What's Hot in Diagnostic Microbiology 2016: Transformation

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Learning Objectives

- Describe the transformative change taking place in your laboratory
- Outline current and upcoming technology changes
- Discuss the impact of new information generation for your practice

Overview

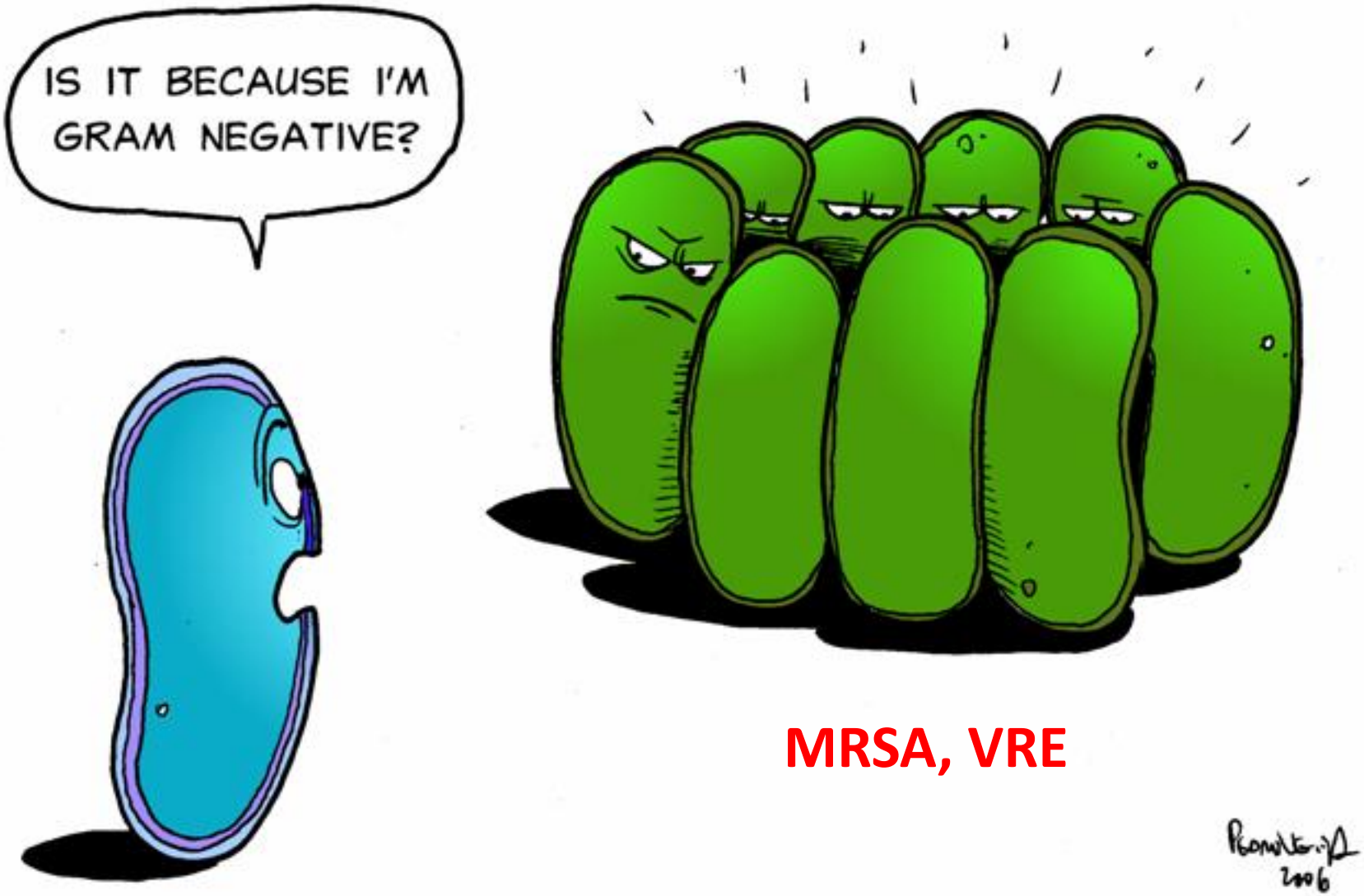
- Introduction
- Microbial transformation
  - Worldwide Dissemination of CREs
- Transformation of Laboratory Practice
  - New Technology
  - The Emergence of Big –Omics Data
  - Point-of-Care Testing
- Summary

Transformation of Microbes and Laboratory Practices

A process of incremental radical change that re-orients how something works while taking it to an entirely different level of effectiveness so that eventually there is little or no resemblance with the past configuration or structure.

Microbes and **Laboratory Practice** are rapidly changing and will continue to change

