%)

RC kit detected the single OXA-48 + specimen

Mid-point, Mortality (416 patients)

National Centre for Infectious Diseases

Rapid Pathogen Identification From Blood Cultures (RABbIT) (ClinicalTrials. gov identifier NCT02743585).

- All patients: Study arm (9.9%), Control arm (15.5%) (*P* = 0.087)
- Gram negative rods only (n=220): Study (10.3%), Control arm (16.3%) (*P* = 0.189)
- Non-GNRs (n=196): study (9.4%), Control arm (14.4%) (*P* = 0.277)

Biomarkers to guide who should get blood cultures or direct from blood rapid diagnostics?

National Centre for Infectious Diseases

- Use of host biomarker* to predict bacteremia and guide the use of blood cultures or other diagnostic tests (e.g., direct-from-blood molecular assays)
- No single marker has been found to be consistently and sufficiently sensitive
- One study limiting blood cultures to patients with a procalcitonin of

  >0.1 mcg/L -- 99% sensitivity for bacteremia (24.4% specificity), reducing blood culture sampling by 20%

  >0.5 mcg/L -- 71.2% sensitivity for bacteremia (73.3% specificity), reducing blood culture sampling by 20%

  Further studies which account for practicalities such as the need for timeliness of results and administration of antibiotics in a septic patient are needed

Paul M, Clin Infect Dis 2006 42:1274–1282

*e.g., procalcitonin, CRP, IL-6, soluble urokinase plasminogen activator receptor (suPAR) levels, blood indices including the neutrophil-lymphocyte count ratio (NLCR), predictive scores, and machine learning algorithms)

Diagnostic Stewardship in Respiratory Tract Infections

National Centre for Infectious Diseases

- CAP vs HAP/VAP, different pathogens, differentiate colonization from infection, properly collected specimens important (e.g. reject if >10 squames per HPF; perform good quality Gram stain)

- No clear evidence from meta-analyses of randomized trials that performing quantitative cultures via invasive sampling techniques significantly improves clinical outcomes (e.g., antibiotic changes, mortality, length of ICU stay, ventilator-days)

- Differences in management guidelines in North America (ATS/IDSA) and Europe (ERS/ESICM/ESCMID/ALAT) on role of invasive sampling in VAP

  two randomized clinical trials reported differences in the role of invasive quantitative cultures in decreasing antibiotic exposure which has resulted in differences in management guidelines

Canadian Critical Care Trials Group. A randomized trial of diagnostic techniques for ventilator-associated pneumonia. N Engl J Med 2006; 355:2619–30.
Fagon JY, Chastre J, Wolff M, et al. Invasive and noninvasive strategies for man- agement of suspected ventilator-associated pneumonia. A randomized trial. Ann Intern Med 2000; 132:621–30.

Diagnostic Stewardship in Respiratory Tract Infections

National Centre for Infectious Diseases

Molecular microbiology

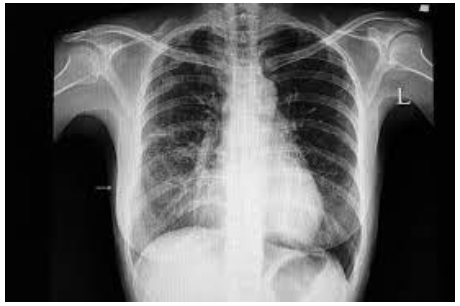
- Multiplex Respiratory PCR panels –

  In one study: The Biofire Respiratory panel found 875 additional targets in 1,764 patients with valid results. Of the **875 additional targets, 25% were positive on culture but below quantitative cut-offs, and the remaining 75% were determined to be true positives through a second molecular test**

  In another study - **sensitivity of 91.7-100% compared to routine microbiology at 27-69%**

  One retrospective multi-center evaluation : 159 pneumonia episodes, results from the Biofire FilmArray Pneumonia Panel potentially would have led to antibiotic de-escalation in 40% of patients, escalation in 22%, and increased appropriateness of therapy to 87% (versus 77% for routine microbiology)

  Well conducted real world RCTs/trials on impact are lacking however.

Murphy CN, 2020. J Clin Microbiol 58:1–20.
Enne VI, 2022. Thorax https://doi.org/10.1136/thoraxjnl-2021-216990
Monard C, 2020. Crit Care 24:1–11.

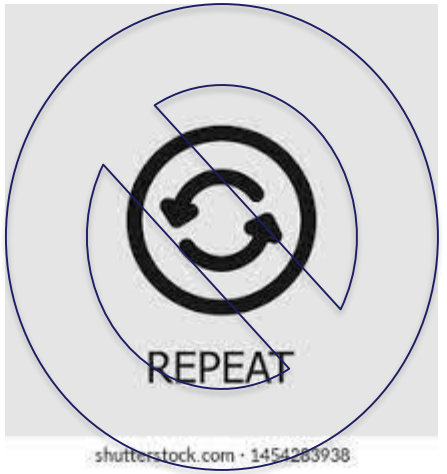
Diagnostic Stewardship in Respiratory Tract Infections

Molecular microbiology

National Centre for Infectious Diseases

- **Avoid unnecessary repeat performance of a multiplex respiratory virus PCR** (e.g., Biofire FilmArray Respiratory Panel) within a 12-hour period yielded an additional 5.6% discordant results, of which only 0.9% (4 of 462 repeats) changed clinical management.

- **Utilization of a negative MRSA nares screen (by either culture or NAAT) to de-escalate empiric anti-MRSA therapy** (e.g., with vancomycin).
  - NPV of 98.1% in CAP and HAP combined compared to 94.8% in VAP.
  - Specificty of 92% versus 88% by NAAT vs culture

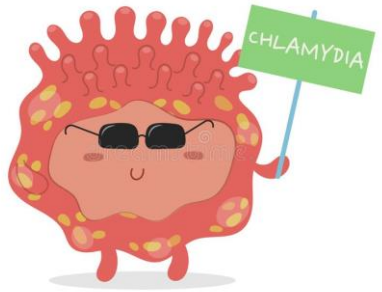


Baghdadi JD, 2022. Diagn Microbiol Infect Dis https://doi.org/10.1016/j.diagmicrobio.2021.115629.
Parente DM, 2018.. Clin Infect Dis 67:1–7.

Diagnostic stewardship in Sexually Transmitted Infections

National Centre for Infectious Diseases

Molecular Microbiology



- **Testing of multiple anatomic sites exposed (rather than a single site)** – one study found that 50% of STIs (chlamydia, gonorrhea, and Trichomonas vaginalis) would have been misse with urogenital testing alone without rectal testing
- strategy incorporating **Be aware of differences in test-of-cure recommendations betwe different guidelines**

US CDC - Not routinely recommended for urogenital or rectal gonorrhea or chlamydial infection after appropriate treatment. Re-test at 3 months as reinfection rates high. Test of cure only if:

- symptoms persist,
- suspicion of poor adherence to treatment regimen,
- suspected re-infection,
- antimicrobial resistance with treatment failure,
- if non-standard treatment regimens are used,
- pharyngeal gonorrhea, pregnancy

European and Australian guidelines recommend a test for cure for all cases of gonorrhea to detect treatment failure and possible antimicrobial resistance . For chlamydia recommend against routine test-of-cure unless:

- first-line treatment regimens were not used.
- pregnant patients,
- complicated infections,
- non-compliance/re-infection is suspected,
- extra-genital infection (particularly when azithromycin 1 g is used in treatment of rectal infection, where failure rates may be higher)

- **High-risk HPV DNA testing is more effective than cytology for primary screening**, with the screening interval being extended to 5 years, and has been found to provide 60-70% greater protection against invasive cervical carcinoma

Jordan NN, 2020 Sex Transm Dis 47:243–245

Diagnostic stewardship in Gastrointestinal Infections

National Centre for Infectious Diseases

Evaluation of Diarrhea – Key Questions

- 1) is the diarrhea **infectious or not**? (rule out enteral feeds and laxative use causing diarrhea prior to microbiologic investigations);
- 2) is the diarrhea **acute or chronic**? (most pathogens identified with routine stool cultures and multiplex gastrointestinal panels represent acute etiologies);
- 3) **are risk factors for C. difficile infection present**? (e.g., if nosocomial onset, admission >72 hours and recent antibiotic receipt);
- 4) **is the diarrhea inflammatory** (e.g., dysenteric) and/or is the patient **ill or at risk of severe illness** (e.g., dehydrated or septic)?; and
- 5) are there **specific host or seasonal factors which are associated with particular etiologies**? (e.g., travel or immunocompromise may be associated with certain parasites).

Diagnostic stewardship in Gastrointestinal Infections

Evaluation of Diarrheae

National Centre for Infectious Diseases

- **Stool cultures or molecular panels should be restricted** to those who have **acute, community-onset diarrhea within <72 hours** of admission given the
    - Lower diagnostic yield beyond that period (~1.4% by culture based methods, and ~3% by molecular methods) , except for certain special hosts (e.g., the immunocompromised)
- **Avoid testing if patients have received laxatives, oral contrast, or been commenced on enteral tube-feeds in the preceding 48 hours**. CDSS or electronic prompts have been successfully deployed to facilitate such diagnostic stewardship efforts
- **New-onset diarrhea who have been hospitalized for >72 hours, should be evaluated for C. difficile by a directed C. difficile assay** given that community causes of diarrhea are unlikely

Specific Gastrointestinal Pathogens

National Centre for Infectious Diseases

- **Diarrheagenic Escherichia coli**

  Shiga-toxin (*stx1*/*stx2*) producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC).

