Apports des nouvelles techniques d'identification des agents infectieux

Jean-Paul Mira

Medecine Intensive Réanimation - Cochin Hospital – Paris –Fr

jean-paul.mira@aphp.fr

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

Institut Cochin

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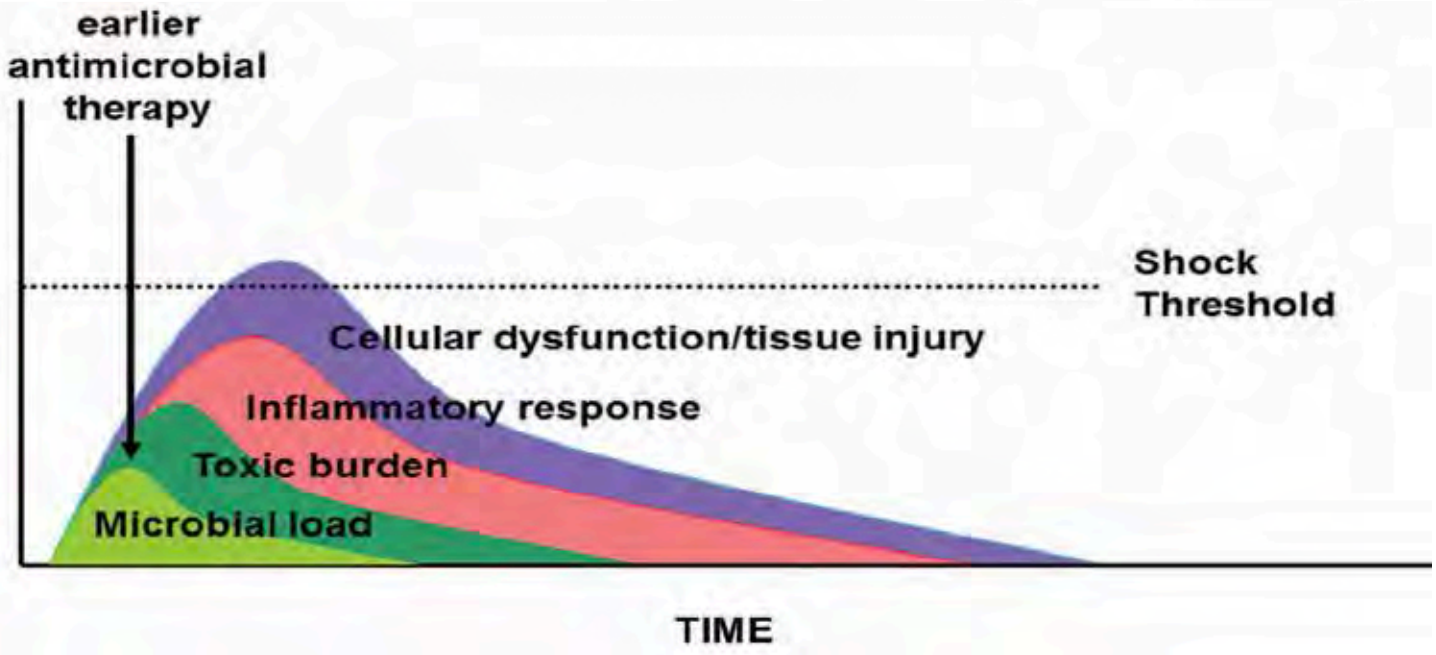
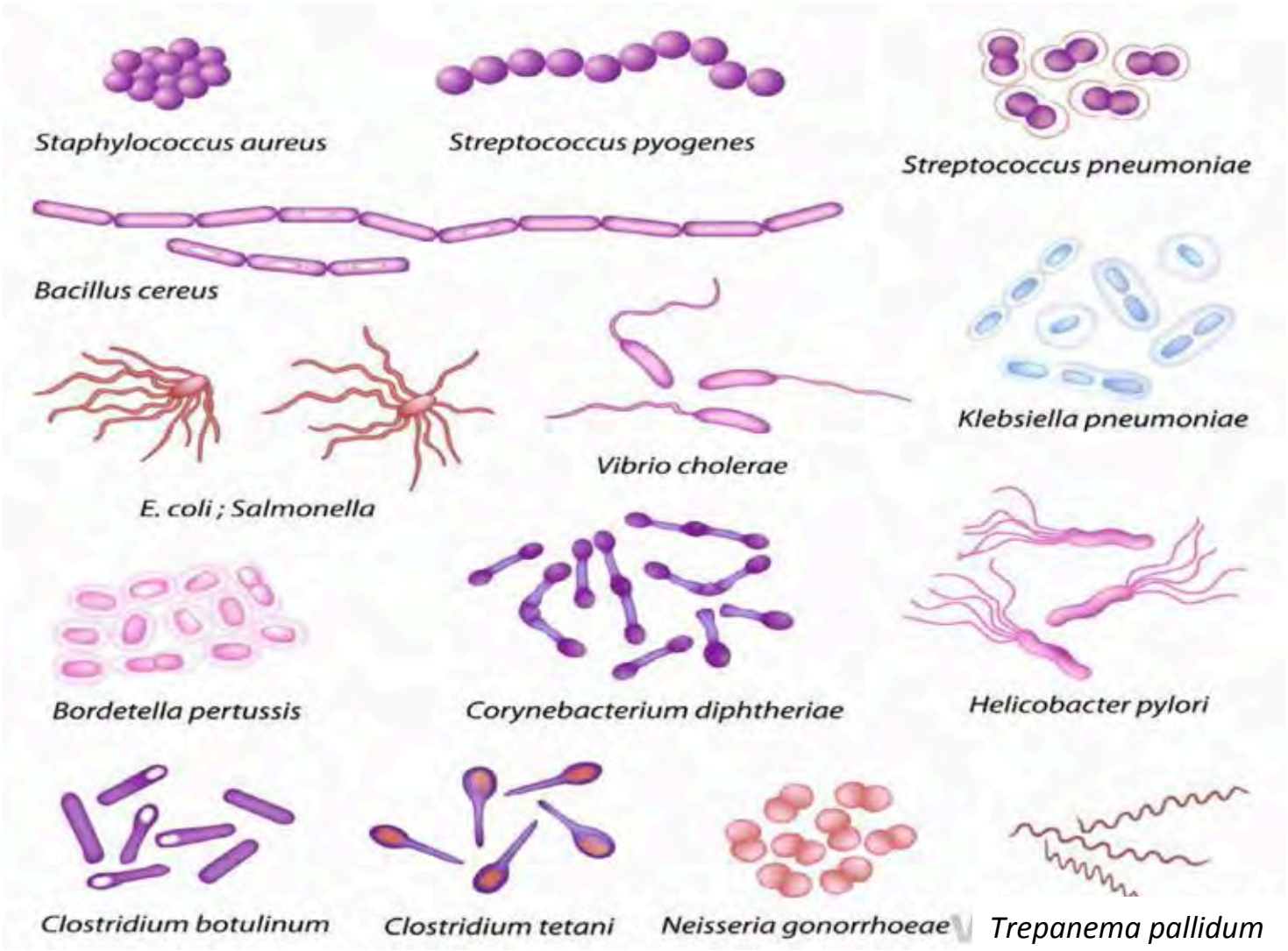
Conflict of interest