The Gram-Negatives Come to the Forefront

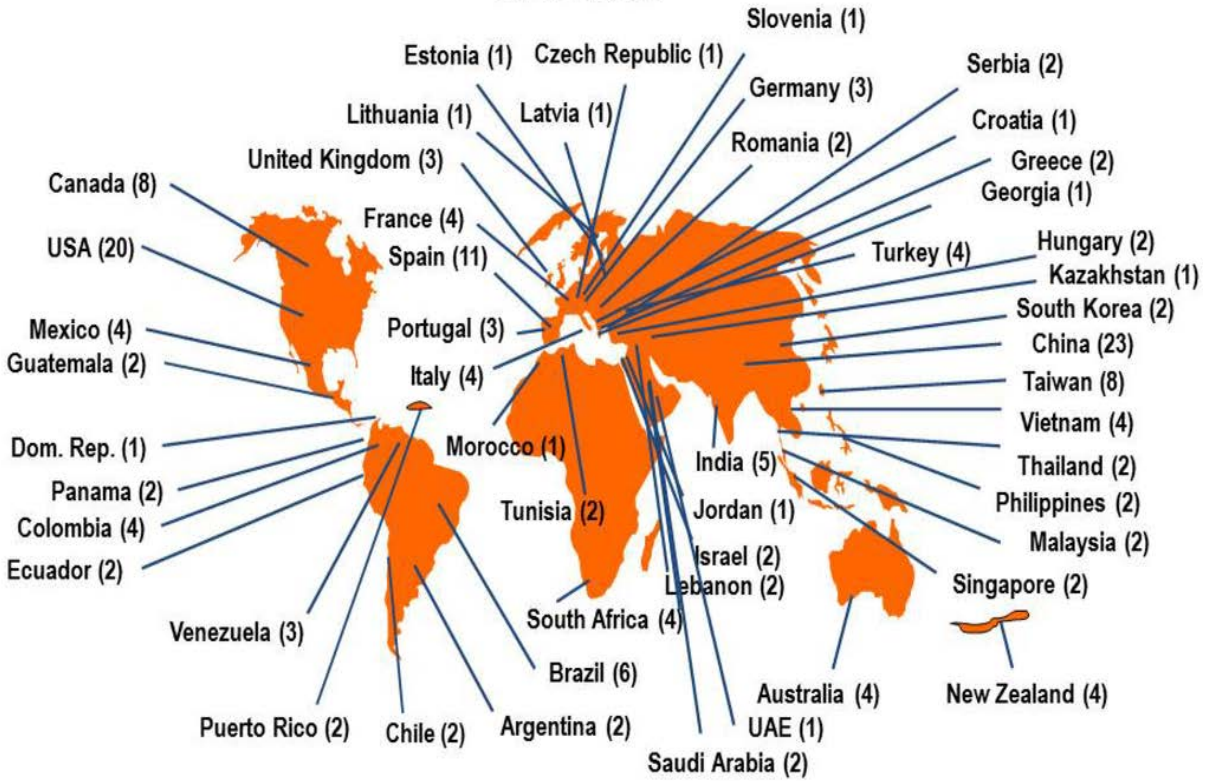


SMART

Study for Monitoring Antimicrobial Resistance Trends

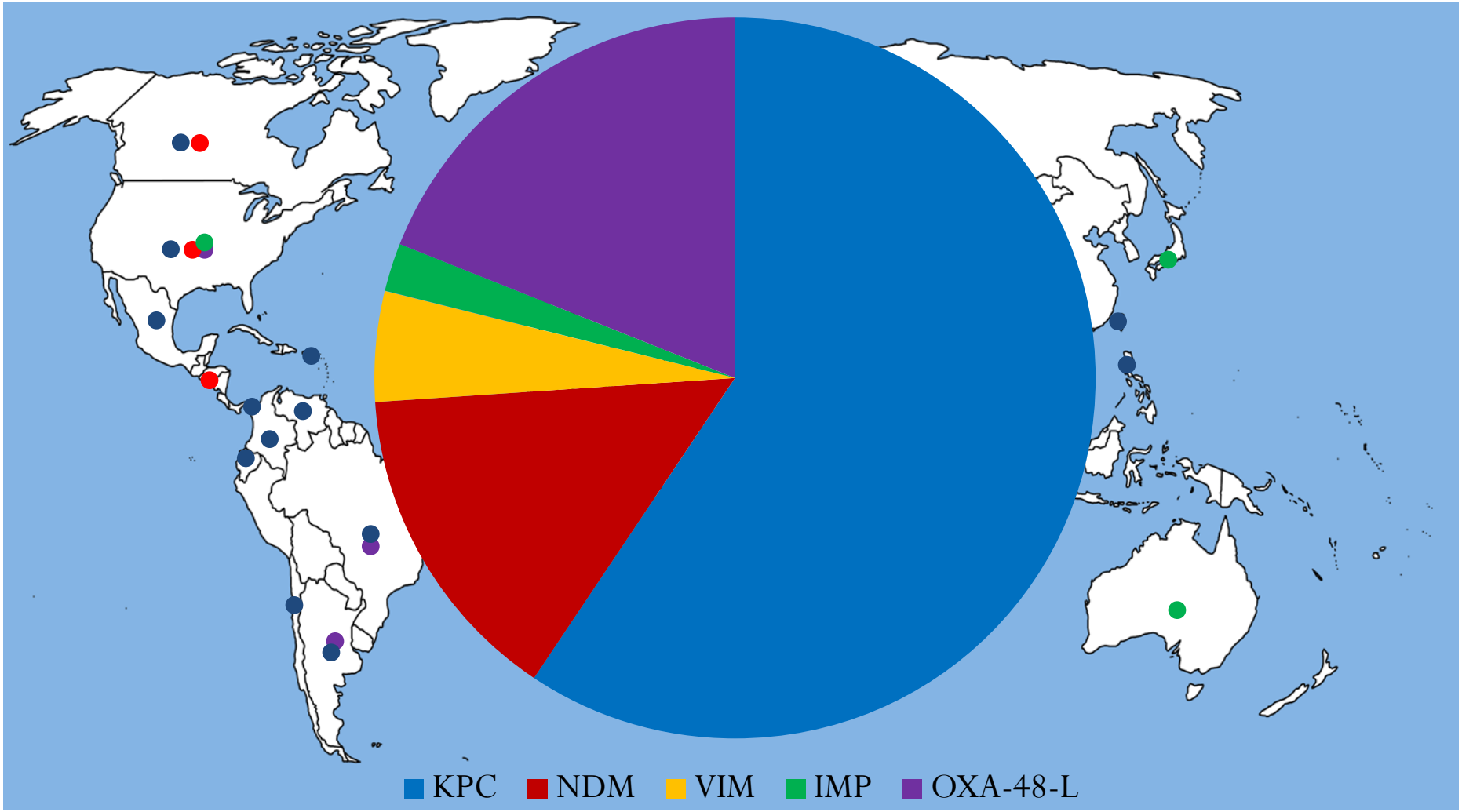
SMART: Worldwide Reach 2011

179 Sites



Special thanks to Krystyna Kazmierczak from IHMA

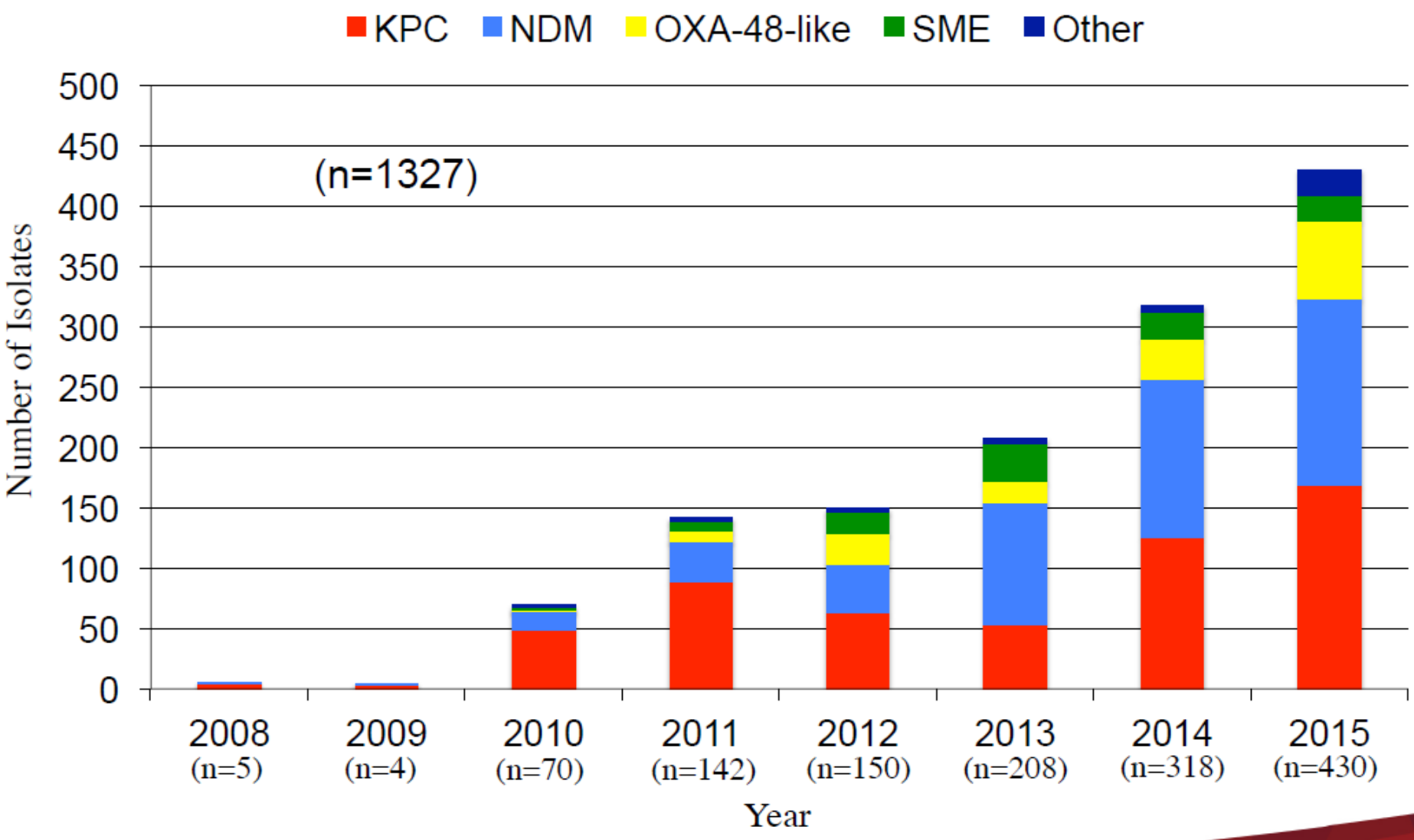
K. pneumoniae (n=1113)



What is the most common type of carbapenemase detected in CRE in Canada?

1) OXA-48 like enzymes
2) SME
3) VIM
4) NDM
5) KPC

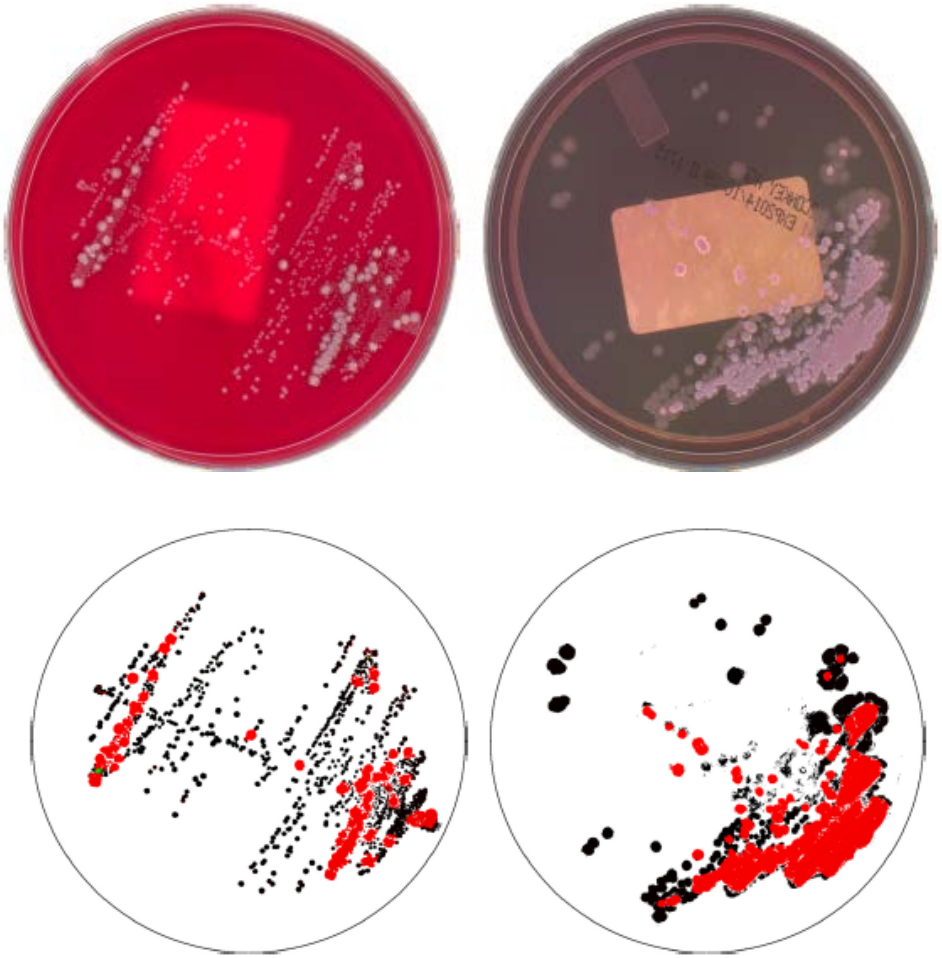
CPE in Canada: CPHLN Data



Transformation of Laboratory Practice – The Tea Toss

Michael H. Davies, 2016, Pangnirtung, Nunavut

Image Analysis Device – Screening Urine Cultures



APAS, LBT Laboratories Ltd., South Australia

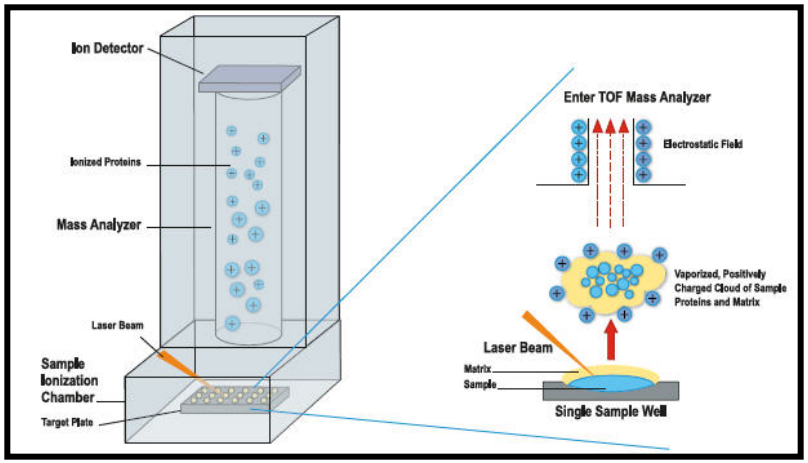
TABLE 2 Organisms detected by APAS compared with those by the routine laboratory reports

Organism	No. of cases detected by APAS	No. of cases reported by the laboratory
Escherichia coli	339	341
Enterococcus faecalis	38	38
Klebsiella pneumoniae	21	21
Proteus mirabilis	19	19
Pseudomonas aeruginosa	18	19
Staphylococcus saprophyticus	14	14
Klebsiella oxytoca	8	8
Staphylococcus epidermidis	7	7
Streptococcus agalactiae	6	6
Enterobacter aerogenes	5	5
Citrobacter koseri	5	5
Enterobacter cloacae complex	3	3
Morganella morganii	3	3
Viridans streptococci	3	3
Candida albicans	2	2
Citrobacter freundii	2	2
Staphylococcus, coagulase negative	2	2
Acinetobacter spp.	1	1
Aerococcus urinae	1	1
Candida spp.	1	1
Enterococcus faecium	1	1
Raoultella spp.	1	1
Serratia liquefaciens	1	1
Serratia ureilytica	1	1
Staphylococcus aureus	1	1
Staphylococcus haemolyticus	1	1
Staphylococcus hominis	1	1
Streptococcus dysgalactiae	1	1
Total	506	509

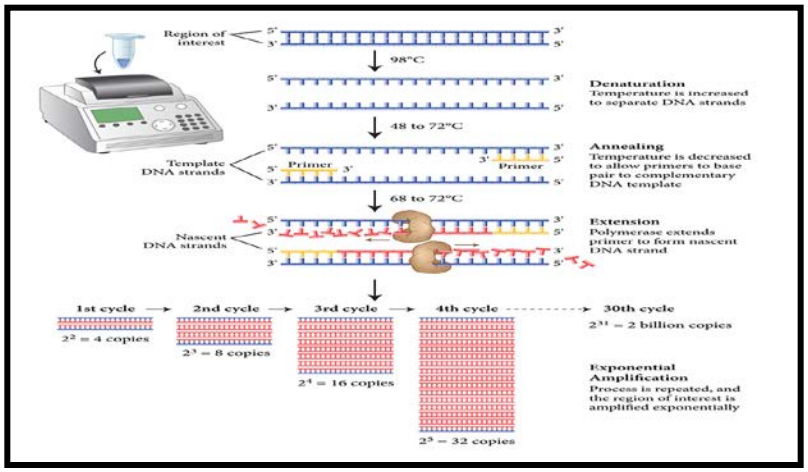
Glasson J et al. JCM 2016; 54:300

Whole Cell Detection to Molecular Methods

Proteomics Detection & Identification



Genomics Detection & Identification



Multiplex Syndromic Testing

- Rapid commercial development of large multiplex panels
  - Causes of infection are broad and diverse and infectious agents might be bacterial, viral or fungal
  - Co-infections may be more common than previously detected with a singleton approach
  - Symptoms of infection may be non-specific, and patients get immediate broad antimicrobial treatment, often before samples are collected
- Initially target serious invasive infections with potentially poor outcomes
  - **Pneumonia**
  - **Gastro-enteric (GI) infections**
  - **Bloodstream infections**
  - **Meningitis**

Should Multiplex Panel Be the First-Line Test for Pneumonia?