  Rapid detection of STEC

  - Avoidance of antibiotic treatment (risk of HUS)
  - Avoidance of other unnecessary treatments (e.g., surgery or corticosteroids for STEC cases which may mimic a surgical abdomen or inflammatory bowel disease),
  - Timelier public health actions
- **Implications of organisms such as EAEC, EPEC, EAEC, ETEC, and EIEC** – Difficult

  Detection may not necessarily indicate causation, especially if multiple potential pathogens are detected

  May trigger unnecessary treatment

  Interpretive comments should be considered to guide clinicians, for example when ETEC is detected the laboratory report may comment that this is usually self-limited and that antibiotic therapy may not be indicated.
- **Low prevalence organis**ms – need culture confirmation (e.g. toxR gene and *Vibrio cholerae)* - non-cholera *Vibrio* sp may possess the *toxR* homologue

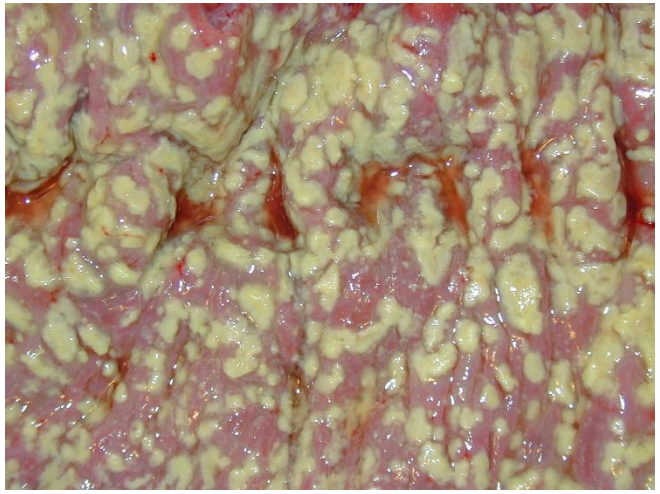
Specific Gastrointestinal Pathogens

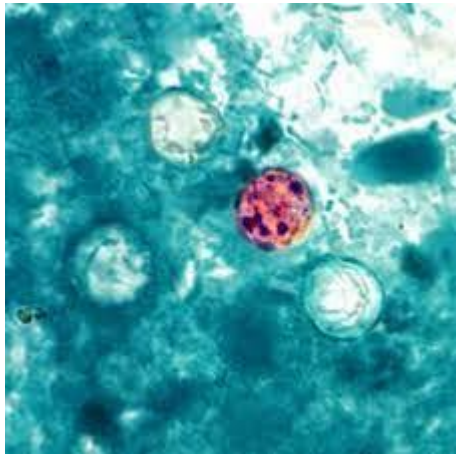
National Centre for Infectious Diseases

- C difficile algorithms : GDH/Toxin EIA +/- → NAAT, vs NAAT upfront
- Multiplex gastrointestinal panels good to excellent sensitivity for panel-included parasites

  may help to detect unexpected outbreaks (e.g., the mid-Western Cyclospora outbreak of 2018) because routine parasitic testing (or specific stains for some parasites) is not performed, or may be helpful when expertise with conventional microscopic methods is lacking.

Bateman AC, 2018. J Clin Microbiol 58:2019–2020.





Diagnostic Stewardship Central Nervous System Infections

National Centre for Infectious Diseases

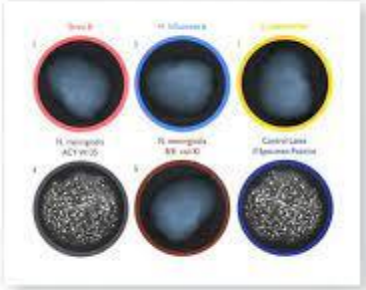
- The laboratory should discourage the use of insensitive tests and encourage providers to use appropriate tests for CNS infection

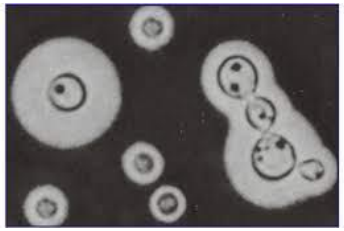
  Latex agglutination (Bacteria) – not sensitive esp with Abx pre-treatment and should not be used

  India Ink stain for Cryptococcus should be sun-set and replaced with Ag tests

  WNV PCR on CSF should be replaced by MAC-ELISA/serology

  - Analytically sensitive (limit of detection 10-100 copies/ml), it is clinically insensitive (4- 7%) due to the short-lived duration of viremia and low levels of WNV in CSF
  - Removal of WNV PCR – one study decrease of 93.5% in test spending, with an increased diagnostic yield from MAC-ELISA when clinicians were using the appropriate test (0 cases diagnosed via NAAT during the study, versus 8 cases diagnosed by MAC-ELISA after the intervention)

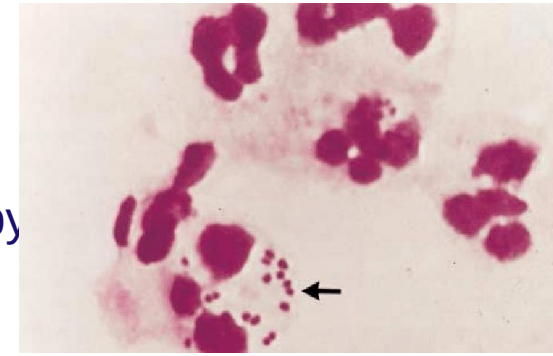




Karaba AH, 2019. T Open Forum Infect Dis 6:2018–2020.

Diagnostic Stewardship Central Nervous System Infections

National Centre for Infectious Diseases



- The European Monitoring Group on Meningococci (EMGM) recommends PCR as essential for the diagnosis of **meningococcal disease**

  Up to 57.1% of 1925 cases of invasive meningococcal disease were only identified by PCR in a study from a UK meningococcal reference unit

- Multiplex PCR (e.g. FilmArray) are sensitive and specific, rapid but …**false-positives and false-negatives have been reported.**

  False-positives : contamination in the pre-analytic phase (e.g., during collection and processing in specimen preparation areas), and included reports of *S. pneumoniae*, *S. agalactiae*, and *Haemophilus influenzae*

  False-negatives have been reported possibly due to in-pouch reagent degradation, antimicrobial treatment (e.g., *Cryptococcus* antigen positive, PCR-negative CSF specimens for patients on antifungal treatment) or a possible higher limit of detection for certain analytes (e.g., HSV)

  HSV sensitivity : a lower sensitivity for HSV-1 at 75-82% for Filmarray : Consider repeat, or use secondary assay if suspicion high

Heinsbroek E. 2013. J Infect 67:385–390.

Lindström J, 2022. Clin Microbiol Infect 28:79–8
Trujillo-Gómez J. 2022. eClinicalMedicine 44..

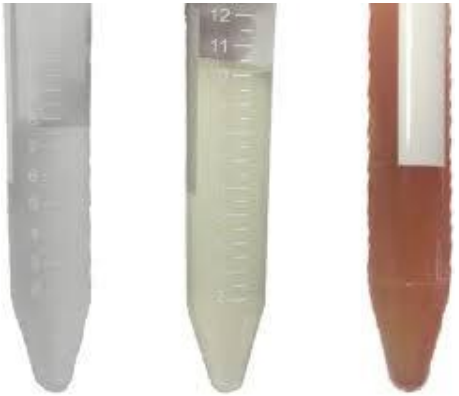
Diagnostic Stewardship Central Nervous System Infections

National Centre for Infectious Diseases

- Selecting appropriate patients for multiplex PCR testing

  In children > 2 years and adults, one study : immunocompetent patients with <10 WBCs in their CSF had a 2% (4/184) positivity rate (1 HHV-6, 1 enterovirus, 2 VZV in patients with concurrent shingles), all of which were not considered clinically significant

  May use - cut-off of >10 cells/mm3 in those aged >2 years and the non-immunocompromised, with the proviso that the clinician is able to still contact the laboratory to request testing if the clinical suspicion remains high

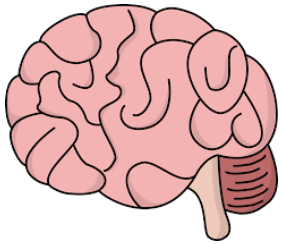


McCreery R, 2022. Diagn Microbiol Infect Dis 102:115605.