- **None**


earlier
antimicrobial
therapy
Shock
Threshold
Cellular dysfunction/tissue injury
Inflammatory response
Toxic burden
Microbial load
TIME


Staphylococcus aureus
Streptococcus pyogenes
Streptococcus pneumoniae
Bacillus cereus
Klebsiella pneumoniae
Vibrio cholerae
E. coli ; Salmonella
Bordetella pertussis
Corynebacterium diphtheriae
Helicobacter pylori
Clostridium botulinum
Clostridium tetani
Neisseria gonorrhoeae
Trepanema pallidum

Apports des nouvelles techniques d'identification des agents infectieux


Surviving
Sepsis
Campaign
2017
6

BUNDLES 2016

TO BE COMPLETED WITHIN 3 HOURS:

1) Measure lactate level.
2) Obtain blood cultures prior to administration of antibiotics.
3) Administer broad spectrum antibiotics.
4) Administer 30 ml/kg crystalloid for hypotension or lactate ≥4mmol/L.

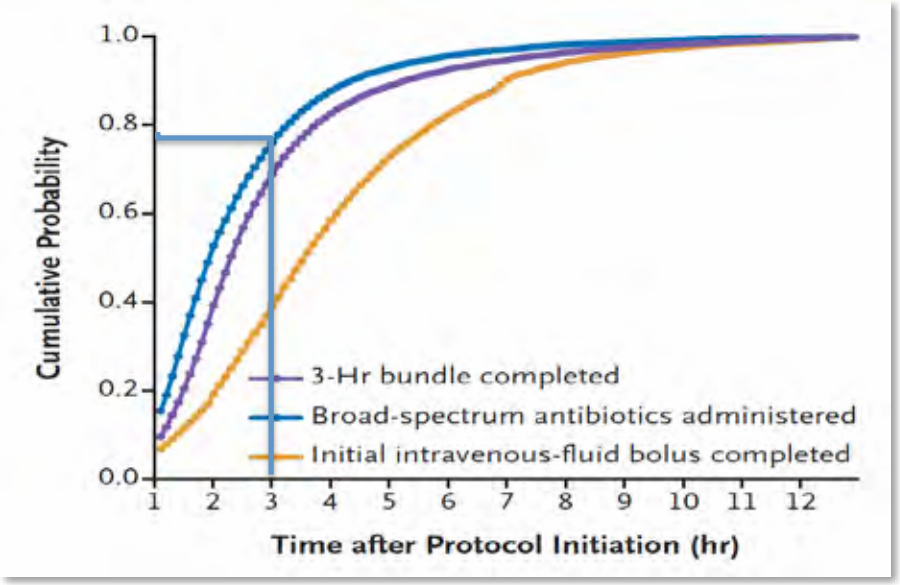
"Time of presentation" is defined as the time of triage in the emergency department or, if presenting from another care venue, from the earliest chart annotation consistent with all elements of severe sepsis or septic shock ascertained through chart review.

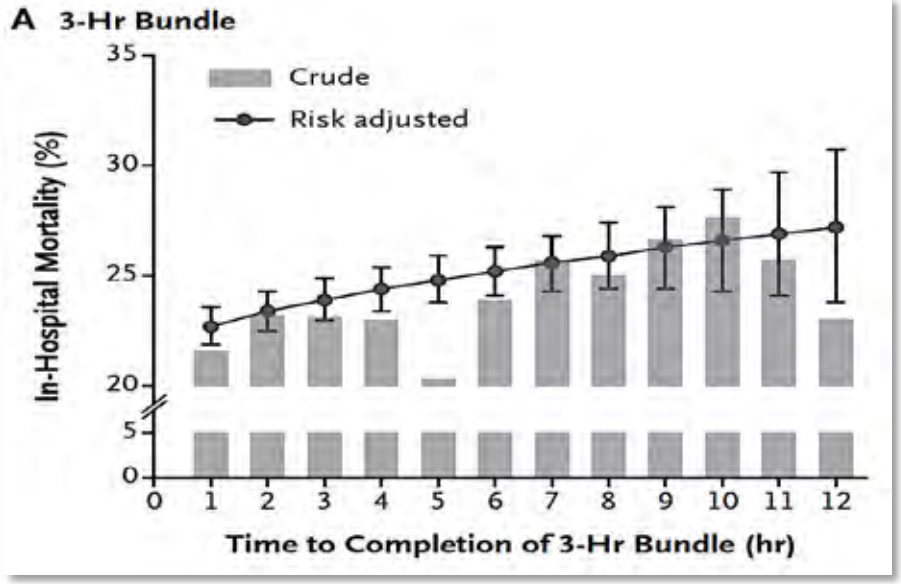
TO BE COMPLETED WITHIN 6 HOURS:

5) Apply vasopressors (for hypotension that does not respond to initial fluid resuscitation) to maintain a mean arterial pressure (MAP) ≥65 mm Hg.
6) In the event of persistent hypotension after initial fluid administration (MAP < 65 mm Hg) or if initial lactate was ≥4 mmol/L, re-assess volume status and tissue perfusion and document findings according to Table 1.
7. Re-measure lactate if initial lactate elevated.

