Diagnostic Solutions For The Clinician: Newer Paradigms in the Management of Infections

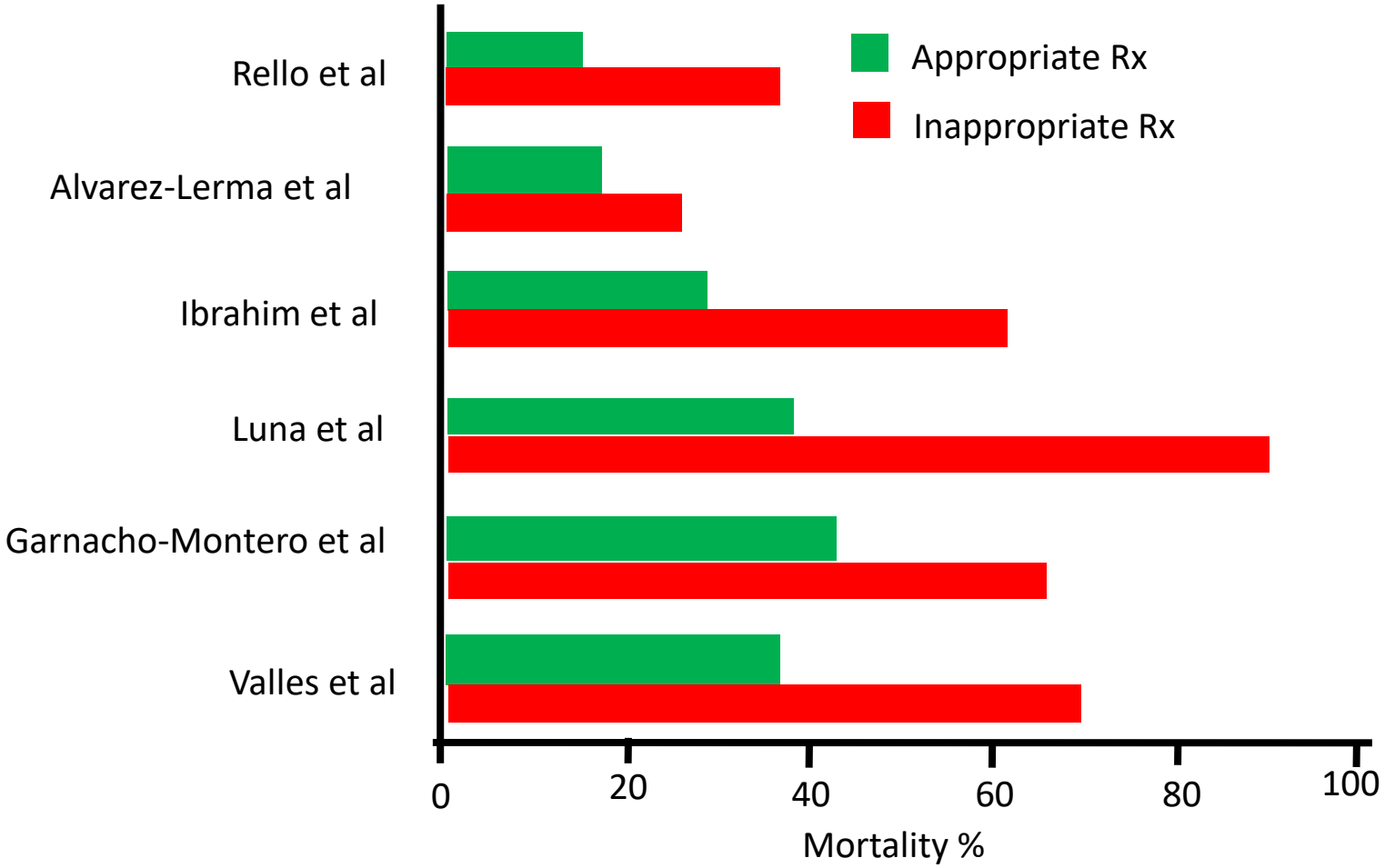
Dr Ramasubramanian

3rd Edition 2024
CAHOLABCON

Disclosures…

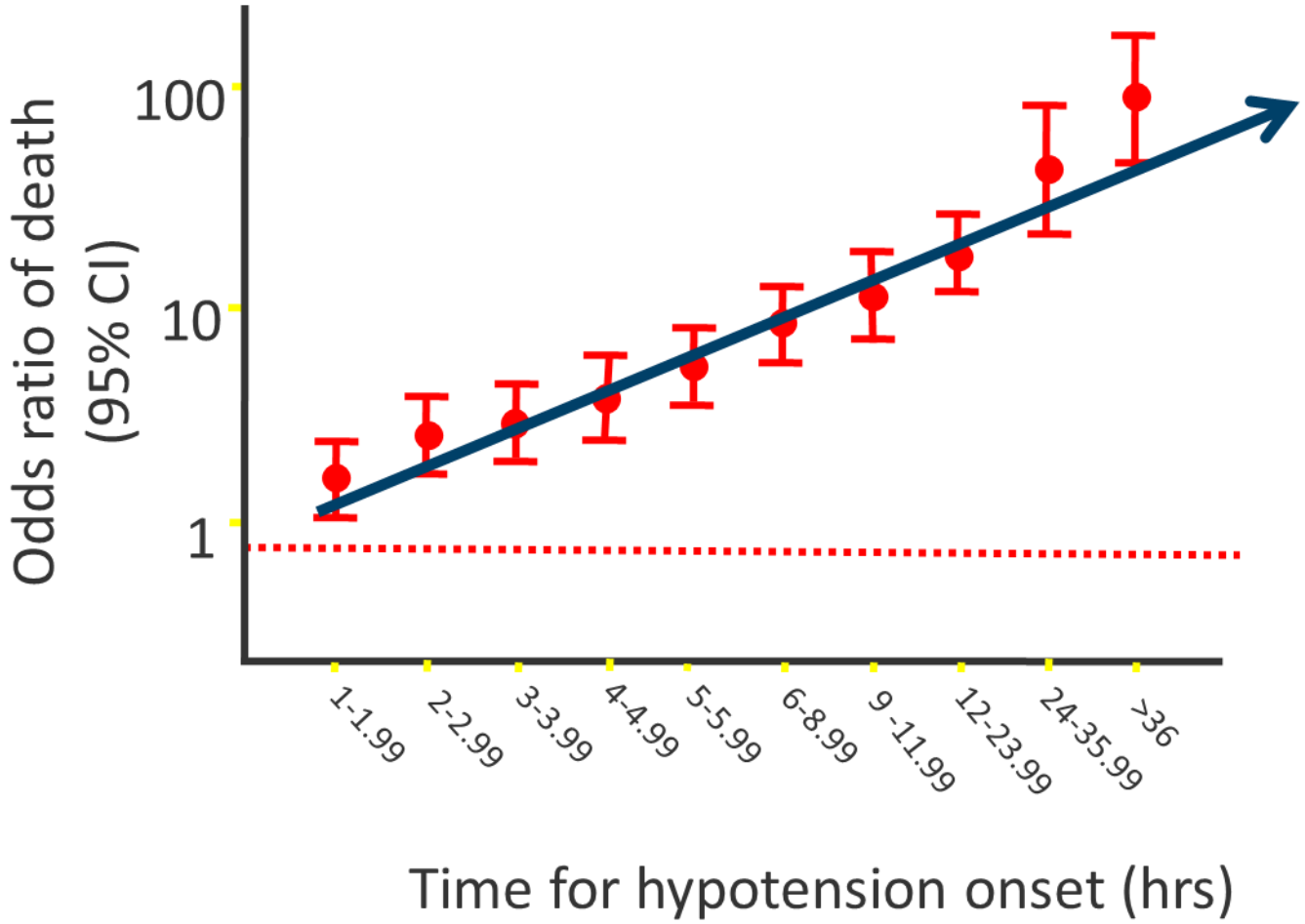