Diagnostic Stewardship Central Nervous System Infections

National Centre for Infectious Diseases

Molecular Testing



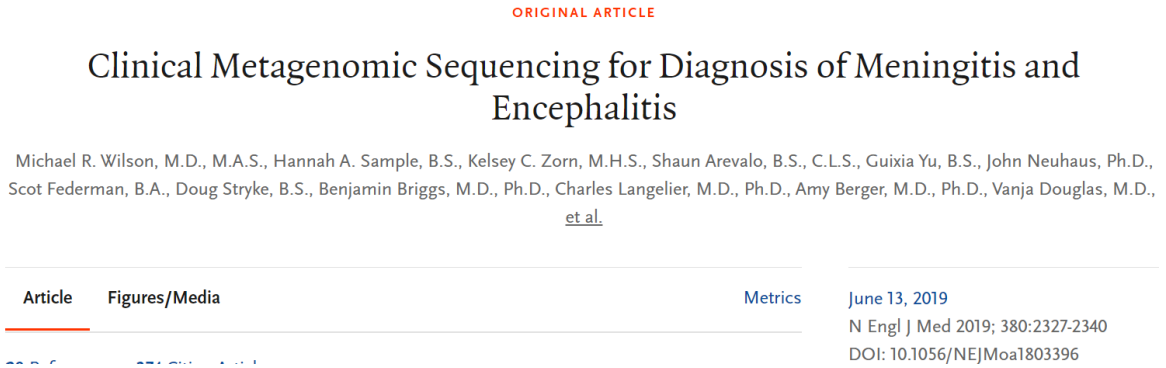
- One study: FilmArray ME Panel with real-time antimicrobial stewardship decision support (weekdays, 8-5 pm) found that :
  - time-to-optimal antimicrobial therapy fell from 28 to 18 hours,
  - time-to-results from 9.6 to 4.8 hours, intravenous antimicrobial duration decreased from 36 to 24 hours, and
  - rates of pathogen identification increased from 10% to 15%,
  - without changes in time-to-effective antimicrobials, admission rates, length of stay, or total hospital costs (although cost of testing increased
- ‘Universal’ approach to using multiplex PCR panels for meningitis : such an approach will likely lead to overuse (up to one-third of ME panels were requested in patients with low likelihood of CNS infection in one study)
- An approach guided by diagnostic stewardship utilizing clinical, demographic, and CSF parameters is likely to be more optimal although further studies are needed.
- multiplex PCR panels cannot be stand-alone tests for meningitis because cultures are required for AST for bacterial causes of meningitis.

Messacar K, 2022.. J Pediatr https://doi.org/10.1016/j.jpeds.2022.02.002.
Duff S, 2019. Infection 47:945–953.
Naccache SN,. 2018.. J Clin Microbiol 56:1–11.

Diagnostic Stewardship Central Nervous System Infections

National Centre for Infectious Diseases

Metagenomic next generation sequencing



ORIGINAL ARTICLE

Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis

Michael R. Wilson, M.D., M.A.S., Hannah A. Sample, B.S., Kelsey C. Zorn, M.H.S., Shaun Arevalo, B.S., C.L.S., Guixia Yu, B.S., John Neuhaus, Ph.D., Scot Federman, B.A., Doug Stryke, B.S., Benjamin Briggs, M.D., Ph.D., Charles Langelier, M.D., Ph.D., Amy Berger, M.D., Ph.D., Vanja Douglas, M.D., et al.

Article Figures/Media Metrics

June 13, 2019
N Engl J Med 2019; 380:2327-2340
DOI: 10.1056/NEJMoa1803396

- Trial of 204 patients with meningoencephalitis without a clear etiology on standard testing,
- 58 infections detected in 57 patients,
- **a mNGS approach identified a pathogen in 13 (22%) infections that was not detected by standard testing**, impacting treatment decisions for about half of these patients
- **However, in the trial, 26 (45%) infections were detected by conventional testing only and missed by mNGS.**

  Of these, 8 misses were secondary to low titers of organism, below the detection threshold of mNGS.
- While promising as a strategy, further work is required to optimize the performance of mNGS and diagnostic stewardship should play an important role to ensure its appropriate use and interpretation (e.g., via mNGS sequencing expert boards)

Wilson MR, 2019. Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis. N Engl J Med 380:2327–2340.

Other areas in diagnostic stewardship where rapid or molecular testing may be deployed

National Centre for Infectious Diseases

Pathogen Specific considerations

- Anaerobic Cultures and Antimicrobial Susceptibility Testing
- Mycobacterial Infections
- Parasitology (e.g. Blood and Tissue Parasites)
- Bloodborne Viral Infections (HBV, HCV, HIV)

Diagnostic Stewardship and the Antimicrobial Stewardship Committee

Diagnostic Stewardship in Infection Prevention and Control and Environmental Testing

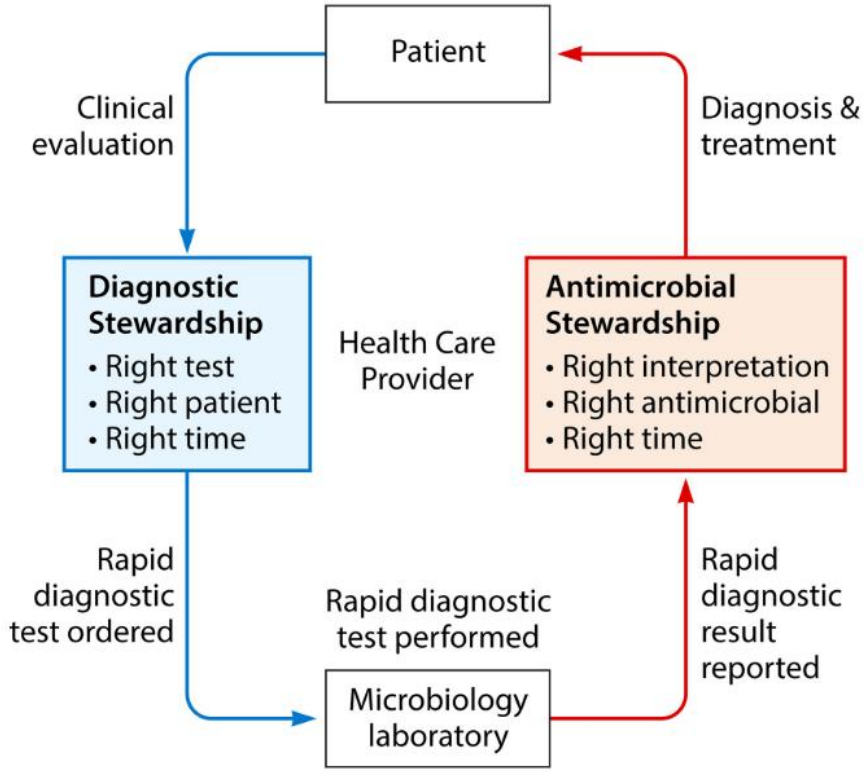


FIG 1 Roles of diagnostic and antimicrobial stewardship in the implementation of rapid molecular infectious disease diagnostics in the clinical setting.

Messacar J Clin Microbiol 55:715–723. https://doi.org/10.1128/JCM.02264-16.

Example of Myanmar HCV treatment programme

National Centre for Infectious Diseases

- Study in Myanmar, community setting in Yangon
- 633 participants
- Rapid POC HCV Ab and RNA testing (Xpert)
- 633 participants, 606 HCV pos (96%), of which 88% RNA pos
- 489 (91%) eligible for HCV DAA
- Retention in care excellent – 477 (98%) completing therapy, 92% SVR

Thingangyun Township, one of the study sites

Even in a resource-limited setting, rapid testing can facilitate retention in care and successful treatment, which should be the end-goal of a successful DSP

Draper BL, et al 2021. Outcomes of the CT2 study: A ‘one-stop-shop’ for community-based hepatitis C testing and treatment in Yangon, Myanmar. Liver Int 41:2578–2589.

Example of diagnostic stewardship research / evaluation @ NCID/TTSH : Detection of CPGNB from surveillance specimens in a Health Systems Development Programme (HSDP) Grant

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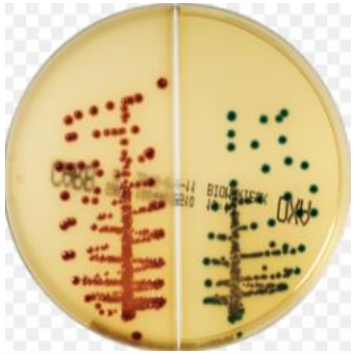

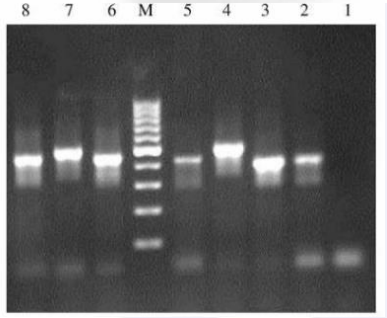

Culture followed by conventional PCR	Direct from Specimen (e.g. GeneXpert)
Longer turn around time Negatives : 1 day Positives : ~ 4 days	Rapid turn around time ~ 1hr
Longer time to isolation, less contacts generated	Shorter time to isolation, more contacts generated
Longer Hands on time	Short hands on time
$	$$
Lower sensitivity potentially	Superior Sensitivity

Lab Workflow: Parallel Testing of CRE culture and Xpert Carba-R (HSDP Phase I)

National Centre for Infectious Diseases

Screening swabs

Rectal swab (eswab)

HSDP funded

- High risk = charge patient
- Contact = charge IC

1. Xpert Carba-R

2. ChromID Carba SMART

Xpert result to be reported in the same accession # for CRE culture screen. Residual E-swab samples with **DETECTED** genotype(s) will be passed to IDRL

All Gram negatives including ***non-coloured colonies** will be identified by MALDI

*Non-Enterobacteriaceae:
- *P. aeruginosa*
- *Acinetobacter* spp. (i.e. A. baumannii, *A. nosocomialis*, *A. lwoffii*)

MEM 'R' **by disk**

Purity plate passed to IDRL

Enterobacteriaceae

Do Vitek

Carbapenem non-sensitive (**I or R**)

In-house PCR

NEG

POS

Clinical Isolates

- Clinical culture with suspect CPE or confirmed CRE
- Meropenem resistant *P. aeruginosa* & *Acinetobacter* spp.