Time to Treatment and Mortality during Mandated Emergency Care for Sepsis

49,331 patients at 149 hospitals

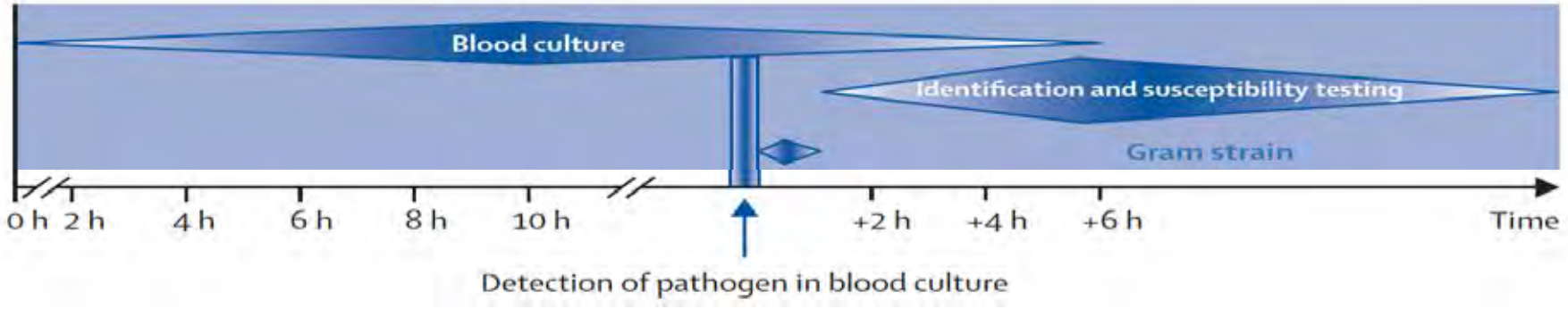


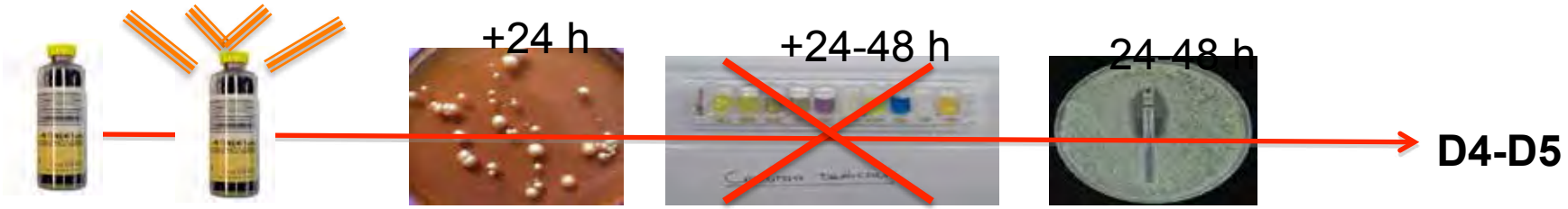


Seymour CW. N Engl J Med 2017;376:2235-44.

2012 RECOMMENDATIONS	2016 RECOMMENDATIONS
C. DIAGNOSIS 1. Cultures as clinically appropriate before antimicrobial therapy if no significant delay (> 45 min) in the start of antimicrobials (grade 1C). At least 2 sets of blood cultures (both aerobic and anaerobic bottles) be obtained before antimicrobial therapy with at least 1 drawn percutaneously and 1 drawn through each vascular access device, unless the device was recently (<48 hrs) inserted (grade 1C). 2. Use of the 1,3-ß-D-glucan assay (grade 2B), mannan and anti-mannan antibody assays (2C), if available, and invasive candidiasis in differential diagnosis of cause of infection. 3. Imaging studies performed promptly to confirm a potential source of infection (UG).	**C. DIAGNOSIS** 1. **We recommend that** appropriate routine microbiologic cultures (including blood) be obtained before starting antimicrobial therapy in patients with suspected sepsis or septic shock if doing so results in no substantial delay in the start of antimicrobials **(BPS)**. **Remarks: Appropriate routine microbiologic cultures always include at least two sets of blood cultures (aerobic and anaerobic).**

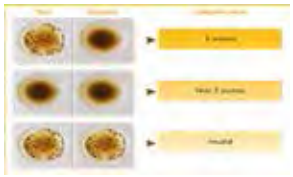
De-escalation of Antibiotics


Blood culture
Identification and susceptibility testing
Gram strain
0 h
2 h
4 h
6 h
8 h
10 h
+2 h
+4 h
+6 h
Time
Detection of pathogen in blood culture