- No Conflict of Interest

Select the Appropriate antibiotic



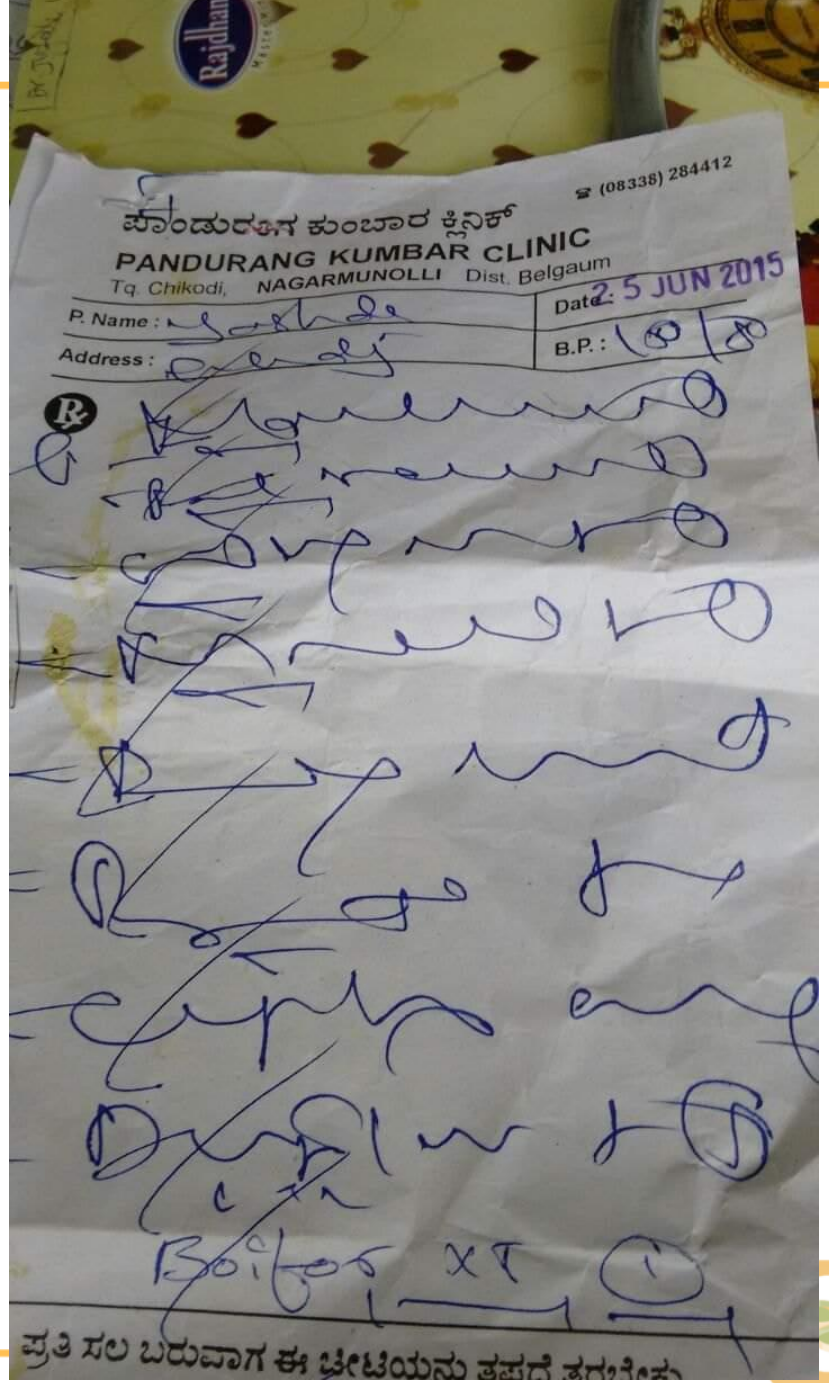
Modified from - Masterton RG. International Journal of Antimicrobial Agents 33 (2009) 105.e1–105.e8