Xpert Carba-R HSDP Funded

POS

NEG

Sent for in-house IMI PCR (done by DLM)

POS

NEG

Purity plate passed to IDRL

PCR positive isolates **OR** Confirmed CRE isolates:
- saved in cryovials for DLM
- Purity plate sent to IDRL

Rev 4.0
31/03//2019

Lab Workflow: Commencement of Phase II with Xpert Carba-R & limited culture screen

Patient / Infection Control to be charged

National Centre for Infectious Diseases

For infection control purposes

Screening swabs

Rectal swab (eswab)

↓

Xpert Carba-R

NEG / POS — **Record under test code MCRES**

Subculture Eswab to ChromID Carba SMART

↓

Identify all Gram negatives including *non coloured colonies by MALDI

*Non-Enterobacteriaceae:
- *P. aeruginosa*
- *Acinetobacter* spp. (i.e. A. baumannii, *A. nosocomialis*, *A. lwoffii*)

↓

MEM 'R' **by disk**

↓

Purity plate passed to IDRL

Enterobacteriaceae

↓

Do MEM disk

↓

MEM zone of inhibition **<28mm** (*EUCAST Epidemiologic cut-off*)

↓

MEM resistant (except VIM): Do in-house PCR targeting the detected Xpert genotype

↓

NEG / POS

NEG → Purity plate passed to IDRL

POS → PCR positive isolates **OR** Confirmed CRE isolates:
- saved in cryovials for DLM
- Purity plate sent to IDRL

Clinical Isolates

- Clinical culture with suspect CPE or confirmed CRE
- Meropenem resistant *P. aeruginosa* & *Acinetobacter* spp.

↓

Xpert Carba-R **HSDP Funded**

↓

POS / NEG / Invalid

POS → PCR positive isolates **OR** Confirmed CRE isolates:
- saved in cryovials for DLM
- Purity plate sent to IDRL

NEG → Purity plate passed to IDRL for IMI follow-up

Invalid → 5-plex in-house PCR

PCR = neg or pos → PCR positive isolates **OR** Confirmed CRE isolates:
- saved in cryovials for DLM
- Purity plate sent to IDRL

Rev 1.0
18/03//2020

Results of HSDP

National Centre for Infectious Diseases

- The Xpert Carba-R was superior to culture in terms of clinical sensitivity
- 24,514 tests from April 2019 – June 2020, and based on a gold-standard of CPGNB positivity defined by a carbapenemase detected by either Xpert Carba-R or the old method of culture followed by conventional PCR
- Clinical sensitivity of the Xpert Carba-R was 94.2% (95% CI 92.1-95.8%), and that for culture was 43.5% (95% CI 39.6-47.4%). The specificity for both was 100% (99.9-100%).

	Chrom +	Chrom =	Total
PCR +	279	326	605
PCR =	37	23,872	37
Total	316	326	642

Table 1. Overall comparison of GeneXpert and CHROMID Carba Smart

Variable	Year 2019									Year 2020						Total
	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	
Xpert detected	39	38	56	54	49	40	40	29	64	68	70	41	17	27	16	648
Xpert Not detected*/but culture positive	3	5	1	3	3	4	2	3	4	2	0	2	0	0	0	32
Culture positive	13	20	26	31	22	24	22	14	34	37	46	18	2	7	1	317
Culture negative but Xpert detected	28	23	32	27	31	20	19	17	34	33	26	15	4	9	6	324
Culture rejected but Xpert detected	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Culture not done but Xpert detected	0	0	0	0	0	0	0	0	0	0	0	11	11	11	9	42

*including Xpert failed, inconclusive, indeterminate

2. Sample-by-sample comparison of discordant results between Xpert and culture method

Table 3. Comparison of discordant results between April 2019-June2020

		CHROMID carba smart-based genotypes																	
		NDM	OXA	IMP	IMI	VIM	KPC	IMP/ NDM	IMP/ OXA48	NDM/ VIM	NDM/ KPC	NDM/ OXA48	VIM/ NDM	VIM/ OXA48	NDM/ VIM/ IMP	NDM/ VIM/ OXA48	Not detected	Others	Culture not done	Total (Xpert)
Xpert result	NDM	45	0	0	1	0	0	0	0	0	0	1	0	0	0	0	114	1#	5	167
	OXA48	0	189	0	0	0	0	0	0	0	0	0	0	0	0	0	77	1	29	296
	IMP	0	1	10	1*	0	0	0	0	0	0	0	0	0	0	0	87	0	2	101
	IMI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	VIM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	0	0	29
	KPC	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	8	0	2	17
	IMP/NDM	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	4
	IMP/OXA	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	6
	NDM/VIM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	NDM/KPC	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2
	NDM/OXA-48	1	6	0	0	0	0	0	0	0	0	9	0	0	0	0	2	0	3	21
	VIM/NDM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	VIM/OXA48	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
	NDM/VIM/IMP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	NDM/VIM/OXA48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	Not detected	3	7	0	18	0	1	0	0	0	0	0	0	0	0	0	Not collected	0	0	32
	Others**	1	2	0	1	0	0	0	2	0	0	2	0	0	0	0	0	0	0	6
	Total (Culture)	51	211	10	21	0	9	0	2	0	0	12	0	0	0	0	326	2	42	686

Culture record of one VIP patient was not accessed

*IMP were noted from culture results from December 2019 onwards

**Others include inconclusive, indeterminate, and failed tests

#VIP patient-unable to access medical record

Results of HSDP

National Centre for Infectious Diseases

Time to detection of CPGNB within 24 hours was achieved for 92%-100% of the time, compared to culture-based methods which took an average of 4 days. Median time to detection (from sample collection till resulting) was in general between 3-4 hours (increasing to ~10 hours during the height of COVID-19), compared to culture ~ 114-117 hours (~4 days).

Time to implementation of isolation precautions : Transfer to single or cohort isolation (from sample collection to resulting) ranged from 20.6-22.8 hours overall for patients who required a transfer.

Technologist Hands on Time: Based on time measurements (3 medical technologists, average timing), the Xpert Carba-R required 2 min and 8 seconds hands-on-time, compared to culture + conventional PCR with a total hands-on-time of 41 min and 51 seconds.

Results of HSDP

National Centre for Infectious Diseases

- Additional contacts reduced by Xpert . Xpert Carba-R averted a total of 7415 contacts (for concordant specimens) and 23,135 contacts (for discordant specimens) from April 2019 to June 2020.

Number of additional contacts prevented by GeneXpert

Table 2. Total additional contact reduced by GeneXpert

Variable	Year 2019									Year 2020						Total
	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	
Additional Contacts until date of culture	550	475	532	773	645	415	436	278	638	756	1030	455	66	268	98	7415
Additional contacts until date of discharge for CHROM ID negative subject (discordant)	2350	1444	2220	1571	1489	672	1261	1450	4501	2287	1660	875	415	537	403	23135

*Between March 2020 and June 2020, 42 patients with CPO by Xpert PCR had no culture done. These patients were excluded from additional contacts analysis

Results of HSDP

National Centre for Infectious Diseases

- CPGNB clinical infection rates estimated from the number of CPGNB positive cultures from clinical samples (hospital-onset) have ranged from 0.6 – 2.0 cases per 10,000 patient days in the HSDP period.

- CPGNB detection rates: From April 2019-June 2019, 1.0-1.4% of all swabs, on a monthly basis, July 2019-June 2020, 0.9-2.4%, and July 2020-June 2021, 0.9-2.4%.

Objectives of the analysis

1. To study the **effectiveness** of rPCR to identify if rPCR screening reduces in-hospital bacteria transmission & infection as compared with conventional culture + PCR screening

2. To analyze the **cost effectiveness** of selective screening for CRE using:

   - rPCR vs.
   - Conventional culture screening vs.
   - No screening

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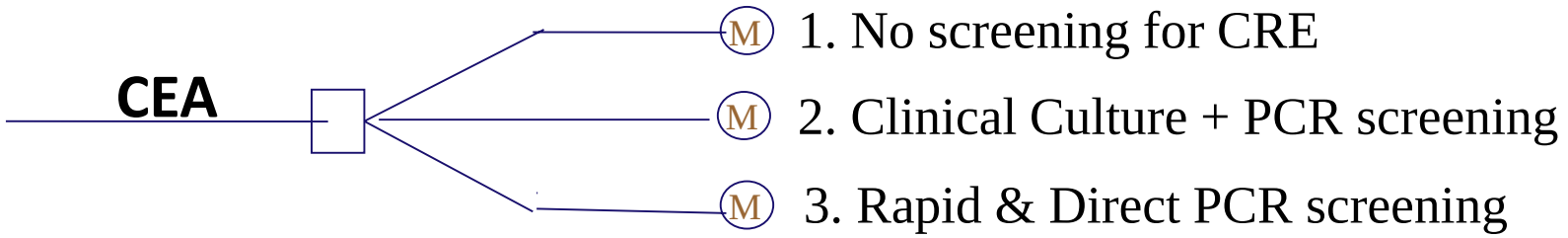
Study method