Point:

Main premise is that other agents (viruses and atypical bacteria) matter to overall clinical and economic outcomes

Reasons:

- Quicker treatment
- Shorter duration of symptoms
- Shorter ED wait times
- Shorter LOS if admitted
- Rapid implementation of IPC measures
- Reduced antibiotic costs
- Improved antibiotic stewardship
- Reduced collateral side effects from antibiotics
- Reduced overall medical costs
- Improved patient satisfaction

Schreckenberger PC and AJ McAdam. JCM 2015; 53:3110

xTAG/NxTAG Respiratory Multiplex Panel – Luminex (ThermoFisher)

- Many new respiratory viruses: Human metapneumovirus (HMP), the sudden acute respiratory syndrome-associated coronavirus(SARS), avian influenza virus H5N1, coronaviruses NL63 and HKU1, human bocavirus, H1N1, paraechoviruses, MersCoV
- Performance varies by analyte, system, seasonality, timing of collection
- Limited evaluations of head-to-head commercial assays or randomised control trials

Many more products/test systems

Viral Targets
Influenza A
Influenza A H1
Influenza A H3
Influenza A 2009 H1N1
Influenza B
Respiratory Syncytial Virus A (RSV A)
Respiratory Syncytial Virus B (RSV B)
Parainfluenza 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4
Human Bocavirus
Human Metapneumovirus (hMPV)
Human Rhinovirus/Enterovirus
Adenovirus
Coronavirus HKU1
Coronavirus NL63
Coronavirus OC43
Coronavirus 229E
Bacterial Targets
Chlamydophila pneumoniae
Legionella pneumophila
Mycoplasma pneumoniae

Benefits & Drawbacks of Multiplex RVI Panels

TABLE II. Total of Viruses Detected By Each Technique Considering the Criterion of Positivity

Virus	Total	IF/VC	Seeplex®	Clart®
RSV	11	11	7	10
AdV	14	13	11	12
PIV-3	2	2	2	2
hMPV	4	4	1	3
RV	15	2	14	14
EV	4	4	ND	4
Multiple detection	4	0	2	4
Total of virus detected	50	36	35	45
Sensitivity[a]	—	70%	76%	89%

ND, unable to be detected by the corresponding technique.
[a]A total of 46 viruses were considered after exclusion of EV.

TABLE III. All Samples With Multiple Viral Detection. Real Multiple Infections According to the Criterion of Positivity Are Shown in Bold

No	IF/VC	Seeplex®	Clart®
1	RSV	RSV	RSV + HBoV
2	NEG	RV + HCoV-OC43	RV
8	NEG	RV	RV + HBoV + PIV-3
10	NEG	RV	RV + HBoV
13	NEG	Inhibited sample	HBoV + EV + PIV-4
15	**PIV-3**	**PIV-3 + RV**	**PIV-3 + RV**
17	NEG	PIV-3 + RV	Inhibited sample
18	**EV**	**RV**	**RV + EV**
20	AdV	AdV	AdV + RV
21	RSV	RSV	RSV + HBoV
22	RSV	RSV	RSV + HBoV
23	**EV**	**RV**	**RV + EV**
26	PIV-3	PIV-3	PIV-3 + HBoV
27	**NEG**	**AdV + RV**	**AdV + RV + RSV**
29	NEG	NEG	AdV + RV
30	RSV	NEG	RSV + HBoV
36	EV	NEG	EV + RV + HBoV
37	RSV	NEG	RSV + EV + RV + HBoV
38	RSV	NEG	RSV + EV + RV
43	AdV	AdV	AdV + HBoV
47	AdV	AdV	AdV + FLUCV
51	AdV	AdV + RV	AdV
52	AdV	AdV + HCoV-OC43	AdV
55	hMPV	hMPV + HCoV-OC43	hMPV + RV
60	AdV	AdV	AdV + HBoV
63	AdV	AdV + RV	AdV
65	AdV	AdV	AdV + hMPV
67	NEG	Inhibited sample	EV + FLUBV
69	NEG	NEG	hMPV + HBoV
79	EV	NEG	EV + hMPV

- Multiple viruses more efficiently detected
- Both NAATs detected different viruses, and some that cannot be detected by IF/VC
- Detection of viral nucleic acids is not necessarily related to the current clinical syndrome
- Does the 'extra' sensitivity provide more reliable information about infection etiology?
- Lack of a true 'gold standard' to compare methods

Garcia-Arroyo L et al. J Med Virol 2016 88:45-50

Should Multiplex Panel Be the First-Line Test for Diarrheal Illness?

CounterPoint:

- **Panels Leads to exhaustive testing as an end in itself:**
- Testing for some pathogens should be guided by risk factors for infection
  - *C. difficile* should not be combined with other agents
- Testing for common then uncommon pathogens
- Don't test where it is highly unlikely that the pathogen is present

Current Issues:

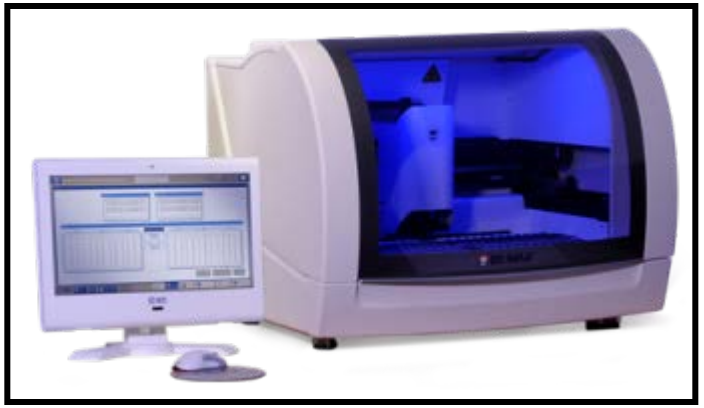
- Laboratories need to publish performance data by analyte. Additional tests may need to be performed.
- What is the right panel composition?
- Automation in a high volume setting
- Re-aligning how diagnostic and public health labs in Canada work together
- What about the public health reporting issues?
- Are there reduced overall medical costs?
- Will this lead to overtreatment with antibiotics?
- Clinical and economic outcomes data is currently limited

Schreckenberger PC and AJ McAdam. JCM 2015; 53:3110

Multiplex Systems – GI Panels

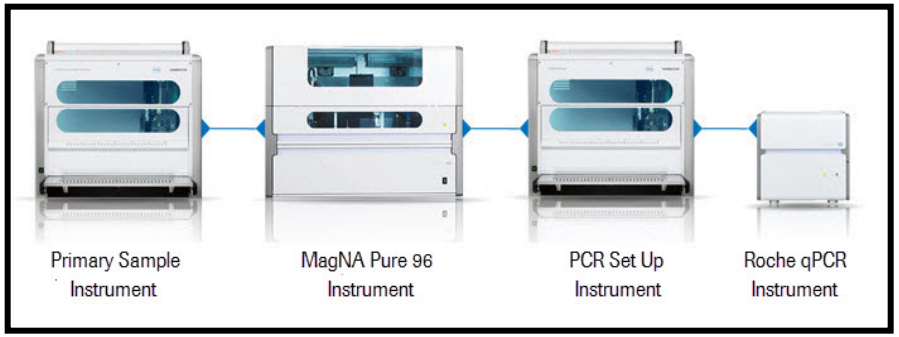
BIOFIRE FilmArray
(bioMérieux)

Seegene

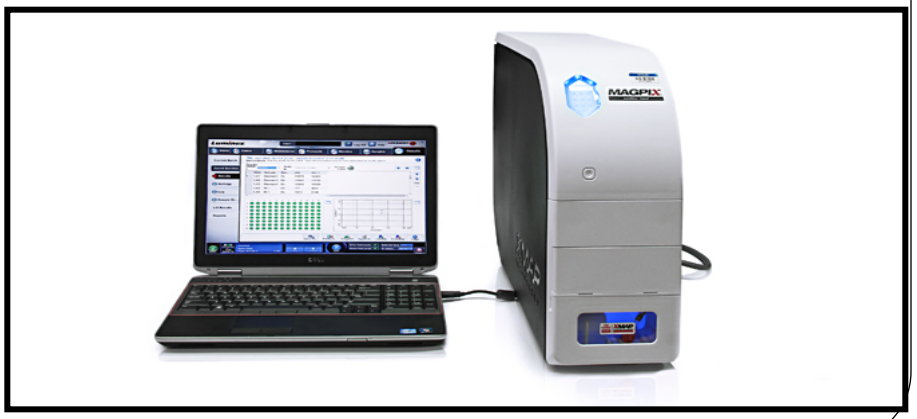


BDMaxx

MAGPIX GPP - Luminex



Roche FlowCell – LightMix Assays



Case-Control Studies - GI Panels

- Only one case-control study to date of patients in the Netherlands
- Cases-controls clinically assessed and completed study questionnaire
- 2710 fecal samples were tested by multiplex PCR for the most common bacteria and 4 protozoa:
  - A total of 54% of the cases and 49% of controls were positive for one or more target organisms
  - Only *D. fragilis* and Shiga-like toxigenic *E. coli* were found less frequently in cases than controls
  - The association between illness and a positive PCR was weakest in children aged 0 to 5 yrs.
  - Cases had higher target loads than controls
  - Does a GI panel need to be quantitative?

Bruijnesteijn Les et al. Clin Microbiol Infect 2015; 21:591e09

Cases and Controls had the Same Positivity

TABLE 3. Overall positivity for the different target organisms

Organism	Case (n = 1515)		Control (n = 1195)		p (case vs. control)
	No. positive	%	No. positive	%	
Campylobacter spp.	154	10.2	33	2.8	0.000
Salmonella spp.	28	1.8	4	0.3	0.000
Pathogenic *Yersinia enterocolytica*	2	0.1	0	—	0.507
Clostridium difficile	64	4.2	21	1.8	0.000
Shigella/EIEC	14	0.9	0	—	0.000
EHEC	2	0.1	2	0.2	1.000
STEC	15	1.0	22	1.8	0.067
ETEC	48	3.2	8	0.7	0.000
EAEC	94	6.2	34	2.8	0.000
Atypical EPEC	144	9.5	84	7.0	0.022
Typical EPEC	10	0.7	10	0.8	0.655
Entamoeba histolytica	0	—	0	—	—
Giardia lamblia	85	5.6	33	2.8	0.000
Cryptosporidium parvum/hominis	46	3.0	10	0.8	0.000
Dientamoeba fragilis	390	25.7	446	37.3	0.000
One or more detections	818	54.0	584	48.9	0.008
Negative	697	46.0	611	51.1	
One or more detections excluding *D. fragilis*	541	35.7	230	19.2	0.000
Negative when excluding *D. fragilis*	974	64.3	965	80.8	
1 target organism excluding *D. fragilis*	417	27.5	204	17.1	0.000
2 target organisms excluding *D. fragilis*	90	5.9	21	1.8	
3 target organisms excluding *D. fragilis*	27	1.8	5	0.4	
4 target organisms excluding *D. fragilis*	7	0.5	0	—	

EAEC, enteroaggregative *Escherichia coli* (with *aggR* and/or *aat*); EHEC, enterohemorrhagic *E. coli* (with *eaeA* and *stx1* and/or *stx2*); EIEC, enteroinvasive *E. coli* (with *ipaH*); EPEC, enteropathogenic *E. coli* (typical with *eaeA* and *bfpA*, and atypical with only *eaeA*); ETEC, enterotoxigenic *E coli* (with *lt* and/or *st*); STEC, Shiga-like toxigenic *E coli* type (with *stx1* and/or *stx2*).

Bruijnesteijn Les et al. Clin Microbiol Infect 2015; 21:591e09

Does your laboratory use a multiplex GI panel for routine testing?