Timing of Antibiotics in the Critically Ill



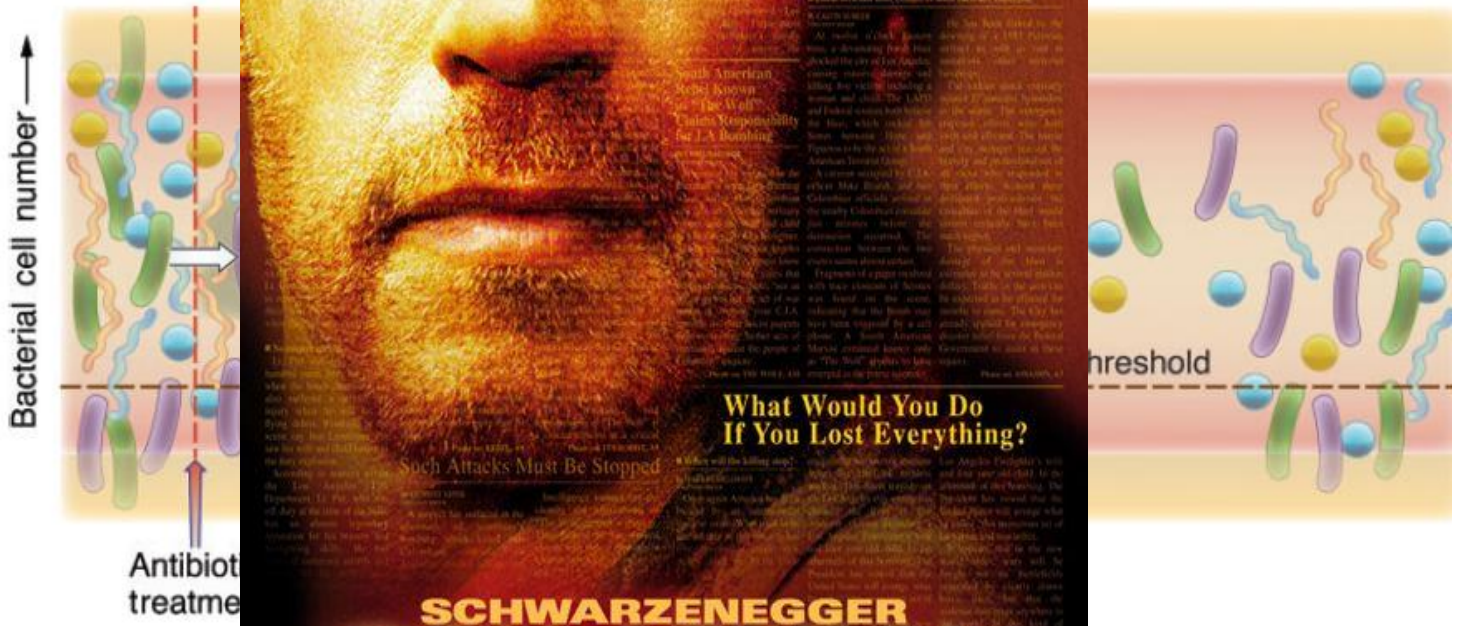
Modified from Kumar A, et al. *Crit Care Med.* 2006;34:1589-1596

Our Response...