Study method: Cost effectiveness analysis (CEA) using a linked infectious transmission model with Markov modeling; and microsimulation for probabilistic sensitivity analysis (PSA):

1. 2-compartment deterministic susceptible-infected (SI) model
2. Markov state transition model
3. Monte Calo microsimulation for patient level sensitivity analysis



Cost analysis: Payer perspective

1. Cost data are charges to patients/payers before subsidy;
2. Only medical cost is included in this analysis

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Study patients

Study patients:

1. Actual cohort to derive simulation parameters: 25K patients in 3 months
2. Simulated cohort of admitted patients in 1 year (~100K)

Selective screening:

1. Upon admission, only patients who meet high-risk criteria are selected for screening
2. During hospital stay, patients identified as contacts of colonized patients will also be selected for screening
3. No screening for known positive patients. Contact precautions applied for them after admission

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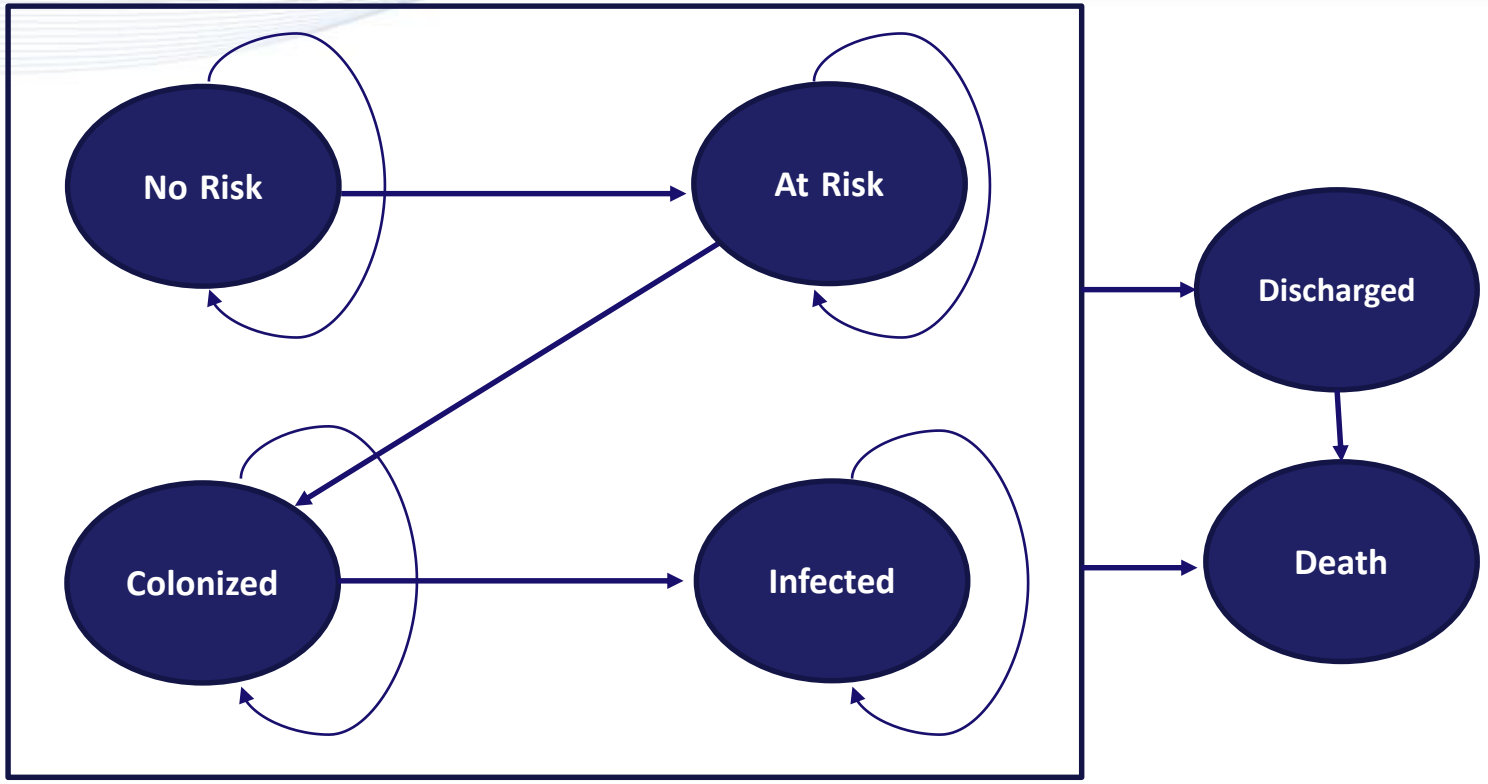
Markov model – dynamic state transition model



States: 6

Modeling period:

1. from admission to either discharge (alive/death); maximum 60 days (99% cases discharged within 60 days) – model cycle: daily
2. From discharge to death (lifelong) – once off modeling

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Model measurements

- **Cost**: S$
- **Effectiveness**:
  - QALY (quality adjusted life years)
  - No of patients screened;
  - No. of colonized and FN colonized patients;
  - No. of hospital acquired infections;
  - No. of deaths due to infection;
- **Cost effectiveness**: ICER (incremental cost effectiveness ratio)= Δcost/ Δeff

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Model parameters: base & range

Prob. of	Base value	Distribution
Prevalence at admission	1.6%	constant
No-risk to at-risk	27.8%	constant
At-risk to colonization	2%	constant
R0	1	Uniform [0.5, 1, 1.5, 2]
Col to infection	3.8%	Constant
Mortality non-infected	1.9%	constant
Mortality infected	15%	Uniform [5%,10%,15%,20%,25%,30%,40%,50%]
LOS non-infected	7 days	Gamma(3.0,2.0)
LOS infected	45 days	Gamma(7.5,1.0) + 18
Sensitivity culture	0.47	Truncated normal (0.44-0.5)
Specificity culture	0.93	Truncated normal (0.89-0.95)
Sensitivity PCR	0.99	Truncated normal (0.98-1)
Specificity PCR	0.96	Truncated normal (0.94-0.98)

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Model parameters: base & range

Cost	Base value	Distribution
Culture screening + PCR confirm	38.4	Constant
PCR screening	91.6	Constant
Contact tracing	20	Uniform [10,20,30,40,50]
General ward	300	Constant
Isolation ward	600	Constant
Colonization precaution	60	Uniform [40,50,60,70,80]
Infection treatment	2000S$	Uniform [1000,2000,3000,4000,5000]
Utility non-infected	0.8	Truncated normal (0.75-0.85)
Utility infected	0.6	Truncated normal (0.55-0.65)
Utility discharge	0.9	Truncated normal (0.85-0.95)

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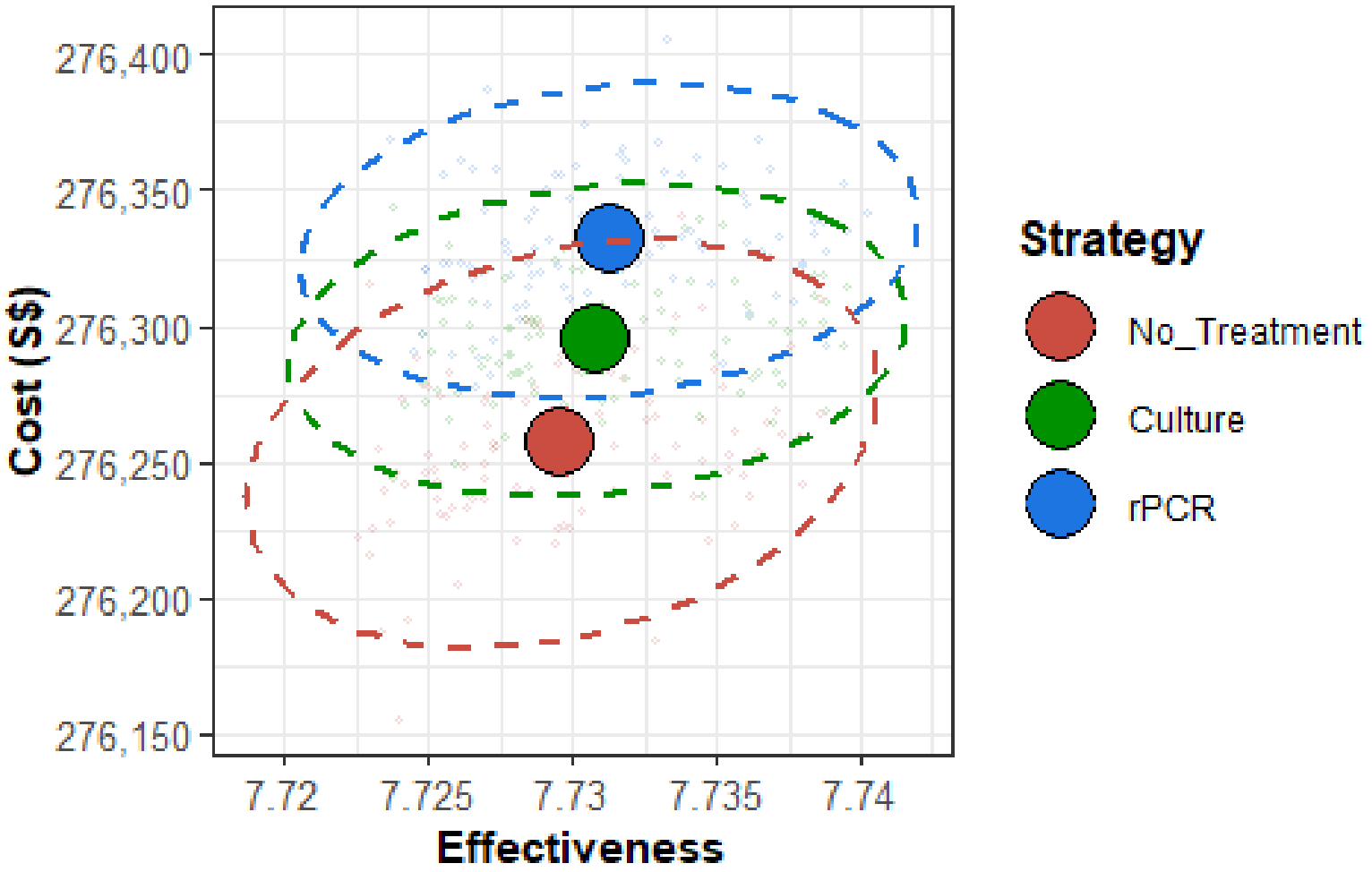
Cost Effectiveness Distribution