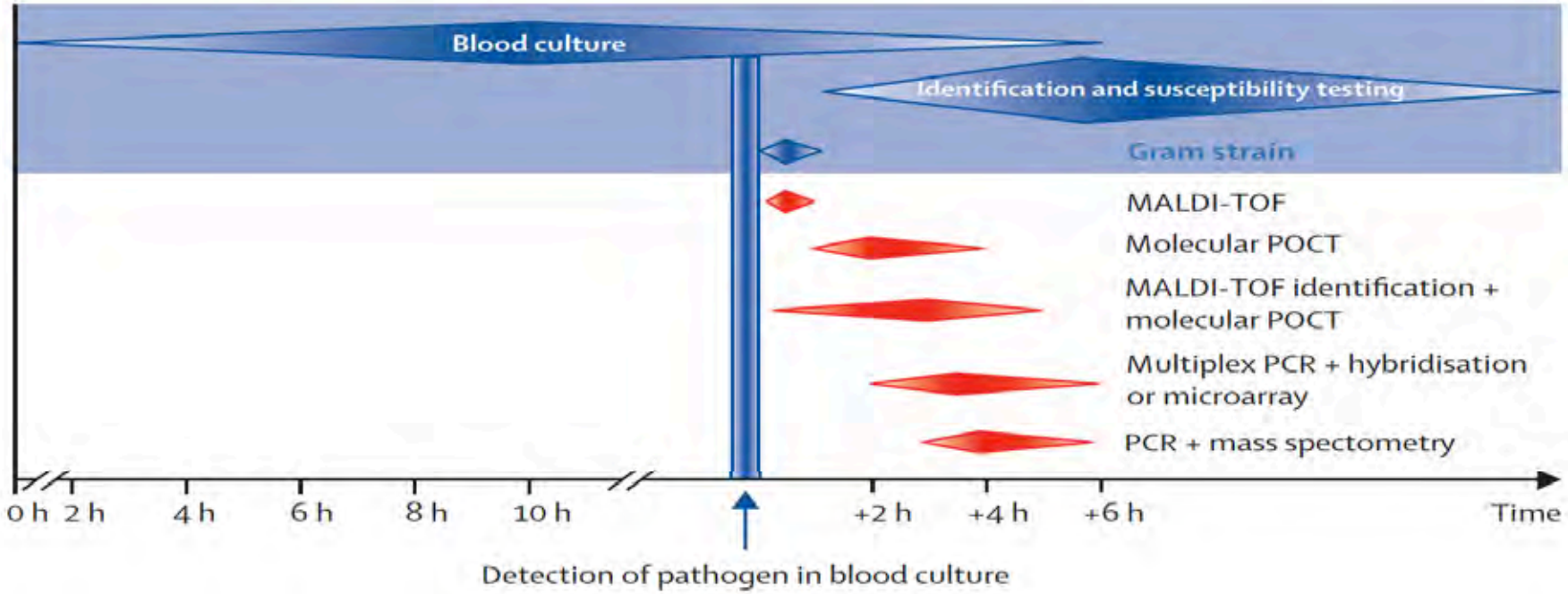


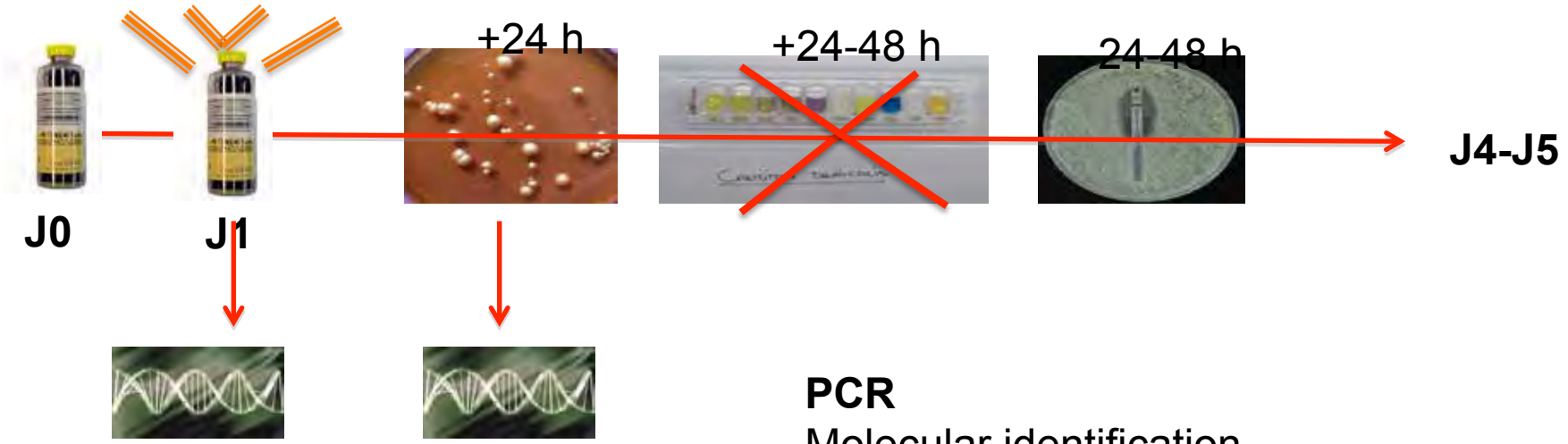
Chromogenic culture

Agglutination tests

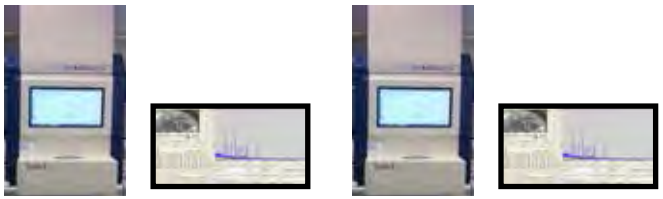


Decreased time for identification
C. albicans, C. krusei, C. glabrata


Blood culture
Identification and susceptibility testing
Gram strain
MALDI-TOF
Molecular POCT
MALDI-TOF identification + molecular POCT
Multiplex PCR + hybridisation or microarray
PCR + mass spectometry
0 h
2 h
4 h
6 h
8 h
10 h
+2 h
+4 h
+6 h
Time
Detection of pathogen in blood culture