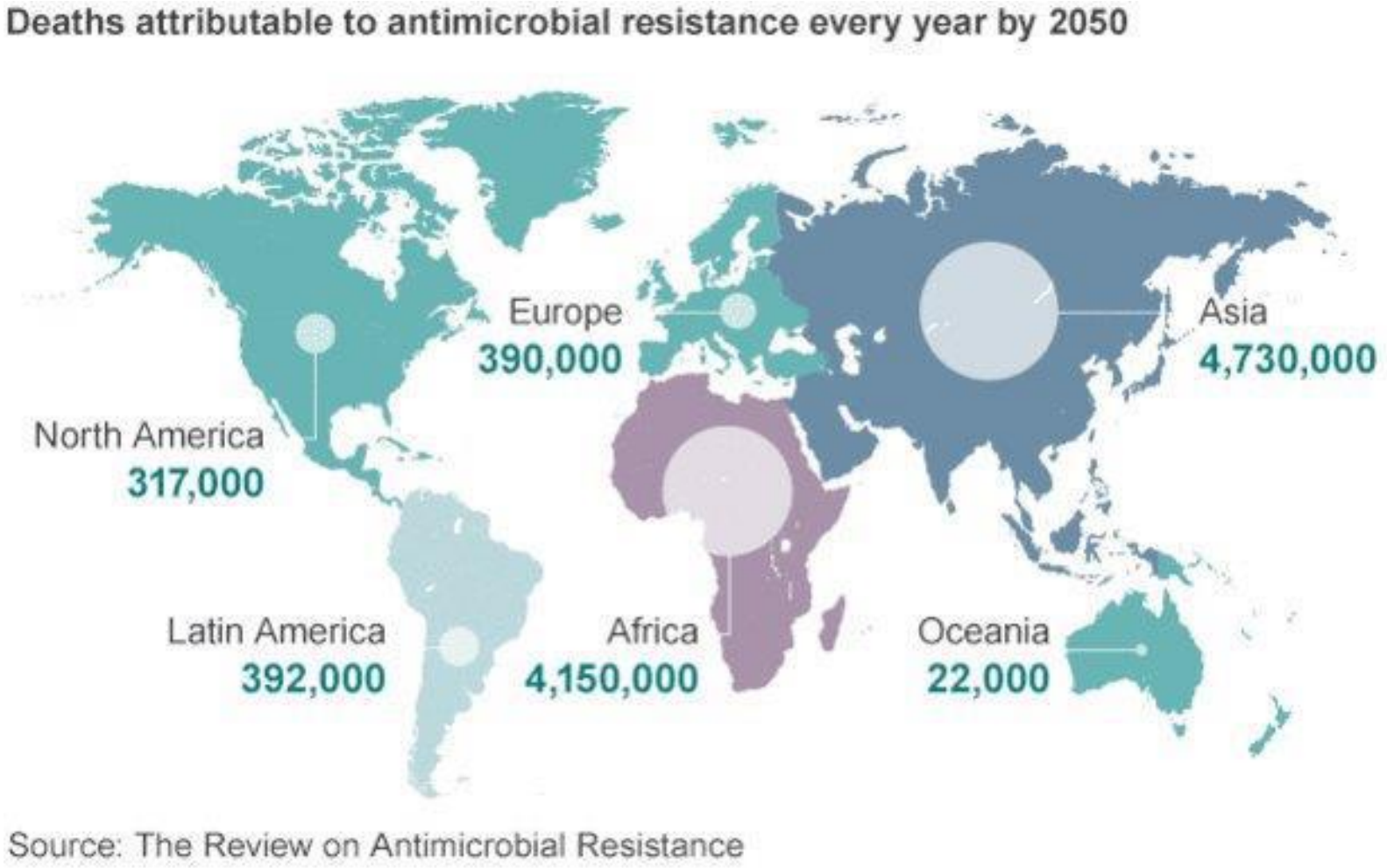


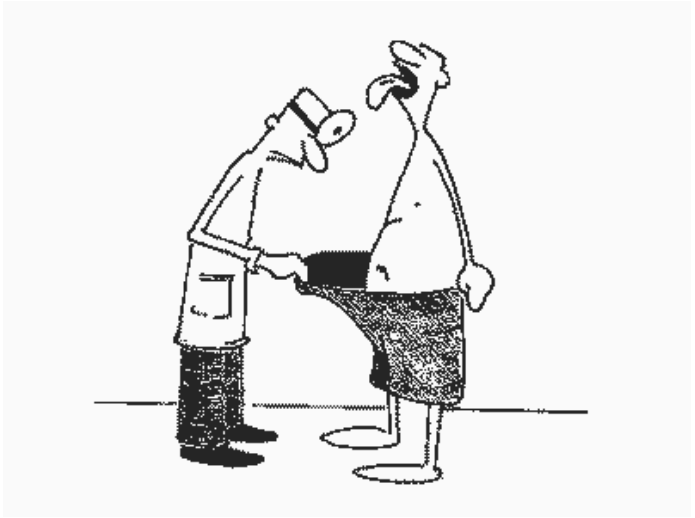
Sepsis in India: Outcomes

Laws of u ence



The Imapct…

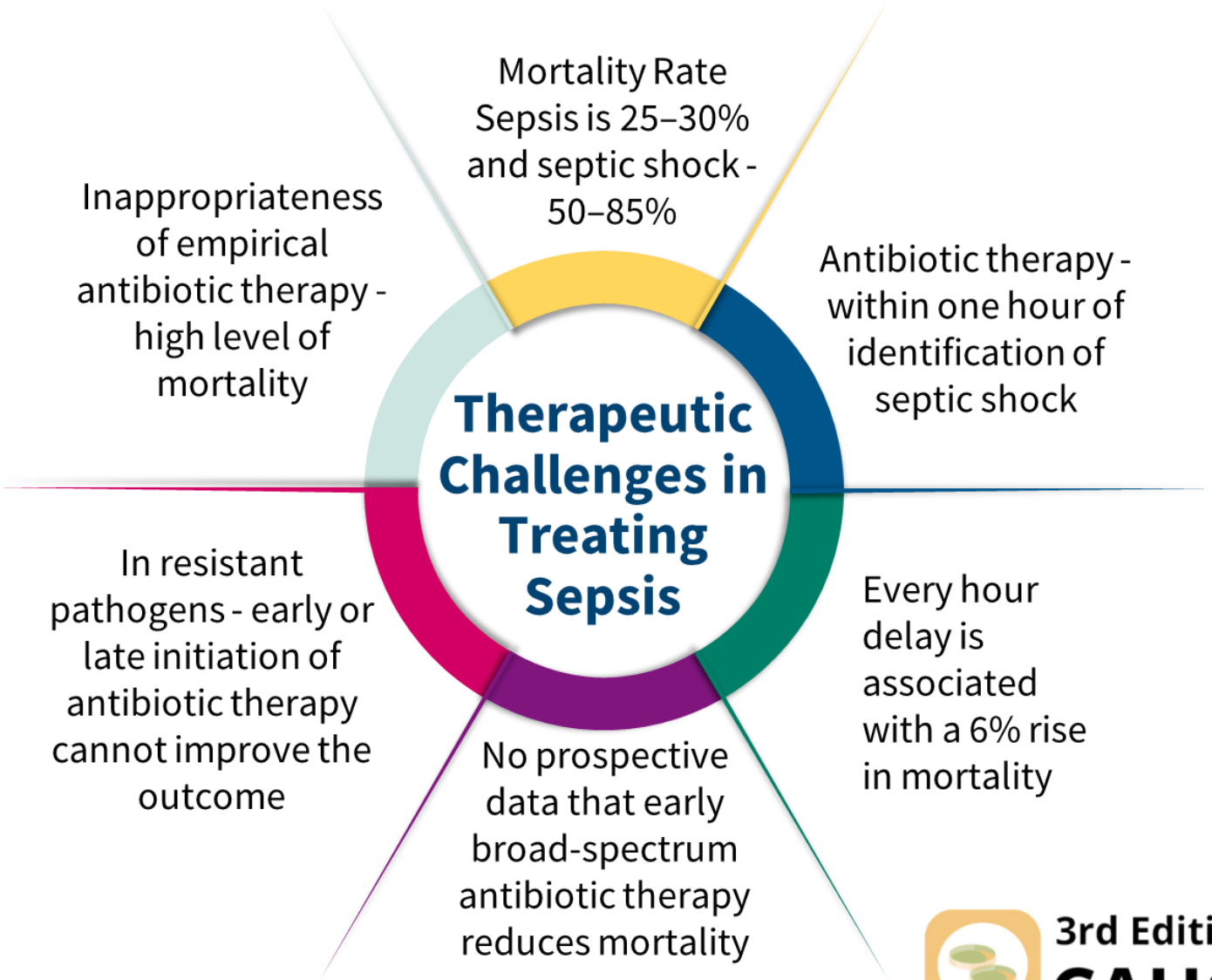

Deaths attributable to antimicrobial resistance every year by 2050
Europe
390,000
Asia
4,730,000
North America
317,000
Latin America
392,000
Africa
4,150,000
Oceania
22,000
Source: The Review on Antimicrobial Resistance