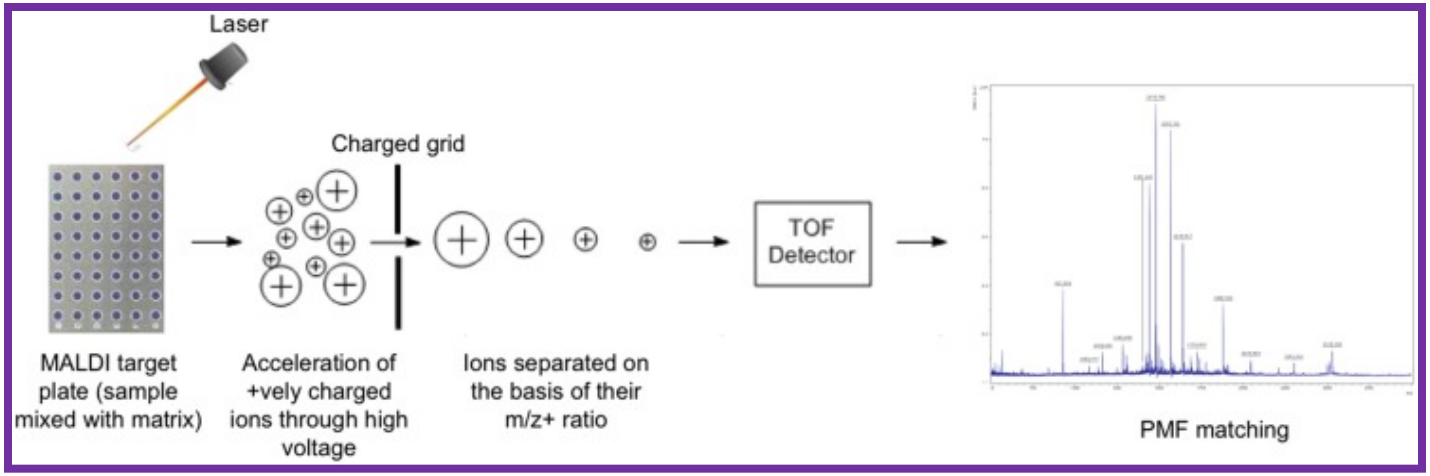
1) A multiplex GI panel is used to detect common enteric protozoa (*Giardia*, *Cryptosporidium*, *Entamoeba histolytica/dispar* and/or *Dientamoeba fragilis*)
2) A multiplex GI panel is used to detect common enteric bacteria
3) A multiplex GI panel is used to detect >20 diarrheal pathogens including parasites/viruses/enteric bacteria and *C. difficile*?
4) No multiplex GI panels are used – we still rely on microscopy and culture

The –Omics Revolution

New Proteomics and Genomics Technology

MALDI-TOF MS ANALYSIS ("The Answer Box") WORKFLOW

MATRICES:
- CHCA (α-cyano-4-hydroxycinnamic acid
- DHB (2,5-dihydroxy benzoic acid
- (Sinapinic acid) 3,5-dimethoxy-4-hydroxycinnamic acid



- Bruker (BD)/Vitek MS (bioMérieux) instruments identify (>95%) commonly encountered aerobic bacteria
  - Less robust for anaerobes, unusual coryneform-GPBs
  - Nocardia, Mycobacteria and fungi need specialized extraction protocols
- Direct analysis of positive blood cultures
  - Extraction protocol dependent
  - Some organisms problematic (i.e., viridans streptococci)
- Direct detection of antibiotic resistance profiles
  - Testing in the presence and absence of a particular antibiotic; detection of a specific peak for *bla*-KPC containing plasmid

Singhal N et al. Front Microbiol 2015; Aug 5. Youn J-H et al JCM 2016;54:35-42

Have you implemented any of these MALDI-TOF MS procedures?

1) Identification of Nocardia, Mycobacteria or fungi?
2) Direct detection of pathogens from blood cultures?
3) Direct detection of one or more type(s) of antimicrobial resistance (i.e., ESBL, carbapenemase, MRSA)?
4) We routinely perform (1) and (2) but not (3)
5) All of the above

Limitations of Commercial MALDI-TOF MS

- Whole-organism protein mass spectra must be sufficiently distinct:
  - Only a small portion of the organism's proteome is interrogated
  - Genetic/proteomic homology within key regions (i.e., ribosomal proteins) limits taxonomic separation (i.e., *E. coli* and *Shigella, S. mitis* group, etc.)
- Mass spectra pattern search against a proprietary database from reference strains:
  - Lack of identification if organism not in database or a false-ID will be assigned (*Paenibacillus* spp.)
  - Ongoing development and validation of expanding spectral library
  - Unable to resolve polymicrobial infections – need pure isolate
- User access to proprietary databases for new spectra and advanced applications development is currently limited on commercial instruments

MALDI-TOF and Metabolomics

- Analysis of complex metabolic signatures would allow 500,000 X the resolution of current MALDI-TOF MS instruments
  - Ultra-high performance liquid chromatography (UHPLC) high-resolution mass spectrometry (HRMS) and /or NMR MS
- Rapid metabolomics analysis of samples, pathogens based on metabolic signatures:
  - *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) can be rapidly differentiated by this approach
    - EC and PA metabolizes sugars via the Entner-Doudoroff pathway vs. SA used glycolysis
    - EC preferentially metabolizes hexose sugars (e.g. glucose) whereas PA prefers small organic acids (e.g. malate)
  - Metabolic analysis of the presence of 2-keto-3-deoxy-6-phosphogluconate, and differential utilization of glucose versus malate will conclusively identify these 3 pathogens

Antti H et al 2013 PlosONE; 8(2); Lewis IA et al. PLOS Genetics 2014;10(1).

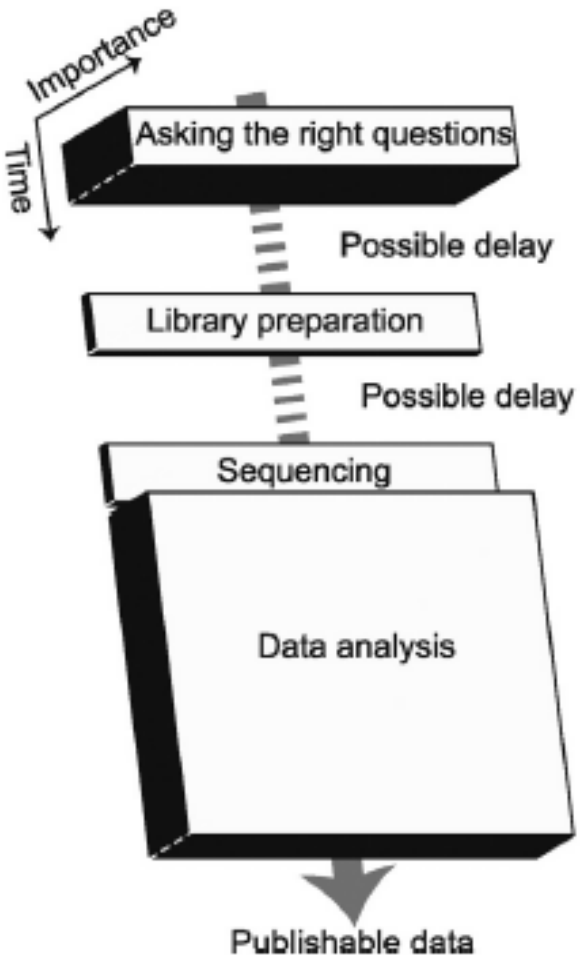
Next Generation Sequencing (NGS)

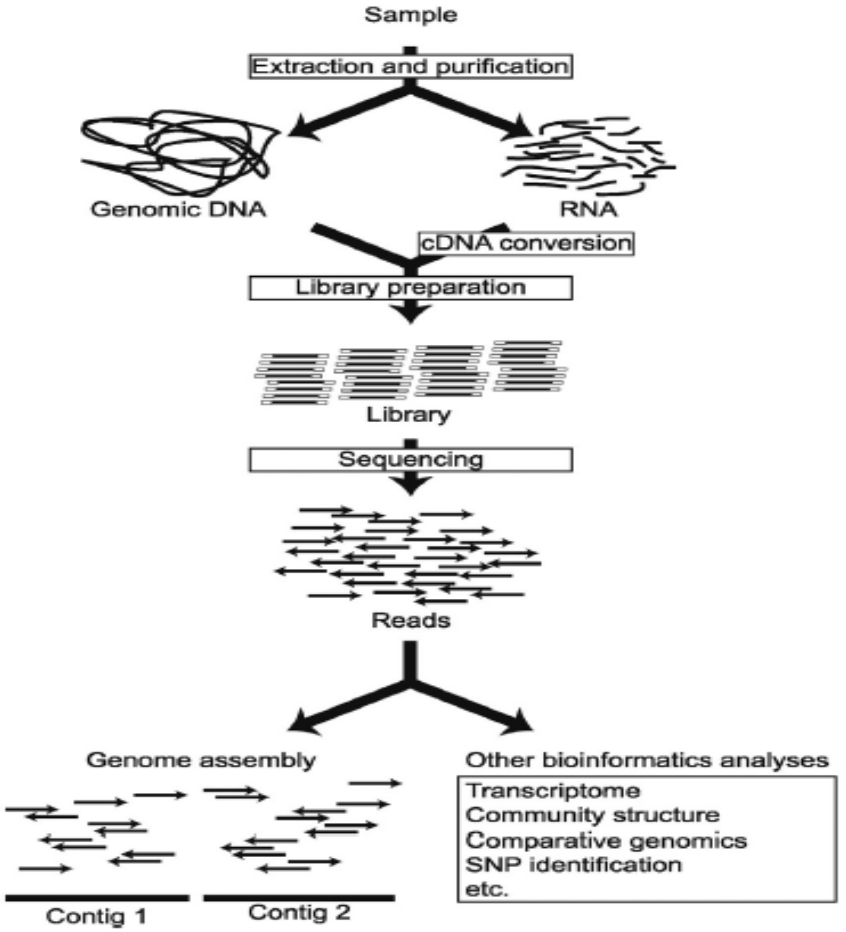
But don't get rid of your capillary sequencer just yet……

Massively Parallel Sequencing

- Capillary sequencing (Sanger) is slow because DNA synthesis and detection occur in two-steps but due to process and longer reads has a high degree of accuracy
- NGS relies on coupling the DNA synthesis and detection steps (sequencing by synthesis) and multiple reactions are occurring at the same time (massively parallel sequencing)
- Desynchronization of reads during NGS synthesis and detection leads to shorter reads and more errors

General Overview of NGS Procedure





Vincent AT et al. J Microbiol Methods 2016 doi.org/10.2016/j.mimet.2016.02.016

Which NGS Sequencer?