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Cohort size: 100K * Bootstrap sampling: 100 = 10 Mil

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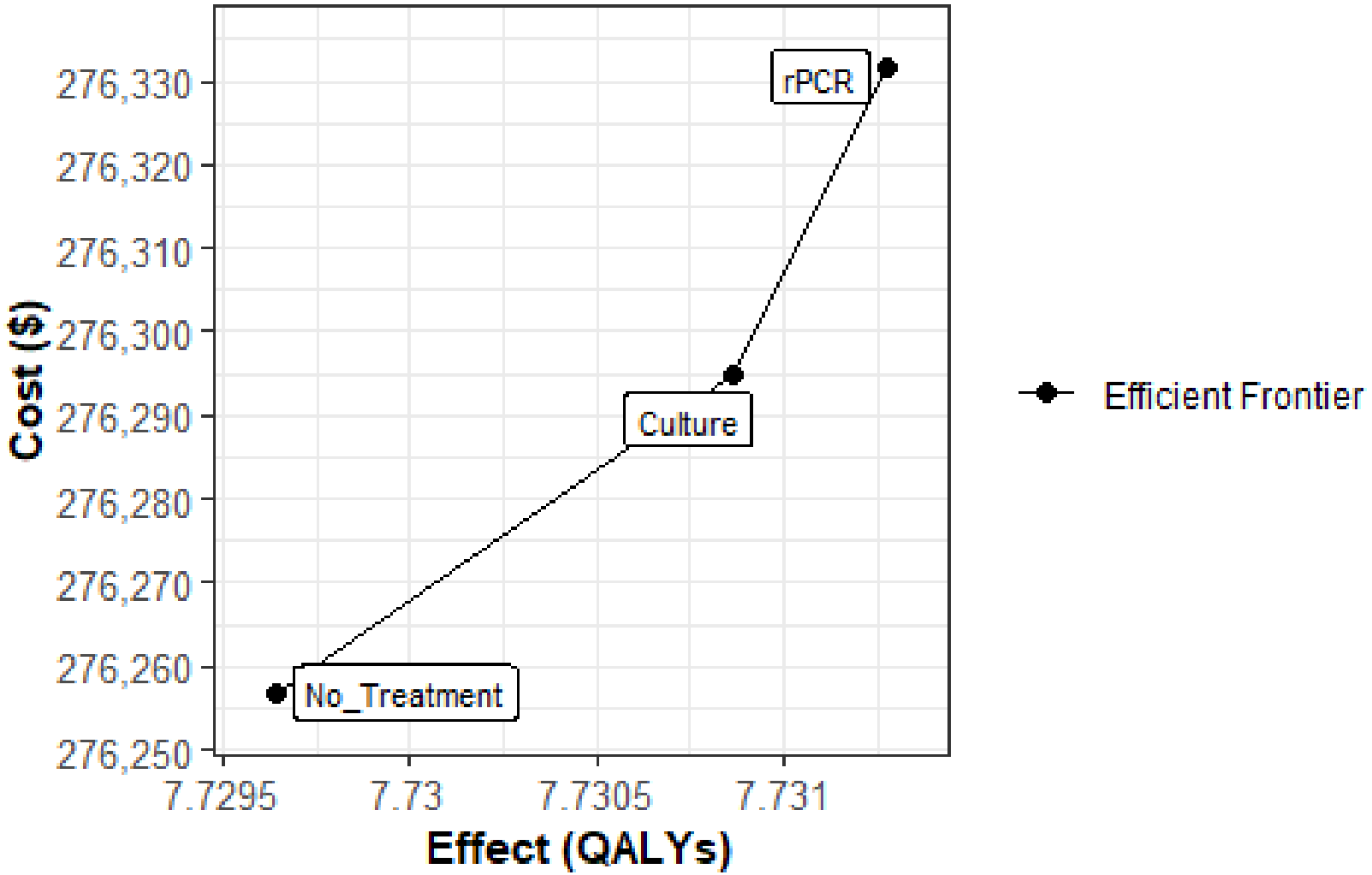
Incremental Cost Effectiveness Ratio (ICER)



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Simulation results with updated base parameters

Approach	Cost per patient	QALYs per patient	Total Psv+	Total coloniz	Total Infect	Total death	ICER (S$/ QALY)
No screening[1]	S$276.26K	7.7296	3947	0	246	2122	
Culture[1]	S$276.30K	7.7309	2631	629	119	2106	**31,184**
rPCR[1]	S$276.33K	7.7313	1228	835	75	2101	**88,406**
Actual cohort in 3 months[2]				171	18	417	
Actual cohort in 1 year reference				**684**	**72**	**1668**	
Difference in 1 year[1] rPCV-Culture	**↑3.66M (tot cost)**		**↓1403**	**↑206**	**↓44**	**↓5**	

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[1]Simulation cohort: 100K with ALOS of ~7 days

[2]Actual cohort of 25K in 3 months with ALOS of ~7 days.

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How to determine the best strategy

1. Cost effective acceptance curve (**CEAC**): measure the proportion of all simulation samples in which a given strategy is cost effective (highest probability of winning)

2. Cost effective acceptance frontier (**CEAF**): usually overlaid on top of CEAC, shows the strategy with the highest expected net benefit as cost effective (highest net benefit)

3. Expected Loss of information Curve (**ELC**) (lowest expected loss when the chosen strategy is suboptimal). It's arguably a more useful representation of uncertainty than CEAC/CEAF.

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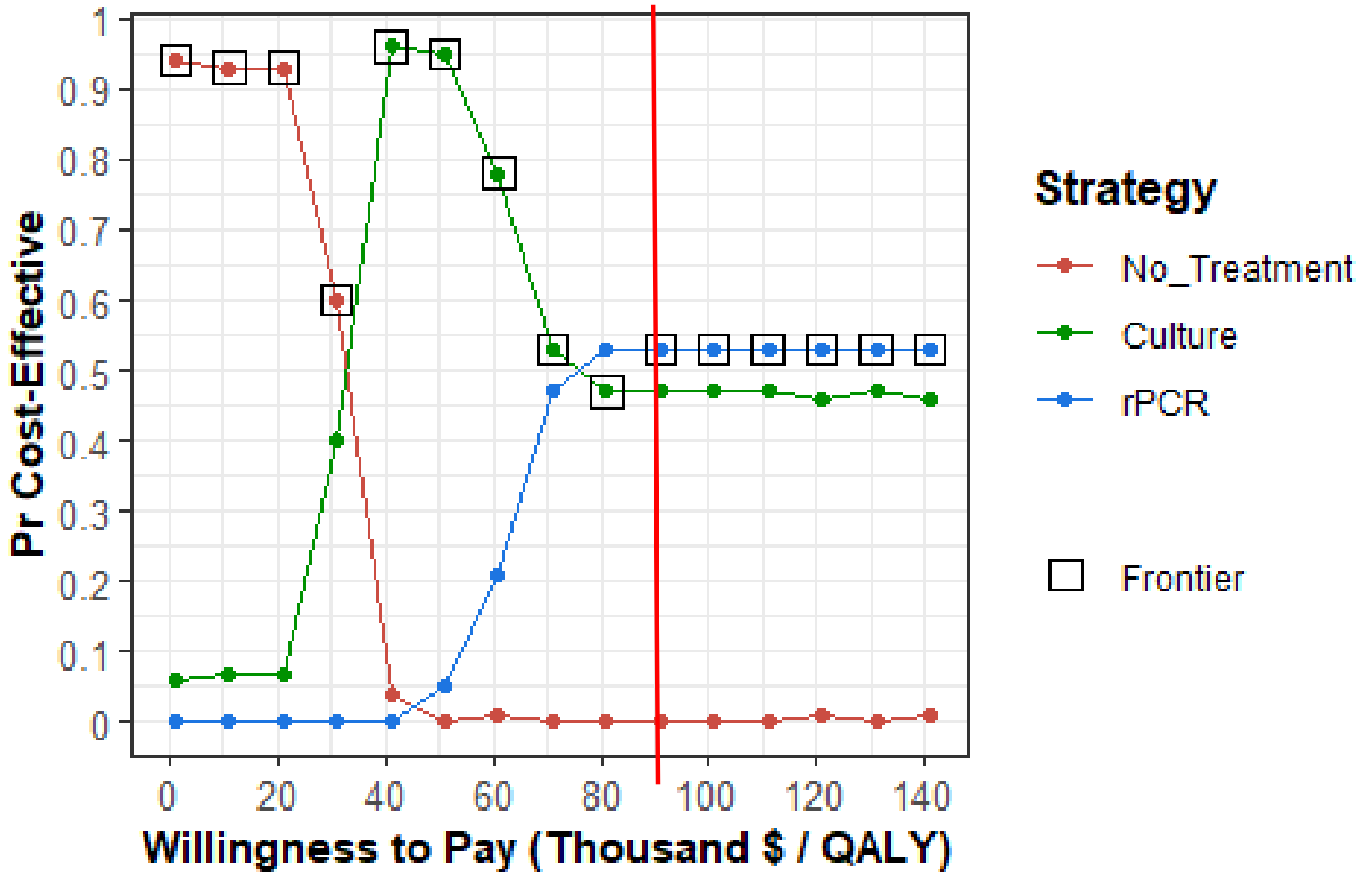
Cost Effectiveness Acceptability Curve (CEAC) and Frontier