Diagnosis is not Everything

It’s the Only Thing…

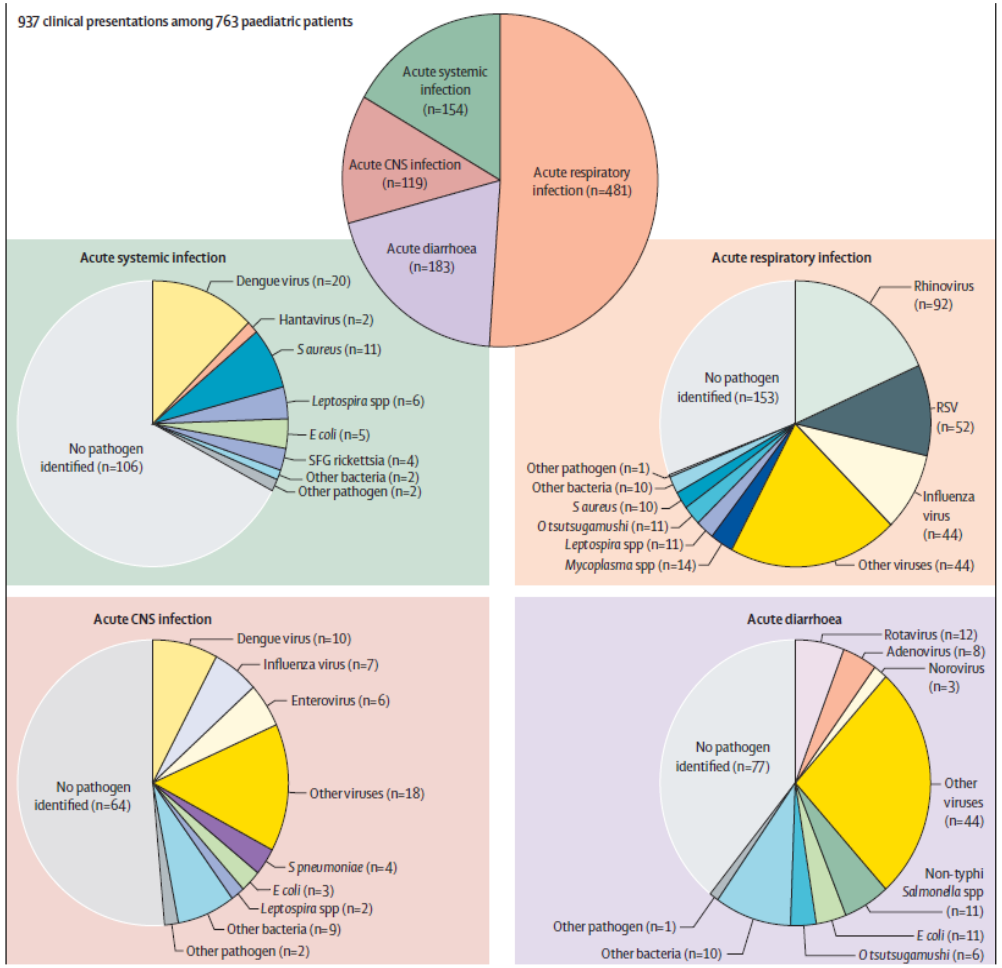
Current Therapeutic Challenges (Sepsis)



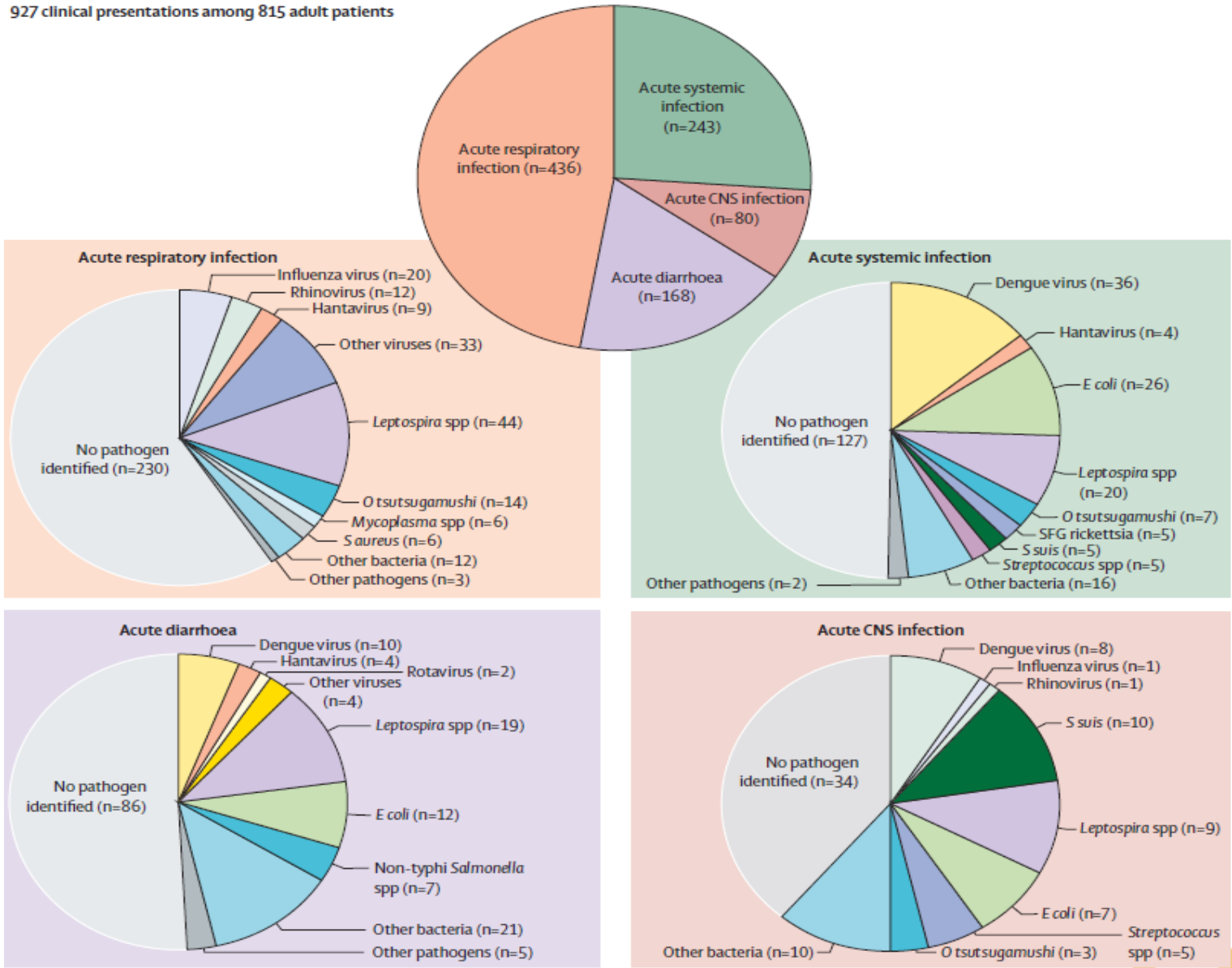
1. Candel FJ. Turning things around. *Rev Esp Quimioter*. 2018;31(4):298-315.
2. Minasyan H. *Scand J Trauma Resusc Emerg Med*. 2019;27(1):19.