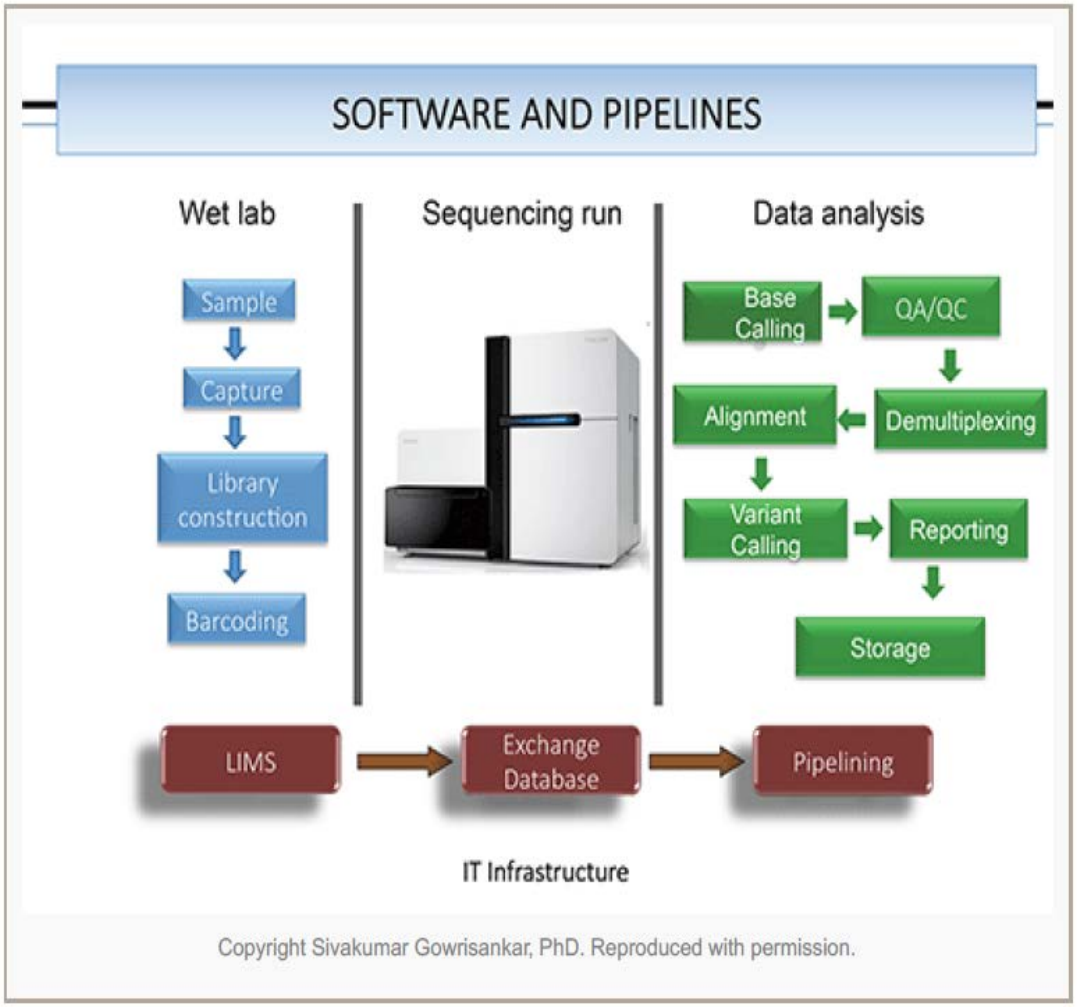
Table 1
Most common applications of next-generation sequencing.

Application	Library type	Relative importance of the sequencer features[a]	Recommended instrument
Genomic diversity and phylogeny	Shotgun	Consensus accuracy*** Throughput** Read length**	All
Structural analysis of genome	Shotgun + mate pairs	Consensus accuracy**** Read length***	MiSeq
Gene expression	Reverse transcription + shotgun	Throughput****** Read accuracy*	HiSeq, Ion Proton
Population diversity studies – species composition	Amplicons	Read accuracy**** Read length***	MiSeq, Ion PGM
Population diversity studies – gene function composition	Shotgun	Read length*** Read accuracy** Throughput**	MiSeq for assembly HiSeq, Ion Proton for quantification
Multi-locus sequence typing	Amplicons	Consensus accuracy**** Read length***	All

[a] Each application is rated with a total of 7 asterisks. The relative importance of a feature is indicated by the number of asterisks.

Vincent AT et al. J Microbiol Methods 2016 doi.org/10.2016/j.mimet.2016.02.016

NGS Clinical Laboratory Infrastructure



CHALLENGES:

- Quality of data
- Assembly of contiguous sequence
- Cross platform analysis
- SNP/mutation detection
- Data annotation – whole genomes
- Efficient data analysis pipeline
- Data storage – terabytes

CAPToday NGS Catching Up with Clinical Demands Nov., 2014

Has your laboratory used NGS for any of these applications?

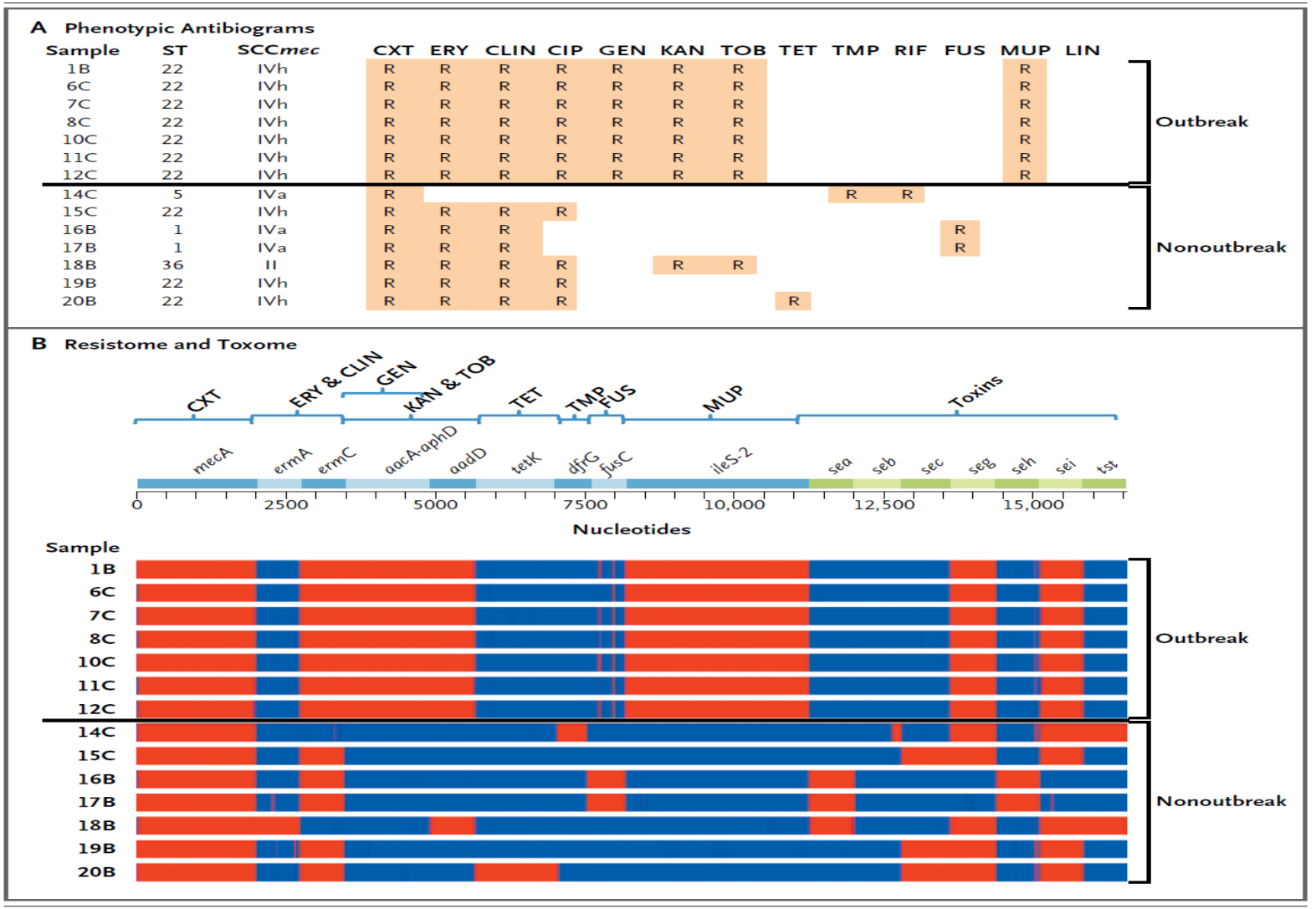
1) Diagnosis
2) Genotyping
3) Resistome studies
4) Plasmid analysis
5) Microbiome analysis

Whole-Genome Sequencing for Outbreak Investigation - Germany

- WGS has been used to delineate public health and nosocomial outbreaks for ~5-years
- May 2011, Germany – 830 HUS and 2,967 non-HUS cases and 46 deaths due to virulent shiga toxin (Stx) producing *E. coli* 0104:H4 (55989)
  - Ion Torrent (Life Technologies) and Optical Mapping done of the outbreak strain and a historic 0104:H4 HUS isolate from 2001. Time to completion of genomes = 62 h.
  - HUS-associated strain carried genes typically found in enteroaggregative *E. coli* (EAEC) and enterohemorrhagic *E. coli* (EHEC)
  - Model proposed in which EAEC 55989 and EHEC 0104:H4 evolved from a common EHEC 0104:H4 progenitor

Mellmann A et al. PLosOne 2011; 6:e27751

Rapid Whole-Genome Sequencing – Neonatal MRSA Outbreak – Cambridge, UK



Koser CU et al. NEJM 2012; 366:24.

Phylogenetics of Outbreak Strains of *S. pneumoniae* 12F

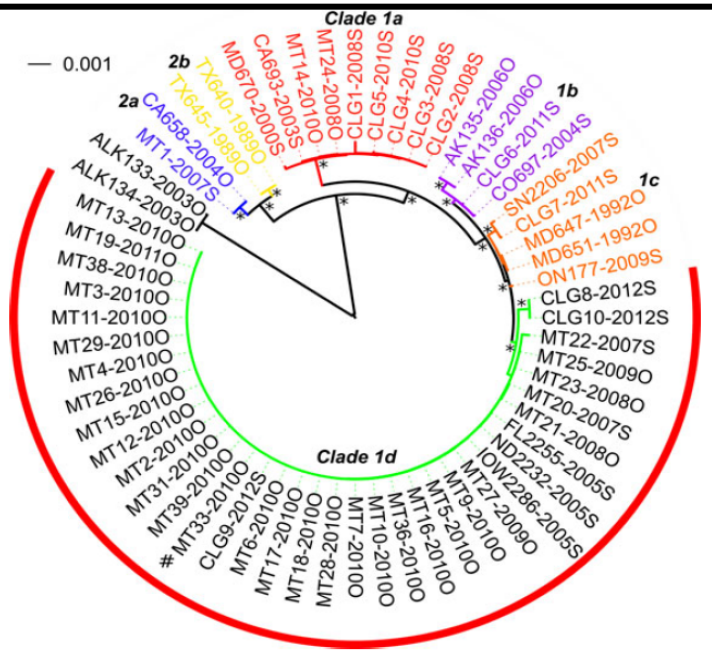


Figure 1. Phylogenetic analysis of 12F-CC218 outbreak and nonoutbreak isolates from Canada, the United States, and Spain between 1989 and 2012. A rooted maximum-likelihood tree (ST1527 isolates AK133 and AK134 as outgroups) based on the core genome with putative recombination sites excluded. Six clades are revealed by the tree: clade 1a (red-colored branch), 1b (purple), 1c (orange), 1d (green), clade 2a (blue), 2b (yellow). All 42 isolates in clade 1d were resistant to macrolides (encircled by red bar); the rest were drug-sensitive (no red bar). *Indicates 100% bootstrap support. Each isolate at the tip was labeled as origin-isolate ID-year of isolation-outbreak or survey. A terminal O refers to outbreak isolate, S refers to non-outbreak survey isolate. Abbreviations: AK, Alaska; CA, California; CLG, Calgary, Canada; CO, Colorado; FL, Florida; IOW, Iowa; MD, Maryland; MT, Manitoba, Canada; ND, North Dakota; ON, Ontario, Canada; SN, Spain; TX, Texas.