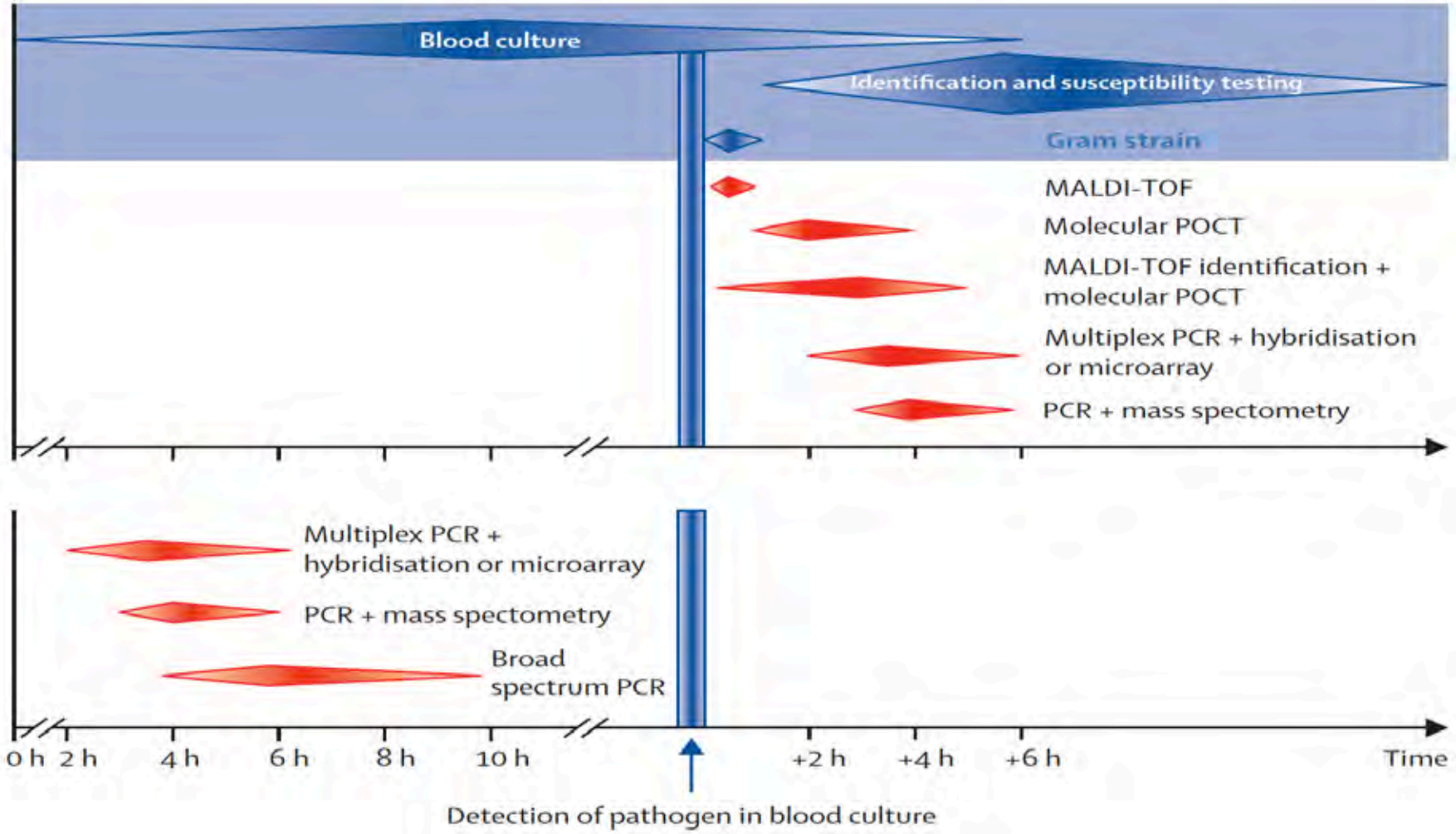


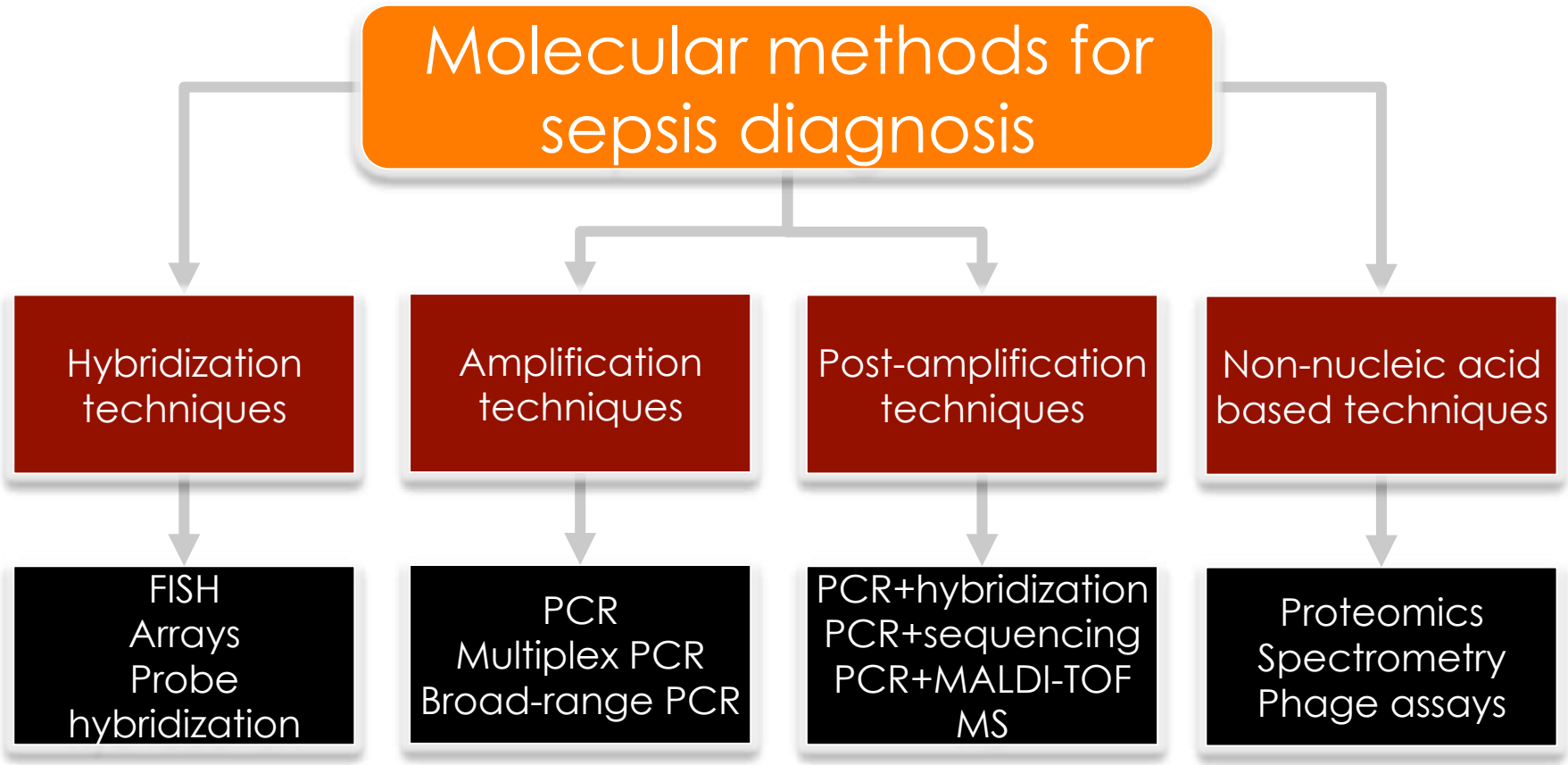
PCR
Molecular identification



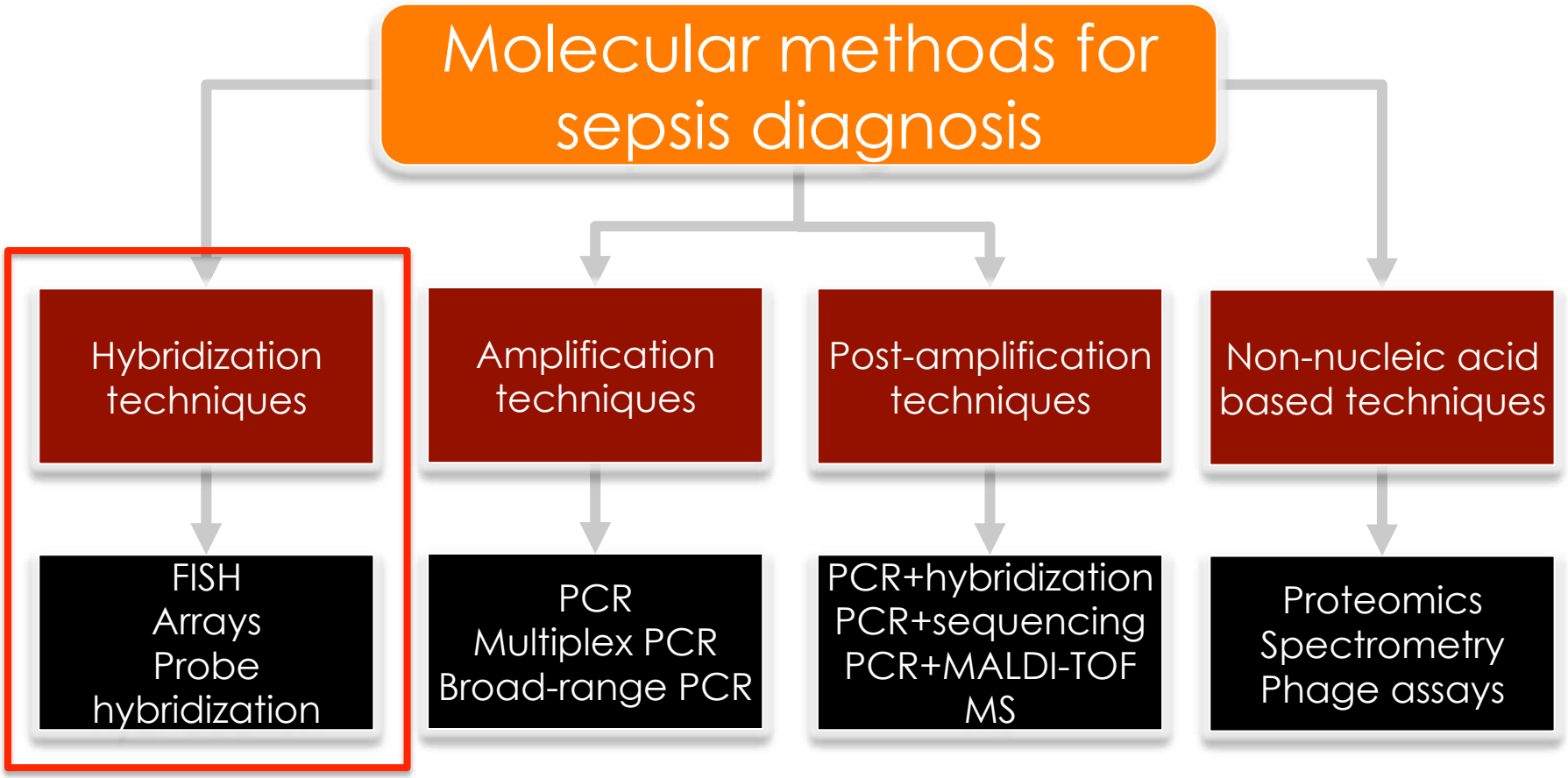
MALDI-TOF
=> Identification

All fungi


Blood culture
Identification and susceptibility testing
Gram strain
MALDI-TOF
Molecular POCT
MALDI-TOF identification + molecular POCT
Multiplex PCR + hybridisation or microarray
PCR + mass spectometry
Multiplex PCR + hybridisation or microarray
PCR + mass spectometry
Broad spectrum PCR
0 h
2 h
4 h
6 h
8 h
10 h
+2 h
+4 h
+6 h
Time
Detection of pathogen in blood culture