Causes and outcomes of sepsis in southeast Asia: a multinational multicentre cross-sectional study

Southeast Asia Infectious Disease Clinical Research Network*



Lancet Global Health 2017:5 e157-167



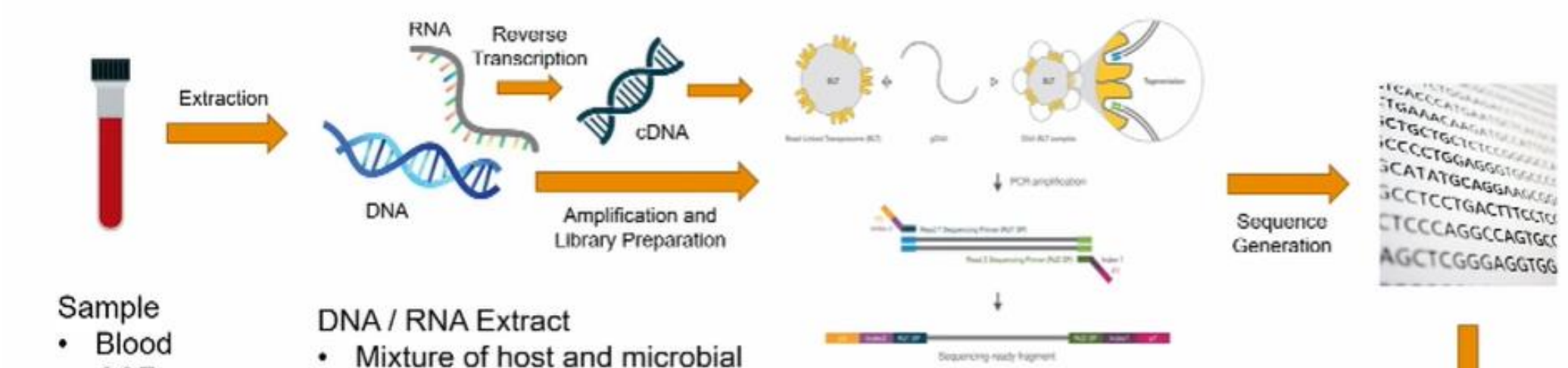
T1 Comparison of Molecular Methods for Diagnosis of Infectious Diseases

	Real-time PCR	Sanger Sequencing	Targeted Next-Generation Sequencing (tNGS	Metagenomic Next-Generation Sequencing (mNGS)
Prior knowledge of the target[1]	Yes	No[2]	No[2]	No
Enrichment of the target	N/A	Yes	Yes	No
Direct detection from clinical sample or microbial isolate required	Direct from sample or microbial isolate	Normally sterile sample2 or microbial isolate	Direct from sample or microbial isolate	Direct from sample or microbial isolate
Turnaround time	< 8h	< 8h	1-7 days	1-7 days
Relative ease of in-house implementation	Low	Low to Moderate	Moderate to High	High
Example of clinical application	Target-specific PCRs (i.e., *Mycoplasma pneumoniae*, methicillin-resistance in *Staphylococcus aureus*)	Microbial identification and strain typing (i.e., 16S rDNA sequencing)	Broad range PCR (i.e., universal fungal PCR)	Unbiased pathogen detection (i.e., Karius)

The need for NGS

- PCR - We need to know what we are looking for
- NGS based tests- an agnostic diagnostic method capable of comprehensive detection of multiple pathogens simultaneously and directly from a patient sample
- Massively parallel sequencing of billions of DNA fragments
- Potential for simultaneous identification and resistance detection
- Clonality/relatedness of suspected outbreak strains
- Prior knowledge of the target organism(s), and thus target-specific primers, is not required
- Obscure, rare pathogens, antimicrobial treated patients

NGS testing workflow



Sample
- Blood
- CSF
- Urine
- Respiratory
- Sterile fluid
- Tissue

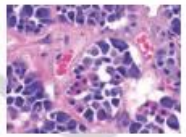
DNA / RNA Extract
- Mixture of host and microbial
- RNA conversion to cDNA

Sequencing Library
- Collection of DNA inserts
- Adaptors – allows sequencing
- Barcodes – allows multiplexing

Bioinformatic Analysis

TISSUE INFECTIONS

- 86% PPA versus 45% by culture and 58% by all other methods[68]
- mNGS can be challenging due to high background; consider 16S rRNA enrichment[69]



JOINT INFECTIONS

- mNGS sensitivity (88-96%) improved over culture (52-80%) in PJI[54-57]
- can assess for antibiotic resistance markers[62]

PNEUMONIA

- increased detection in immunocompromised patients[43, 44]
- can incorporate RNA host response profiles[48, 49]