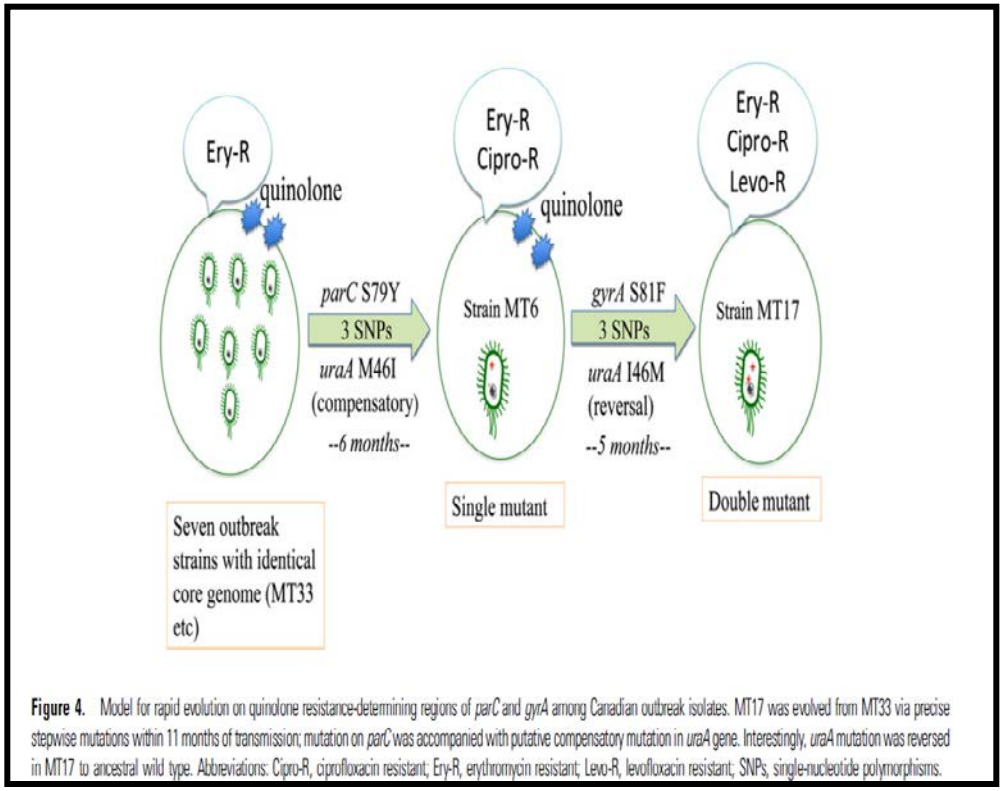
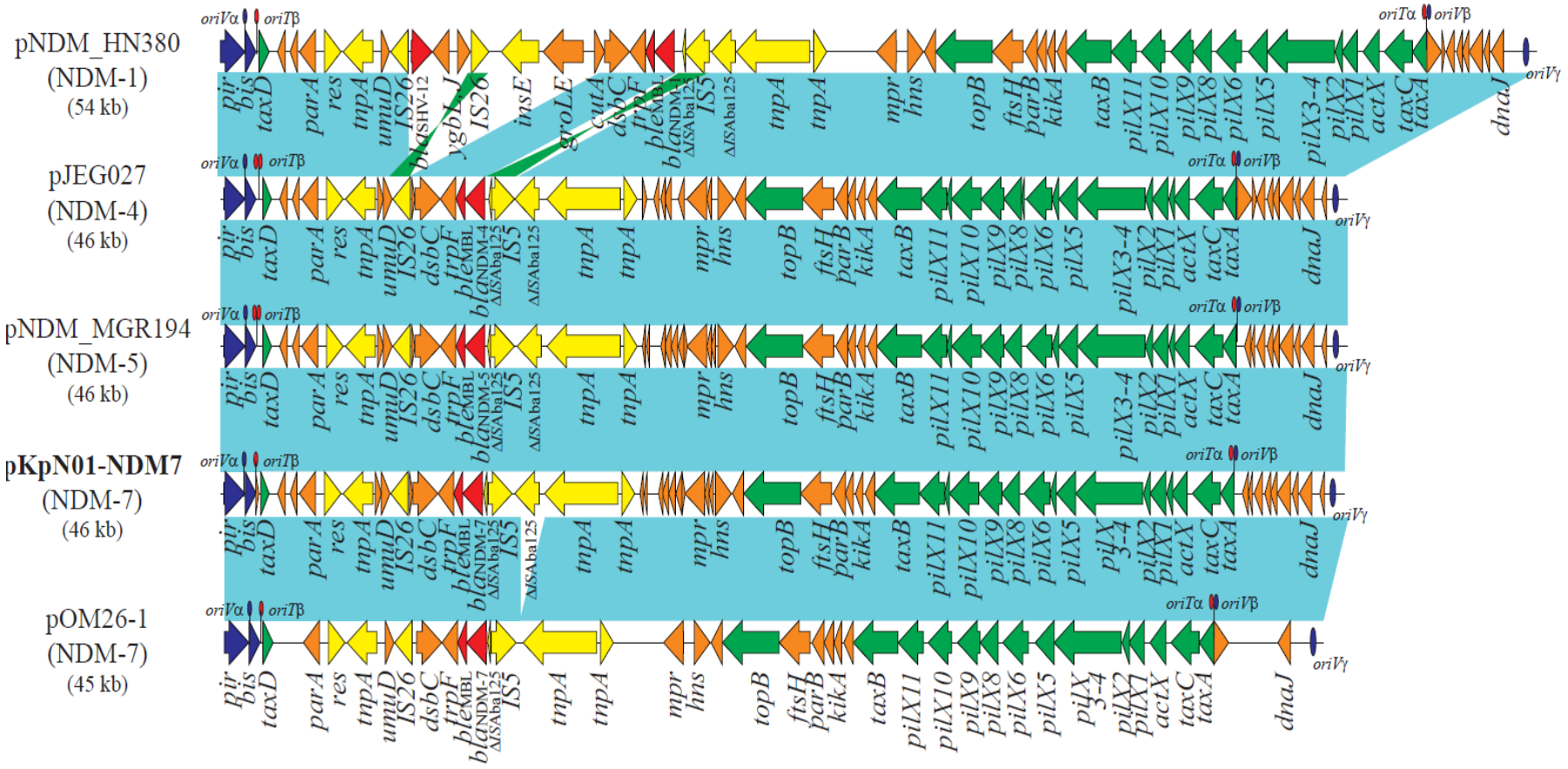


Figure 4. Model for rapid evolution on quinolone resistance-determining regions of *parC* and *gyrA* among Canadian outbreak isolates. MT17 was evolved from MT33 via precise stepwise mutations within 11 months of transmission; mutation on *parC* was accompanied with putative compensatory mutation in *uraA* gene. Interestingly, *uraA* mutation was reversed in MT17 to ancestral wild type. Abbreviations: Cipro-R, ciprofloxacin resistant; Ery-R, erythromycin resistant; Levo-R, levofloxacin resistant; SNPs, single-nucleotide polymorphisms.

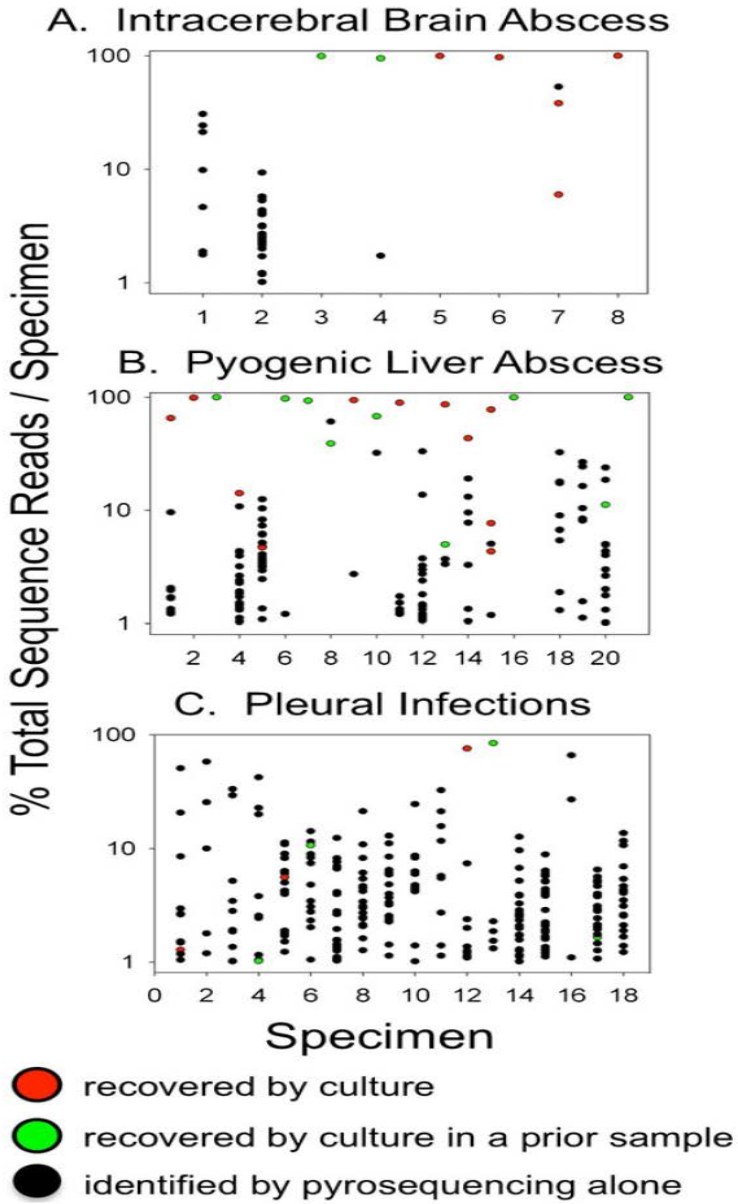
Deng X et al. CID March 2016

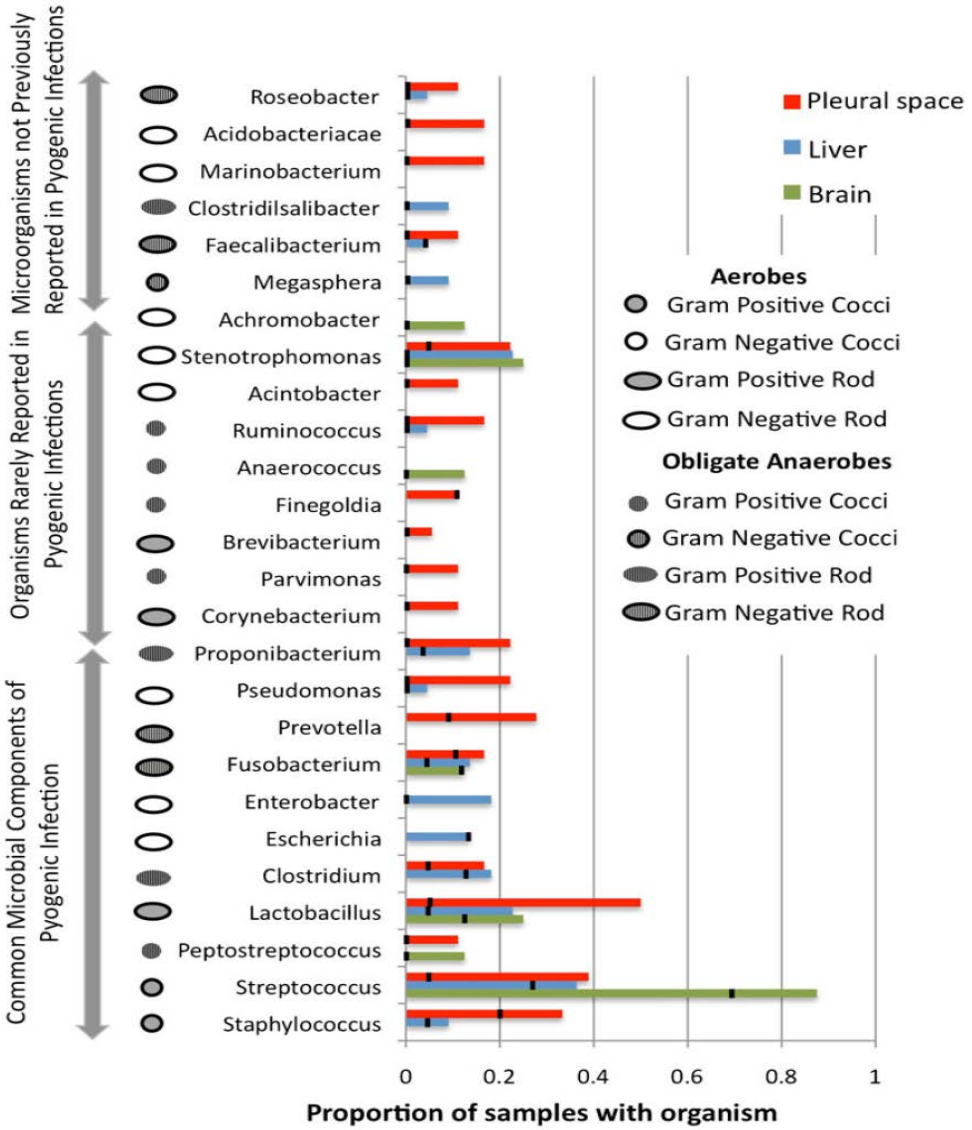
Enterobacteriaceae NDM-7 Plasmid Analysis - Calgary



Chen L et al. AAC 2016; 60:1258-1263.