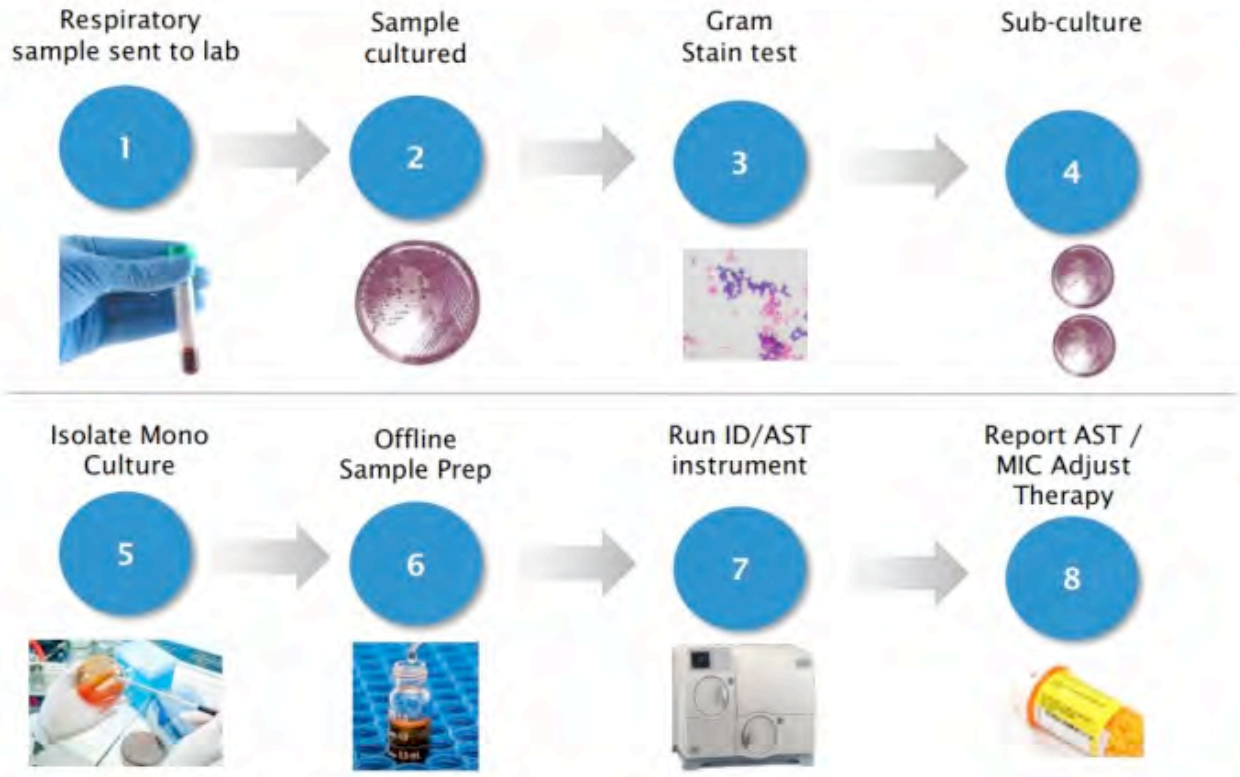


European Journal of Microbiology and Immunology 4 (2014) 1, pp. 1–25



European Journal of Microbiology and Immunology 4 (2014) 1, pp. 1–25

Today's microbiology lab workflow, ~72hrs



Sample to answer BACcel workflow, <6 hrs

Specimen

- Positive Blood
- Bronchoalveolar lavage fluid
- Wound Swab

<6 Hours

Automated Sample Prep *(Patented)*	Automated Imaging *(Patent Filed)*	Computerized Image Analysis *(Patent Filed)*
1	2	3

Patient Report

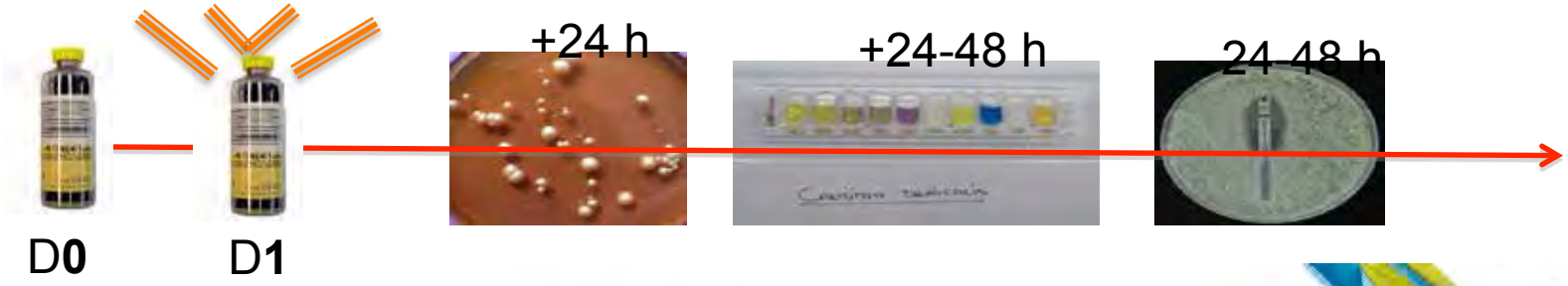
- AST / MIC
- Quantitative ID

Evaluation of the Accelerate Pheno System for Fast Identification and Antimicrobial Susceptibility Testing from Positive Blood Cultures in Bloodstream Infections Caused by Gram-Negative Pathogens

The utilization of the Accelerate Pheno system reduced the time to result for identification by 27.49 h (P 0.0001) and for AST by 40.39 h (P 0.0001) compared to culture-based methods in our laboratory setting. In conclusion, the Accelerate Pheno system provided fast, reliable results while significantly improving turnaround time in blood culture diagnostics of Gram negative BSI.