OCULAR INFECTIONS

- >85% PPA with culture and PCR testing[63]
- diagnosis of atypical pathogens[64-66]

ENDOCARDITIS

- diagnosis of culture-negative endocarditis[36]
- potential low diagnostic specificity from plasma[37]

FEVER OF UNKNOWN ORIGIN

- dengue and Zika virus detection in tropical febrile outbreaks[27, 28]
- identification of disseminated adenoviral and HSV infections in ICU[31]

SEPSIS

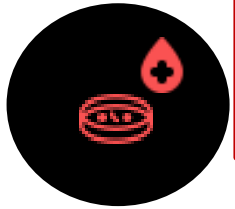
- 94% sensitivity versus blood cultures[23]
- potential pathogen in 15% negative samples[23]

MENINGITIS/ENCEPHALITIS

- 81% PPA, 99% NPA versus other clinical testing[5]
- 22% of cases diagnosed only by mNGS, of which 50% actionable[7]

Blood Culture Vs. NGS

- Overall low positivity rate
- Difficult to cultivate fastidious microbes
- Requires more volume of blood
- Long waiting time for results
- Technically challenging
- Infrastructure required



Blood cultures are inefficient in detecting bacteremia. Cultures collected during 24 hours after admission yielded more positive results than those collected later.[1]

Conventional blood culture is more time consuming and generates results that are false negative in the case of antibiotic pretreated samples as well as slow-growing microbes[3]

	Metagenomics – NGS	Tissue/Fluid Culture	Tissue/Fluid PCR tests	Blood Culture	Blood Serology
Diagnostic Yield	●	○	◑	○	○
Breadth of Pathogens	●	◑	◑	◑	○
Pathogen Detection Post Antimicrobial Therapy	●	◑	◑	◑	●
Time of Results	●	○	○	○	○

Challenge ○ - - - ◑ - - - ● Strength

1. Panday N, et al. PloS one. 2019 Mar 21;14(3):e0214052.
2. Bhattacharya S. Indian journal of medical microbiology. 2005 Oct 1;23(4):220.
3. Gupta E, Saxena J, Kumar S, Sharma U, Rastogi S, Srivastava VK, Kaushik S, Jyoti A. Fast Track Diagnostic Tools for Clinical Management of Sepsis: Paradigm Shift from Conventional to Advanced Methods. *Diagnostics*. 2023; 13(2):277

Why is not more widely used/ available

- Few validated, standardized tests available even internationally
- Limited data in literature on clinical utility
- Large variability in available institution and patient population wise data
- Limited to labs with instrumentation and technical expertise
- Requires robust bioinformatics support expertise
- Interpretation of results
- Cost

Abayasekara et al. BMC Infectious Diseases (2017) 17:631
DOI 10.1186/s12879-017-2727-8

BMC Infectious Diseases

TECHNICAL ADVANCE Open Access

Detection of bacterial pathogens from clinical specimens using conventional microbial culture and *16S* metagenomics: a comparative study

Lalanika M. Abayasekara[3*], Jennifer Perera[1,3], Vishvanath Chandrasekharan[2,3], Vaz S. Gnanam[3], Nisala A. Udunuwara[3], Dileepa S. Liyanage[3], Nuwani E. Bulathsinhala[3], Subhashanie Adikary[3], Janith V. S. Aluthmuhandiram[3], Chrishanthi S. Thanaseelan[3], D. Portia Tharmakulasingam[3], Tharaga Karunakaran[3] and Janahan Ilango[3]

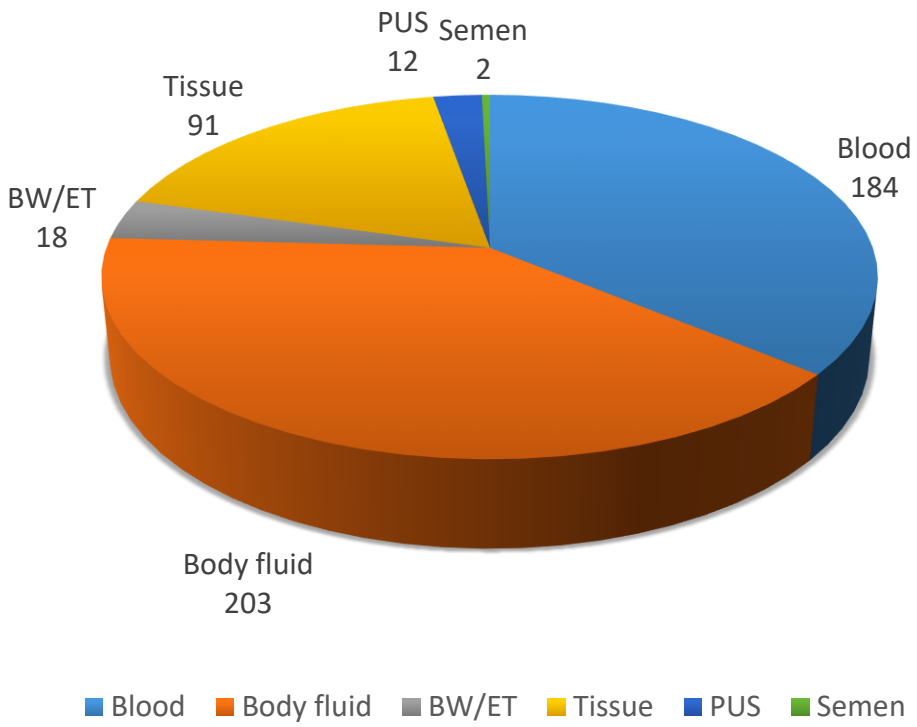
Total Specimens	103			
NGS processed specimens	97			
		Total Specimens	Bacterial PCR Results	
			Negative	Positive
Culture results	No Bacterial Growth	36	19	17
	Culture Positive	61	1	60

Table 2 Comparison of culture results and bacterial metagenomic results

	Culture Negatives (%)	Culture Positives (%)	Total Specimens
Matches with metagenomic results	19 (52.8%)	56 (91.9%)	75 (77.3%)
Conflicts with metagenomic results	17 (47.2%)	5 (8.1%)	22 (22.7%)
Total	36 (100%)	61 (100%)	97 (100%)