Pyrosequencing Analysis of Pyogenic Abscess Samples



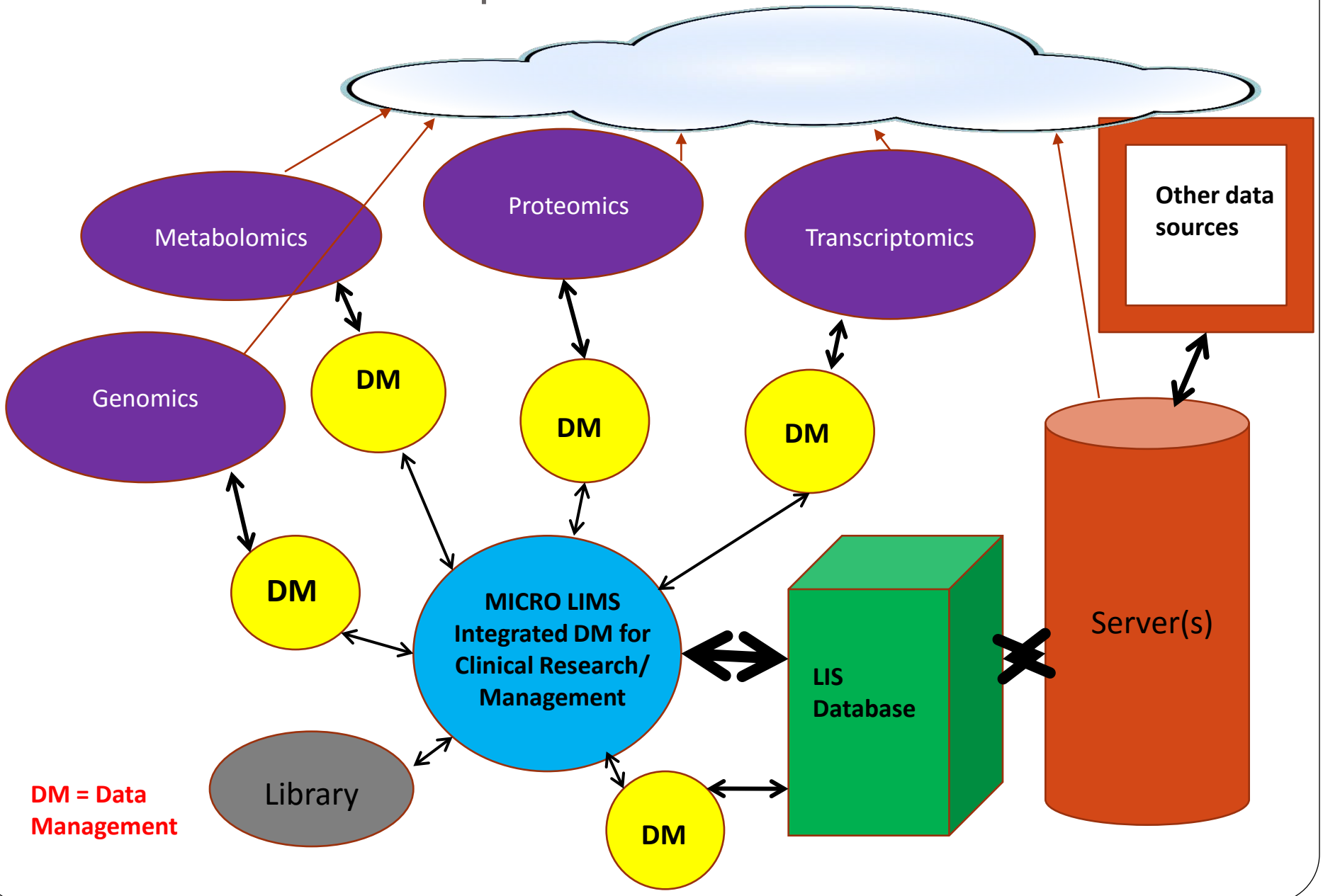


Sibley C et al Eur J Clin Microbiol 2012; 31:2679-91

The Emergence of Big –Omics Databases

- Genetic Code: Gene (Genomics)→*m*RNA (Transcriptomics)→Protein (Proteomics)→Metabolites (Metabolomics) plus environmental regulation and expression determine pathogen differences
- A systems biology approach with integration of all of the –omics data may be required to fully understand the infection process
  - 500-1200 genes in parasitic bacteria,
  - 1500-7500 genes in free-living bacteria
    - *M. genitalium* (470 genes), *E. coli* (4,288 genes)
  - 1500-2700 genes in archaea
- ~1500 different mRNA present at any time in bacterial growth
- Bacterial cells contain between 2-4 X $10^6/\mu m_3$ of metabolites
- A new generation of diagnostics will be developed out of the convergence of –omics information

Future State – Specialized Data LIMS Modules



Biosenors - What if you never had to culture urine?

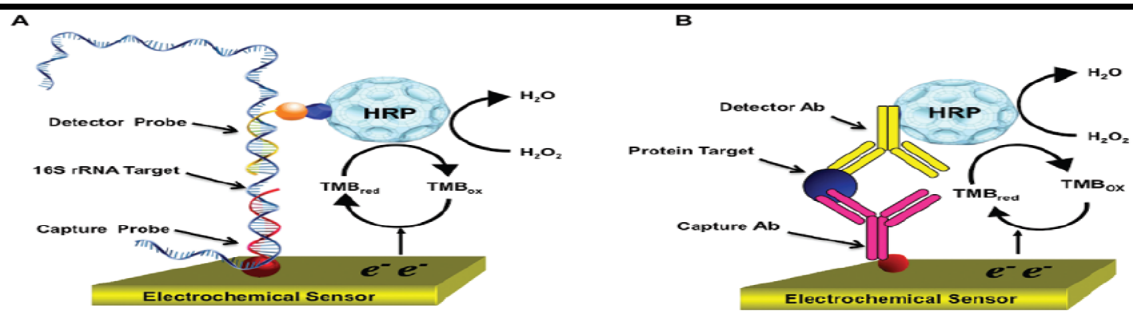


Figure 1. Schematics of urine-based diagnostics for electrochemical biosensor detection of nucleic acids and proteins. (A) Schematic of pathogen identification based on sandwich hybridization of bacterial 16S rRNA with capture and detector oligonucleotide probes; (B) Schematic of immunoassay based on sandwich detection host urinary protein with capture and detector antibodies. The two assays share similar assay parameters, including surface functionalization with biotinylated capture probes/antibodies, probe-target binding at 37°C, and amperometric detection using horseradish peroxidase (HRP) as the signaling enzyme [7,8].
doi:10.1371/journal.pone.0026846.g001

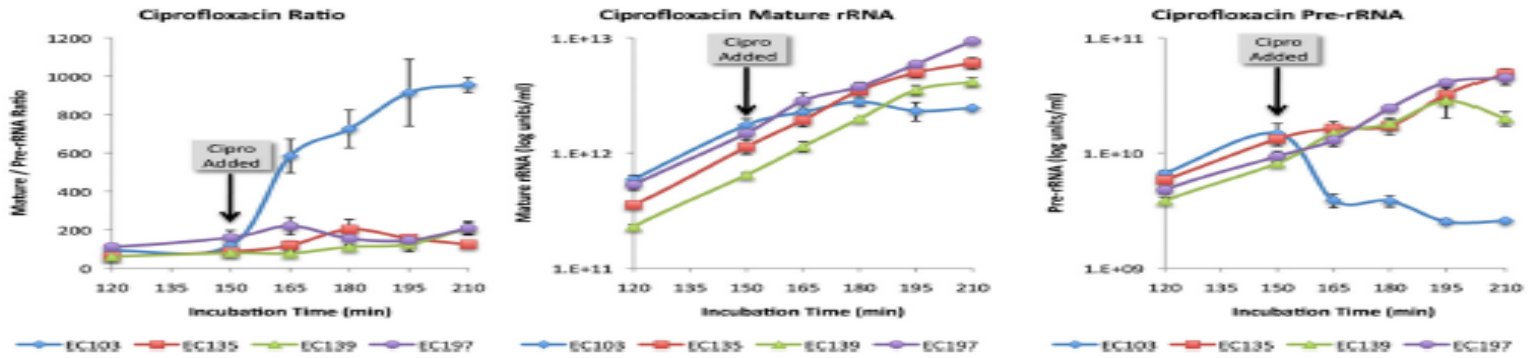
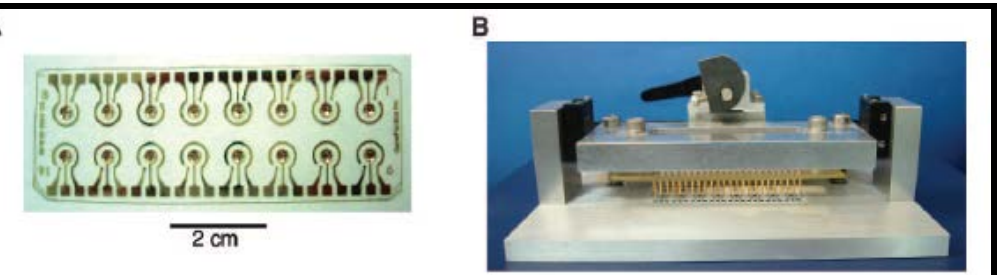


FIG 6 Comparison of ciprofloxacin-susceptible and -resistant *E. coli* strains. rRNA and pre-rRNA were measured in cultures of an *E. coli* clinical isolate susceptible to ciprofloxacin (EC103) and three ciprofloxacin-resistant isolates (EC135, EC139, and EC197). The amount of pre-rRNA in strain EC103 was significantly lower than that of the ciprofloxacin-resistant isolates within 15 min after addition of the antibiotic. Error bars estimated the standard deviations.



www.MICROBEDx.com (2016)
Automated rRNA Hybridization Probe System for Rapid UTI DX
ID/LFT*=30-45 min and AST=90-150 min.
LFT is a molecular marker of pyuria

Liao JC et al. JCM 2006; 44:561-570; Mohan R et al. PLosOne 2011;6:e26846; Halford C et al. AAC 2013; 57:936.