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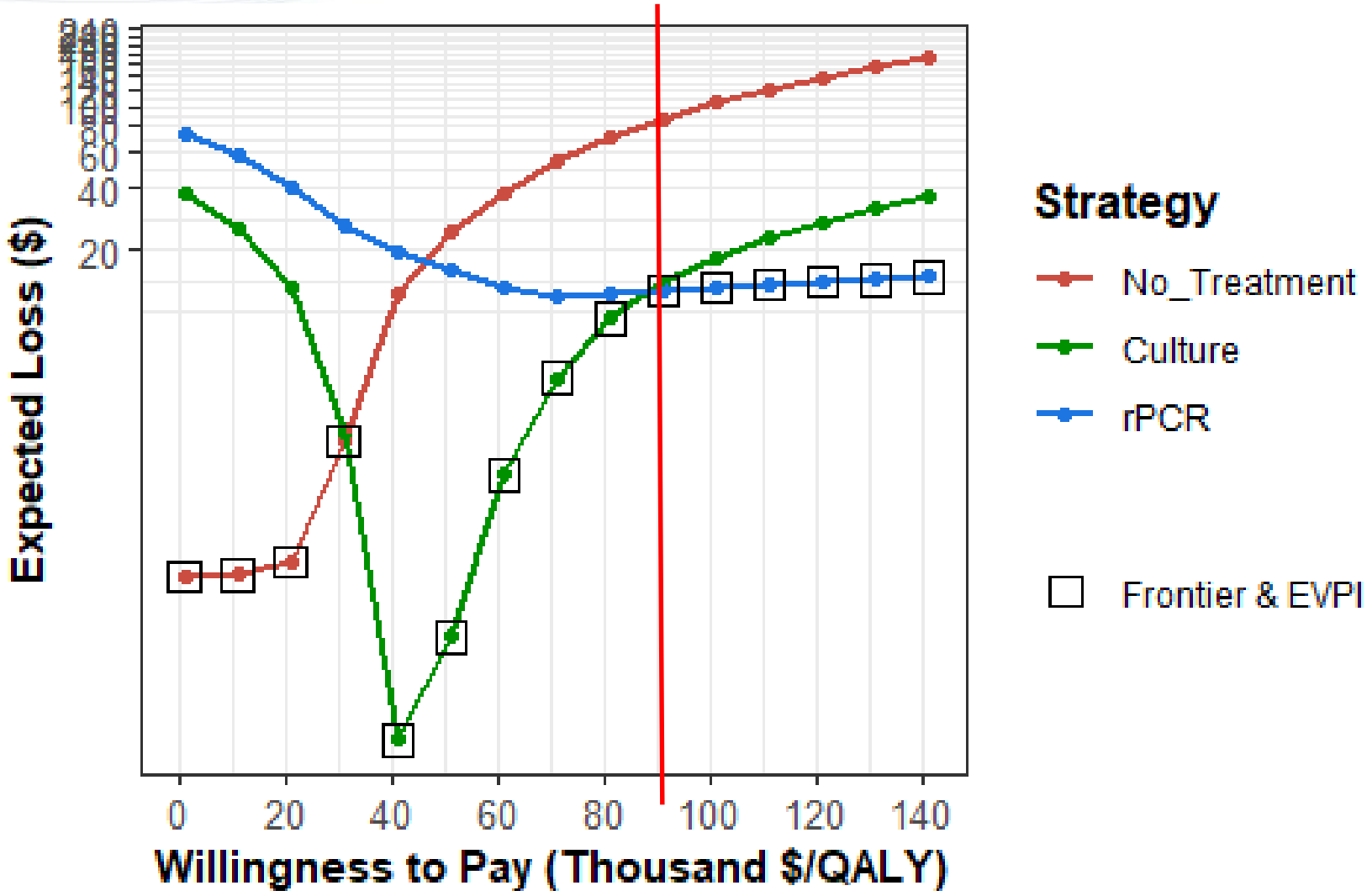
Expected Loss Curve (ELC)



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Simulation results: summary

1. rPCR achieved the best effectiveness, it can reduce ~1403 more positive cases, ~44 more infections and save ~5 more death compared with culture screening at an extra cost of 3.66Mil per year;
2. rPCP screening is the most cost effective approach compared with no screening and culture screening with an ICER of 88,406 S$/QALY gained (given WTP = DGP per capita in SG is S$90K).
3. The cost effectiveness is sensitive to the uncertainty in parameter estimation. Probabilistic sensitivity analysis is recommended.