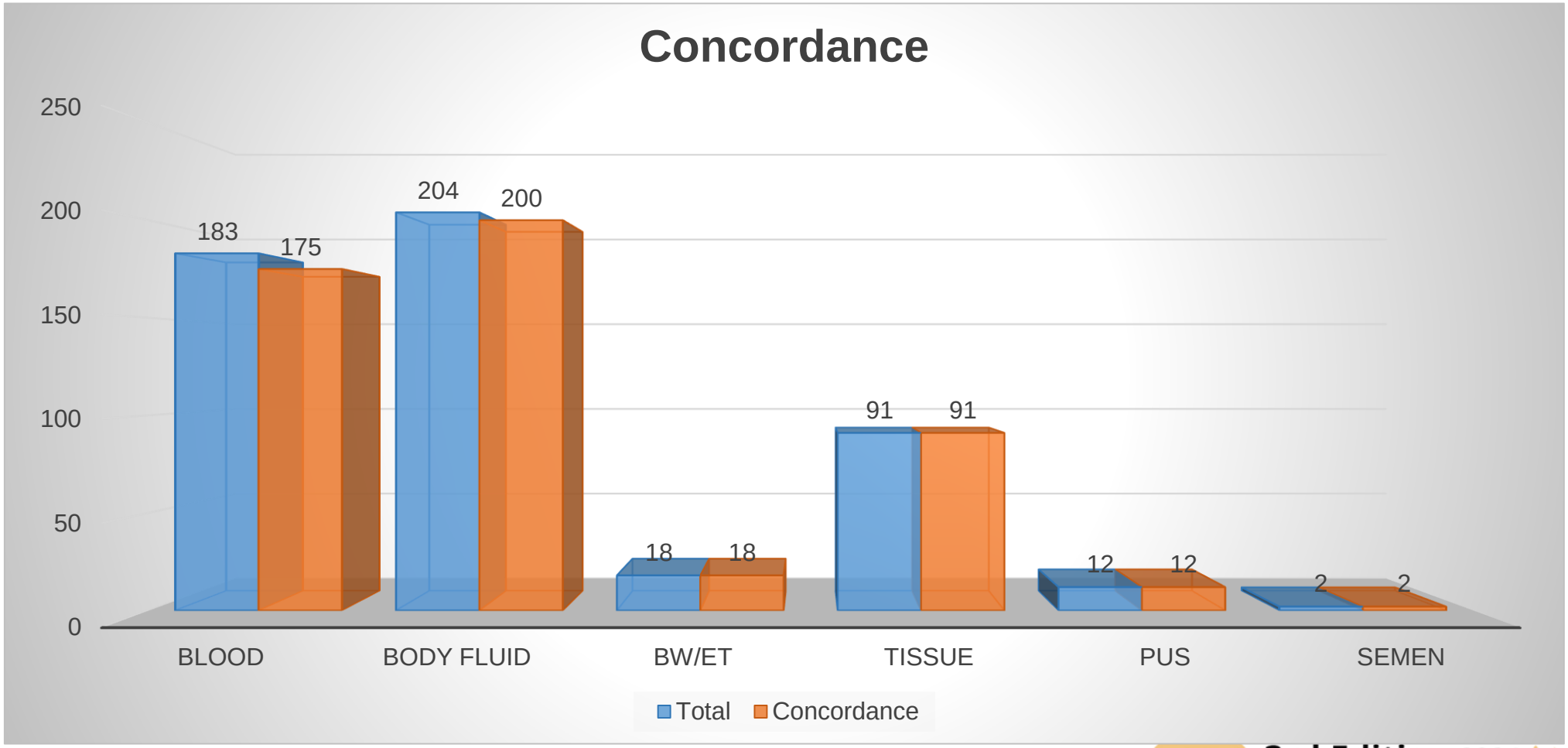
Cross-section of samples for the validation study at Apollo

510 samples processed with BF & FF during a selected period:



Body fluids:
Ascitic fluid
Bile
CSF
Synovial fluid
Pancreatic fluid
Peritoneal fluid
Pericardial fluid
Pleural fluid
BW/ET:
Bronchial wash
Endotracheal wash

Concordance (97.6%) with the culture(498 samples)



Summary of the validation study:

- 51 culture negative -> Positive on BF & FF recorded as clinically significant results by Team
- Culture negative suspected Mycobacterial samples were detected Using BF
- Anaerobic infections (*Bacteriodes spp.*, *Clostridium spp.*) were detected
- Mixed infections were recorded
- This test is applicable to any sample type (Peripheral blood, Sterile Body Fluids, Tissue etc.)
- This test has a clear benefit towards infection control due to a faster turnaround time

When to use

When a large number of microbes can cause a certain infection

Traditional microbiology is unable to identify the pathogen- rare, novel or fastidious

Prior use of antibiotics

Immunosuppressed patients

?To rule out infection

Bactfast®

Identifies all known referenced bacteria to species and subspecies level in any sample. It identifies and isolates, specific infection causing bacteria with 99.7% accuracy. ***It tests for 22,000 types of bacteria*** in a single sample.

Fungifast®

It is a test procedure for identification of infection causing fungi. It is based entirely on the sophisticated analysis of DNA. 99.7% accurate analysis and comparisons are made to isolate findings with the DNA of all known fungi. ***Over 6000 fungi*** could be identified in one test.

dxn1

Virfast®

Identifies all known DNA and RNA viruses in a single test. The application stack allows to seamlessly update new viruses and their variant when the references become available. ***Covering over 9000 references viruses*** Virfast® allows the clinician to identify a virus from any sample. The scalability of a test can cater to seasonal or regional viral outbreaks, endemic or epidemic viral outbreaks.

digitalABST®

Based on the results obtained by the tests for pathogen identification, digitalABST® is a big data and machine learning solution that will identify the resistance or sensitive genes favourable to treatment response.

Take Home Message…