Biosensors - Ultra-Sensitive Malaria Detection

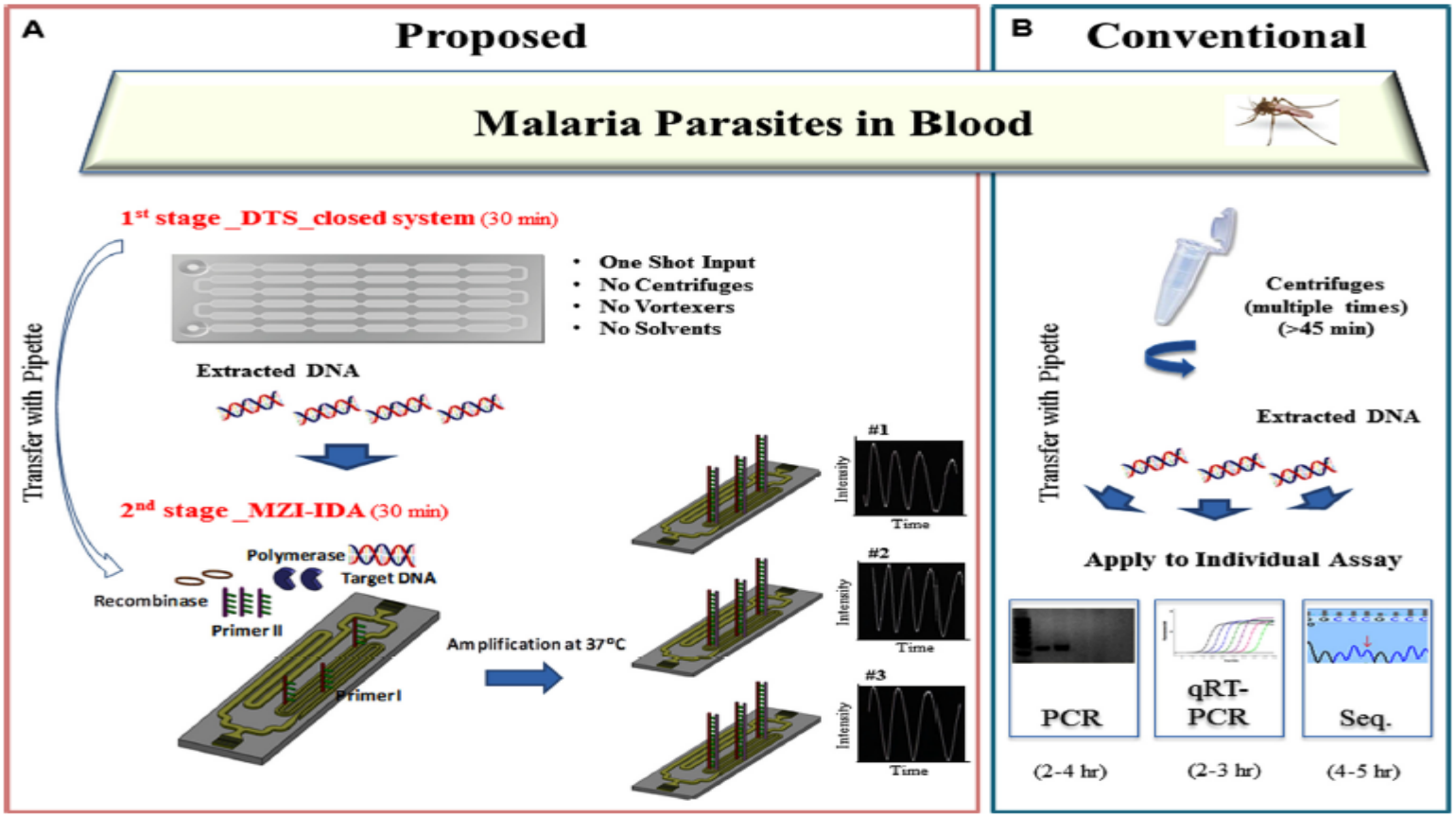


Fig. 1. Schematic representation of the principle of a sample-to-answer system based on nucleic acid amplification approach for diagnosis of malaria. (A) Proposed method that combined DTS (close system) as a sample processing module (1st stage) and MZI-IDA technique as a detection module (2nd stage). Malaria DNA extracted from DTS assay with one-shot input of all mixture solutions, less-instrument, and less hand-on steps followed by MZI-IDA system for the detection of malaria. In addition, the MZI-IDA system can measure at least 3 targets simultaneously. (B) Conventional method that combined Qiagen kit (open system) as a sample processing module and one of the down-stream analysis method as a detection module. Malaria DNA extracted from Qiagen kit with multiple centrifuges and hand-on steps followed by either PCR, qRT-PCR or sequencing assay for detection of malaria.

Liu Q et al. Biosensors and Bioelectronics 2016; 82:1-8

Point-of-Care Testing (POCT)

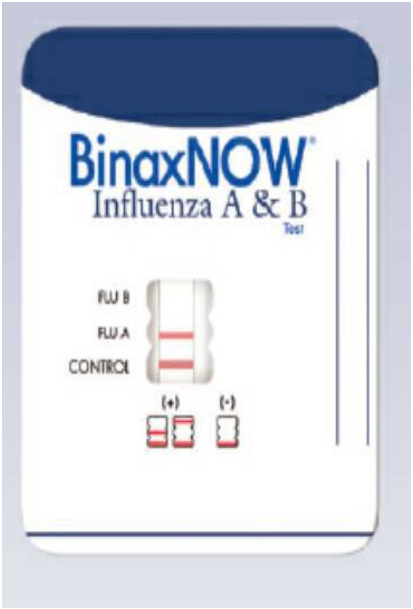
- New and innovative technology permits diagnostic tests to be done near the patient
- POCT traditionally done in RRL
- POCT will now be performed in a variety of patient-care settings, and to patient-based testing
  - ED, ICUs, physicians offices, cruise ships, STARS chopper, airlines, international space station
  - Resource-limited settings – many in Canada due to limits of geography, weather, distances and acceptable transport times for specimens to centralized lab

Wiencek J and J Nichols. Exp Rev Mol Diagn 2016; doi.org/10.1586/14737159.2016.1141678

What test(s) does your laboratory perform as a POCT?

1) Malaria
2) Rapid HIV
3) Influenza and/or RSV
4) Other (Group A Streptococcus, *C. difficile*, *S. pneumoniae* etc.)
5) All of the above

Rapid POCT Detection



Parasite density (n/μl)	Malaria rapid diagnostic test			
	Sensitivity (%)	x^2	*P*-value	Positive predictive value (%)
		47.690	<0.001*	
<100	0			0
100-999	1.9			100
≥1000	100			100

*Statistically significant $P < 0.05$

- Expanding Menu:
- Malaria
- G6PD
- Filariasis
- HIV
- Influenza A/B
- RSV
- *S. pneumoniae*
- *Legionella*
- Group A Streptococcus

Elechi, HA et al. Nigerian Med J 2015; 56:85-90

Rapid POCT Molecular Detection

Alere i

Molecular NEAR (Nicking Enzyme Amplification Reaction) – isothermal method. Molecular beacon with fluorophore detection. Single sample cartridge. Results in min. not hours.

Menu: Influenza A & B, Group A Streptococcus

GeneXpert Omni

Cartridge-based real-time PCR. Small (9 in.) X 2.2 lbs. field instrument. Battery operated, wireless, web-enabled

Menu: TB, MDR/XDR TB, HIV, Ebola

Wireless Multiplex Diagnosis of Infection Using an Integrated Quantum Dot Barcode Smartphone

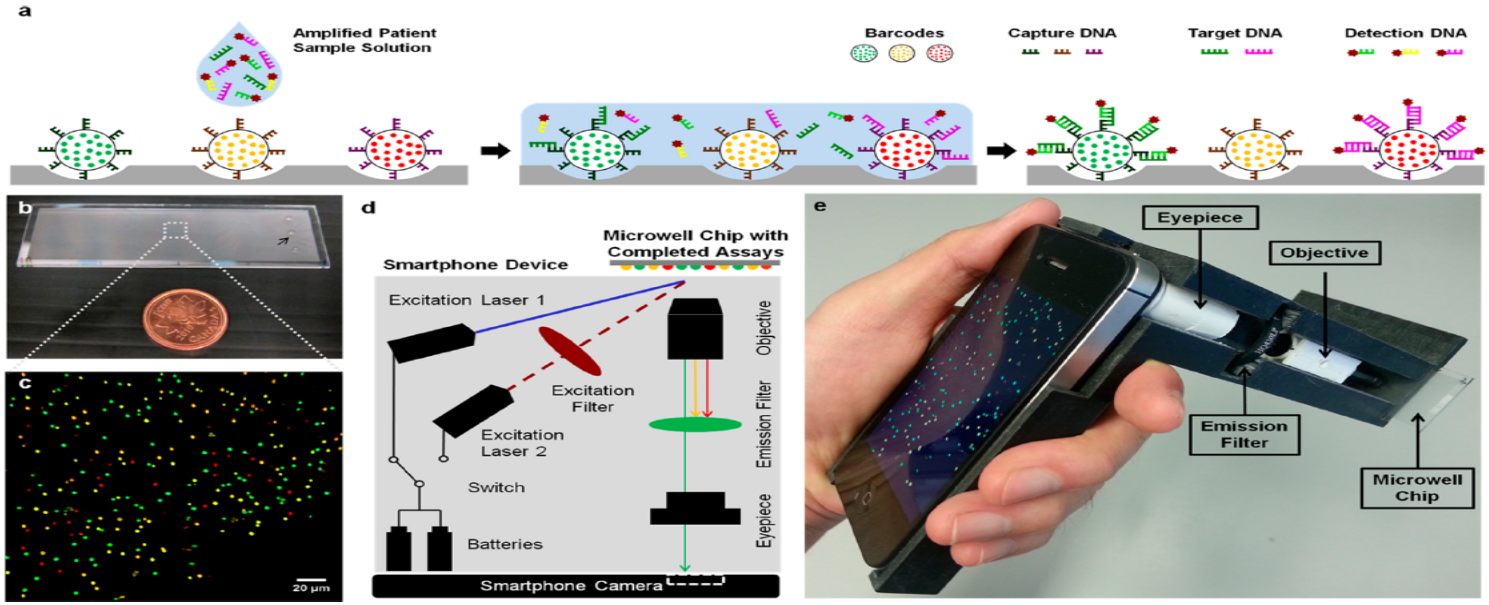
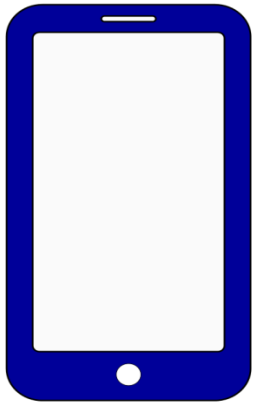


Figure 1. Overview of the smartphone device utilizing quantum dot barcodes. (a) Assay involves the addition of patient samples to a chip coated with microbeads, which are optically barcoded by quantum dots and are coated with molecules that recognize a target analyte. This target analyte joins the barcode to the secondary probe. Since each barcode is conjugated with a known biorecognition molecule for a specific pathogen target, the imaging of the optical signal from the barcode would allow for the identification of the pathogen and whether it is present in a patient sample (*i.e.*, lack of a secondary probe signal indicates no pathogen present, in this case, the yellow microbead). (b) Typical microwell chip containing different barcodes in each well. In a biological assay, we add 20 μL (for multiplexing synthetic targets) or 50 μL (for monoinfection patient samples) sample on the chip (see black arrow), incubate at 37 °C for 20—60 min, rinse, and image. (c) Smartphone camera captures the image of four different quantum dot barcodes arrayed on the surface of the chip. These barcodes are excited with a violet laser source (λ_{ex} = 405 nm, 50 mW), and optical signals are collected by a set of lenses, filtered with 430 nm long-pass filter, and imaged using an Apple iPhone 4S smartphone with an exposure time of 0.05 s. (d) Two excitation sources excite the quantum dot barcoded chip independently. The optical emission is collected by a set of objective and eyepiece lenses, imaged using a smartphone camera, and interpreted as positive or negative detection using a custom-designed algorithm. The images may be sent wirelessly to a centralized facility for further evaluation or for the mapping and tracking of infectious diseases. (e) Image of the smartphone device.

Ming K et al. ACSNANO 2015; 9:3060-3074.

Training of End Users

OLD Way



Summary of Changes

- Microbial threats will continue to emerge and evolve
- Microbiology testing will continue to transform from a 'whole cell' approach to a molecular –omics approach
  - LIMS will evolve to meet the requirements of new technology and handling big data
  - LIMS will evolve to store and integrate more diverse –omics data
- Biosensors will be deployed in new diagnostic devices/instruments in an expanding array of settings
- Microbiology POCT and near-patient testing will become routine
- Effective communication using modern tools and devices is key
- Gram stains and culture will be with us for the foreseeable future

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