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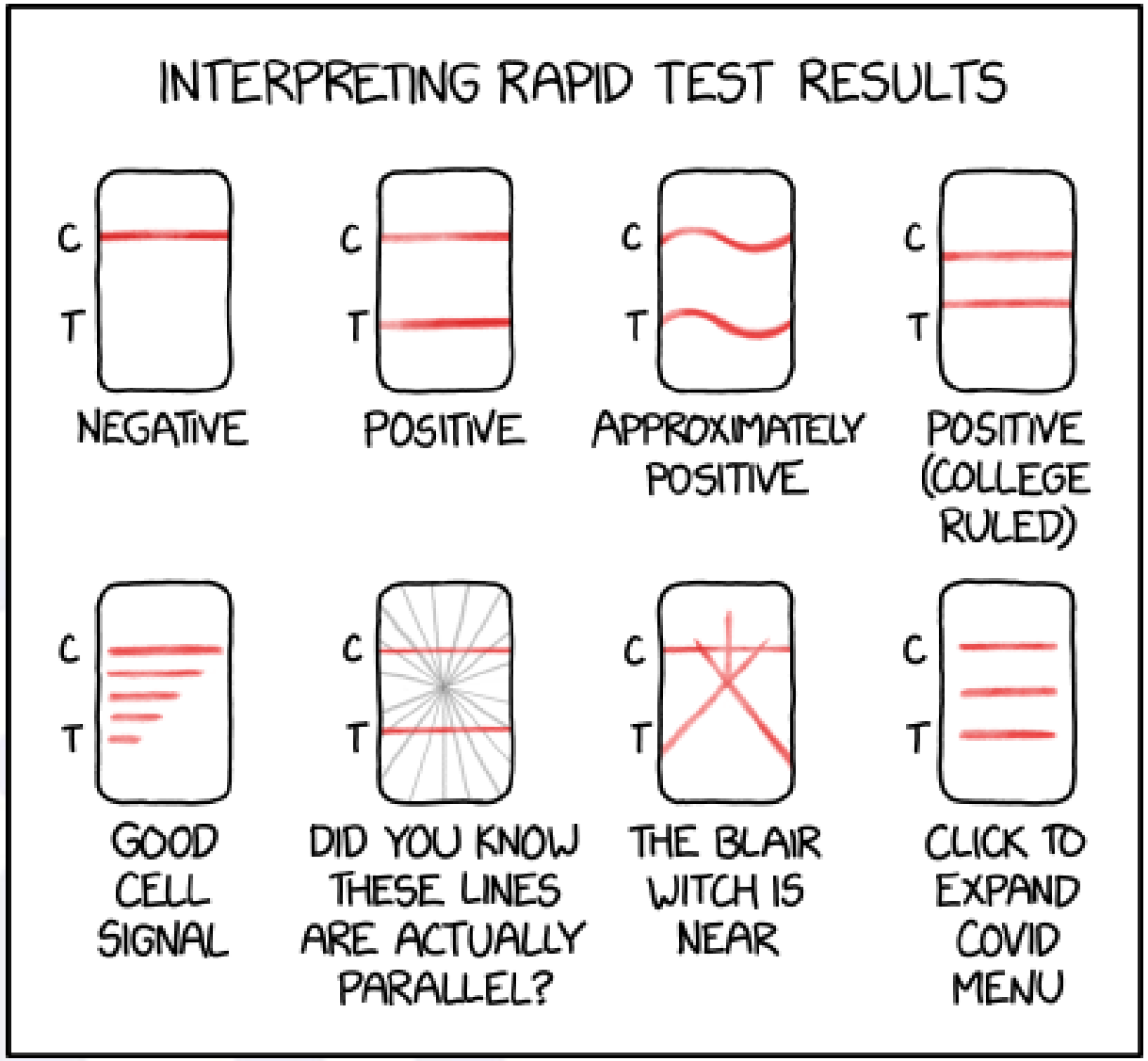
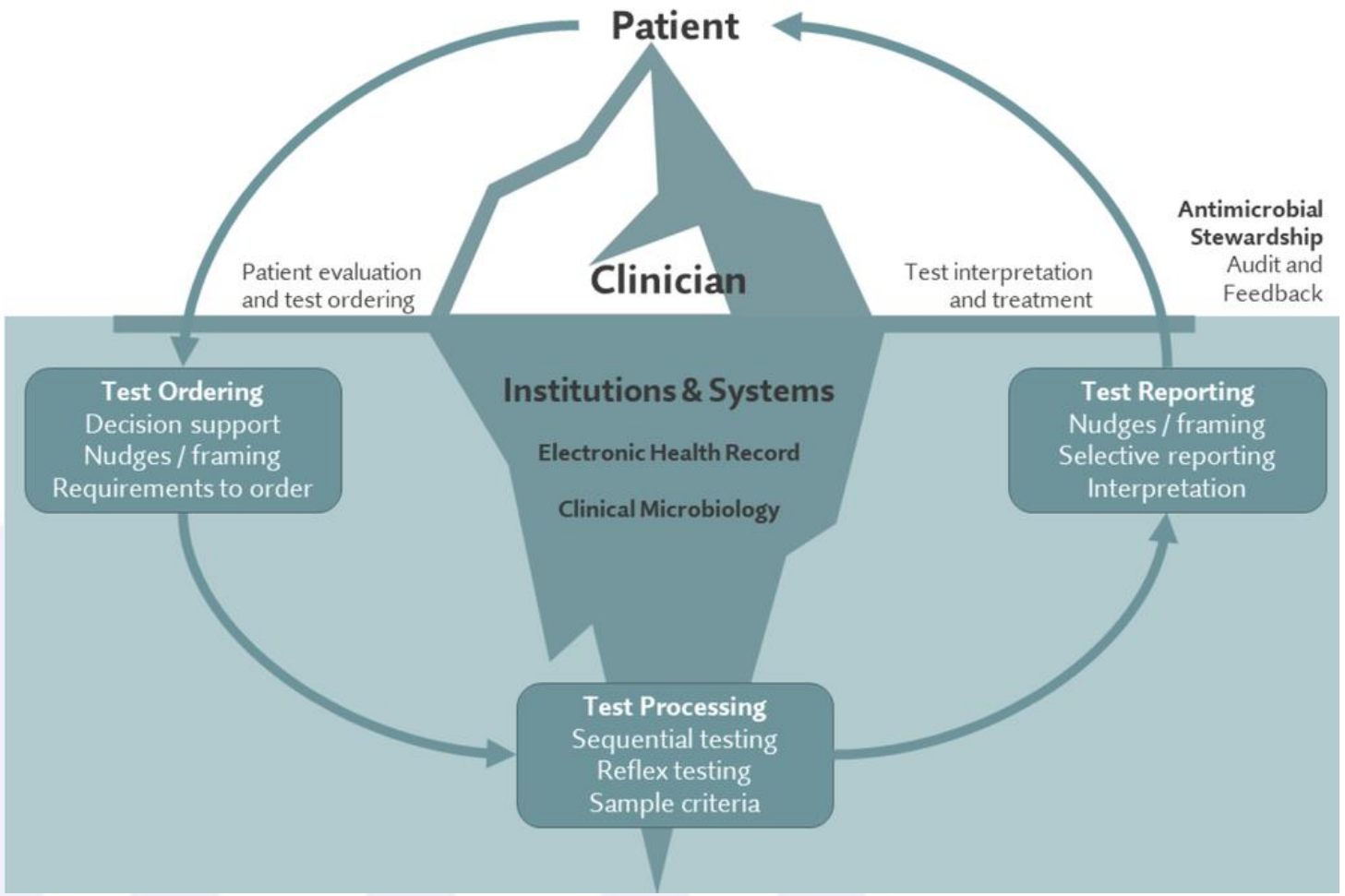


https://www.explainxkcd.com/wiki/index.php/2558:_Rapid_Test_Results

In summary

- (Warning busy slide ahead) ….



https://www.testingwisely.com/diagnostic-stewardship

Facets of a diagnostic stewardship program	Notes
Goals	That the right test is performed at the right time, for the right patient, with the right interpretation and ensuing right action, whilst ensuring value (value may be defined as the measured improvement in health outcomes for the cost spent for that improvement) Implementing evidenced-based practice in clinical microbiology where attention is paid to both accuracy and optimal patient outcomes
Composition	A multidisciplinary team comprising laboratorians, infectious diseases physicians and other clinicians, pharmacists, infection prevention and control (IPC) practitioners, informatics and information technology (IT) specialists

Tools (the 'how')	Preanalytic
	• Removal or introduction of tests
	• Clinical decision support system (CDSS), changing order options
	• Display costs
	• Minimum reordering interval and prevention of duplicate orders
	• Development of diagnostic algorithms
	• Laboratory formularies with test restriction/requiring prior authorization
	• Hold back orders for review prior to authorization
	• Education: formal laboratory communications, informal or ad-hoc communications
	• Laboratory test utilization committees
	• Test reimbursement policies (e.g., insurance reimbursement policies)
	
	Analytic
	• Clear specimen rejection and criterion
	• Reflex testing
	• Reflective testing
	• On-demand vs batch testing
	• Test selection and implementation and factors which influence:
	  • Clinical and analytic sensitivity/specificity
	  • Predictive values
	  • Cost
	  • Test volumes
	  • Feasibility
	• Cascade testing and reporting (e.g., antimicrobial susceptibility testing)
	
	Post-analytic
	• Interpretive comments
	• Templated comments (e.g., about test rejection, test performance)
	• Expert guidance (e.g., infectious diseases physician and/or laboratory consultation, microbial sequencing boards)
	• Provision of utilization report cards
	• Utilization of adjunct tests to distinguish colonization from infection
	• Laboratory notifications of time-sensitive actionable results and reporting times

Interactions	• Institutional leadership • Stakeholders of test(s) (i.e., clinicians and patients) • Microbiologists and laboratory medicine specialists • Infectious diseases physicians • Antimicrobial stewardship program (ASP) teams • Infection Prevention and Control (IPC) teams • Pharmacy • Nursing • Other non-infectious disease diagnostic stewardship programs in the institution • Various sites of implementation (e.g., emergency department, primary care, inpatient care, long-term care facilities)
Priority areas	• Discontinuation of tests of low/no value • High cost or high-volume tests with questionable clinical value • Common clinical syndromes, diseases or pathogens with high clinical impact (e.g., morbidity or mortality) for which an accurate and/or a time-sensitive diagnosis is needed
Measuring impact	• Test utilization rates and appropriateness • Duplicate test rates and minimum re-ordering intervals • Timings (i.e., test turn-around-times but also decreased pre- and post-analytic delays, and time to appropriate down-stream actions such as appropriate therapy or institution of infection control measures, and also timing of diagnostic sampling) • Proportion of patients receiving appropriate therapies • Cost savings from prevention of unnecessary testing • Patient outcomes • Cost-effectiveness, cost-benefit or cost-utility analyses • Reports/dashboards • Dissemination and implementation of a science-based framework for sustainability

CLSI GP49: Laboratory test utilization management program
CLSI. Principles and Procedures for Blood Cultures. 2nd ed. CLSI guideline M47-Ed2.

WHO-GLASS : guide on establishing a DSP in the context of antimicrobial resistance and surveillance

CDC Laboratory Medicine Best Practices (LMBP) initiative's systematic review and meta-analysis on eight key practices impacting test utilization (https://www.cdc.gov/labbestpractices/pdfs/816.full.pdf)

IDSA/ASM guidance on utilization of the microbiology laboratory 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis 67:e1–e94.

Lang T, Croal B. 2021. National minimum retesting intervals in pathology. R Coll Pathol. www.acb.org.uk.

Manual of Clinical Microbiology, 13th Edition (Forthcoming) :New Chapter on Diagnostic Stewardship

Fatemi Y, Bergl PA. 2022. Diagnostic Stewardship: Appropriate Testing and Judicious Treatments. Crit Care Clin 38:69–87
Morgan DJ, Malani P, Diekema DJ. 2017. Diagnostic stewardship - leveraging the laboratory to improve antimicrobial use. JAMA - J Am Med Assoc 318:607–608.
Messacar K, Parker SK, Todd JK, Dominguez SR. 2017. Implementation of rapid molecular infectious disease diagnostics: The role of diagnostic and antimicrobial stewardship. J Clin Microbiol 55:715–723.
Patel R, Fang FC. 2018. Diagnostic Stewardship: Opportunity for a Laboratory-Infectious Diseases Partnership. Clin Infect Dis 67:799–801.
Baird G. 2014. The laboratory test utilization management toolbox. Biochem Medica https://doi.org/10.11613/BM.2014.025.
Dik JWH, Poelman R, Friedrich AW, Panday PN, Lo-Ten-Foe JR, Assen S Van, Van Gemert-Pijnen JEWC, Niesters HGM, Hendrix R, Sinha B. 2016. An integrated stewardship model: Antimicrobial, infection prevention and diagnostic (AID). Future Microbiol 11:93–102.

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Thank you