Marschal M. J Clin Microbiol 2017; 55:2116

	Identification Only	Identification and Susceptibility
Faster	 T2Biosystems  BIOFIRE  Nanosphere  UNYVERO I-4 LYSATOR UNYVERO C8 COCKPIT UNYVERO A80 ANALYZER UNYVERO APPLICATION CARTRIDGES	 ACCELERATE
Slower	BIOMÉRIEUX   BRUKER	 BD  BIOMÉRIEUX



Future MICROBIOLOGY

REVIEW

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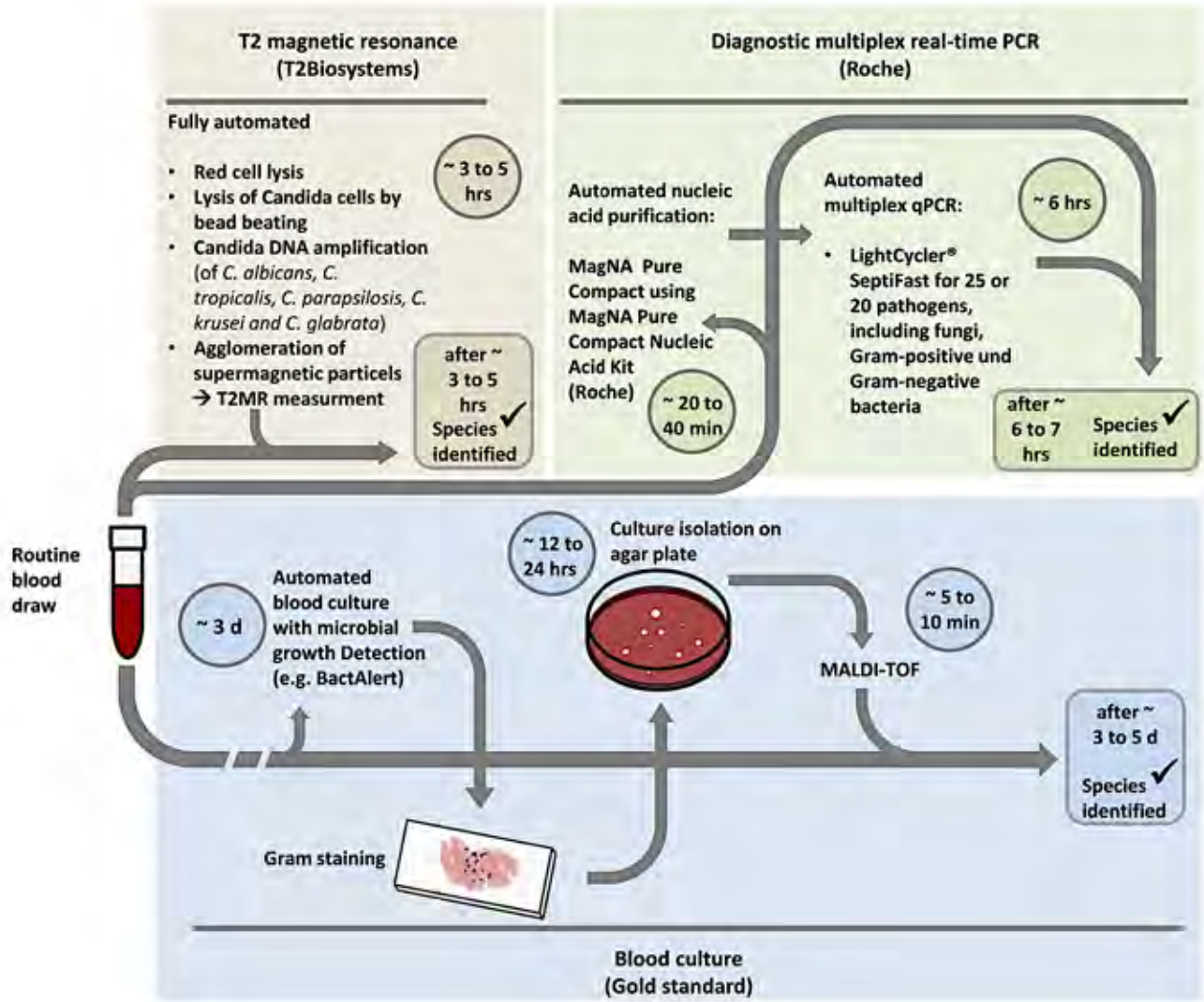
T2MR and T2Candida: novel technology for the rapid diagnosis of candidemia and invasive candidiasis

Michael A Pfaller[*1,2], Donna M Wolk[3] & Thomas J Lowery[1]

T2Biosystems

Pfaller MA. Future Microbiol. 2015; 11, 103


T2 magnetic resonance
(T2Biosystems)
Fully automated
Red cell lysis
Lysis of Candida cells by bead beating
Candida DNA amplification (of C. albicans, C. tropicalis, C. parapsilosis, C. krusei and C. glabrata)
Agglomeration of supermagnetic particels → T2MR measurment
~ 3 to 5 hrs
after ~ 3 to 5 hrs Species identified
Diagnostic multiplex real-time PCR
(Roche)
Automated nucleic acid purification:
MagNA Pure Compact using MagNA Pure Compact Nucleic Acid Kit (Roche)
~ 20 to 40 min
Automated multiplex qPCR:
LightCycler® SeptiFast for 25 or 20 pathogens, including fungi, Gram-positive und Gram-negative bacteria
~ 6 hrs
after ~ 6 to 7 hrs Species identified
Routine blood draw
~ 3 d
Automated blood culture with microbial growth Detection (e.g. BactAlert)
~ 12 to 24 hrs
Culture isolation on agar plate
~ 5 to 10 min
MALDI-TOF
after ~ 3 to 5 d Species identified
Gram staining
Blood culture
(Gold standard)

T2MR and T2Candida: novel technology for the rapid diagnosis of candidemia and invasive candidiasis

Table 7. Summary of T2Candida detection of invasive candidiasis and candidemia.

Disease detected	T2Candida	Blood culture	Total *Candida* infections
Candidemia	31	33	33
Invasive candidiasis	12	0	12
Probable or suspected invasive candidiasis	10	0	10
Total cases	53	33	55
Sensitivity	96.4% (53/55)	60% (33/55)	

Pfaller MA. Future Microbiol. 2015; 11, 103

T2 Magnetic Resonance Assay for the Rapid Diagnosis of Candidemia in Whole Blood: A Clinical Trial

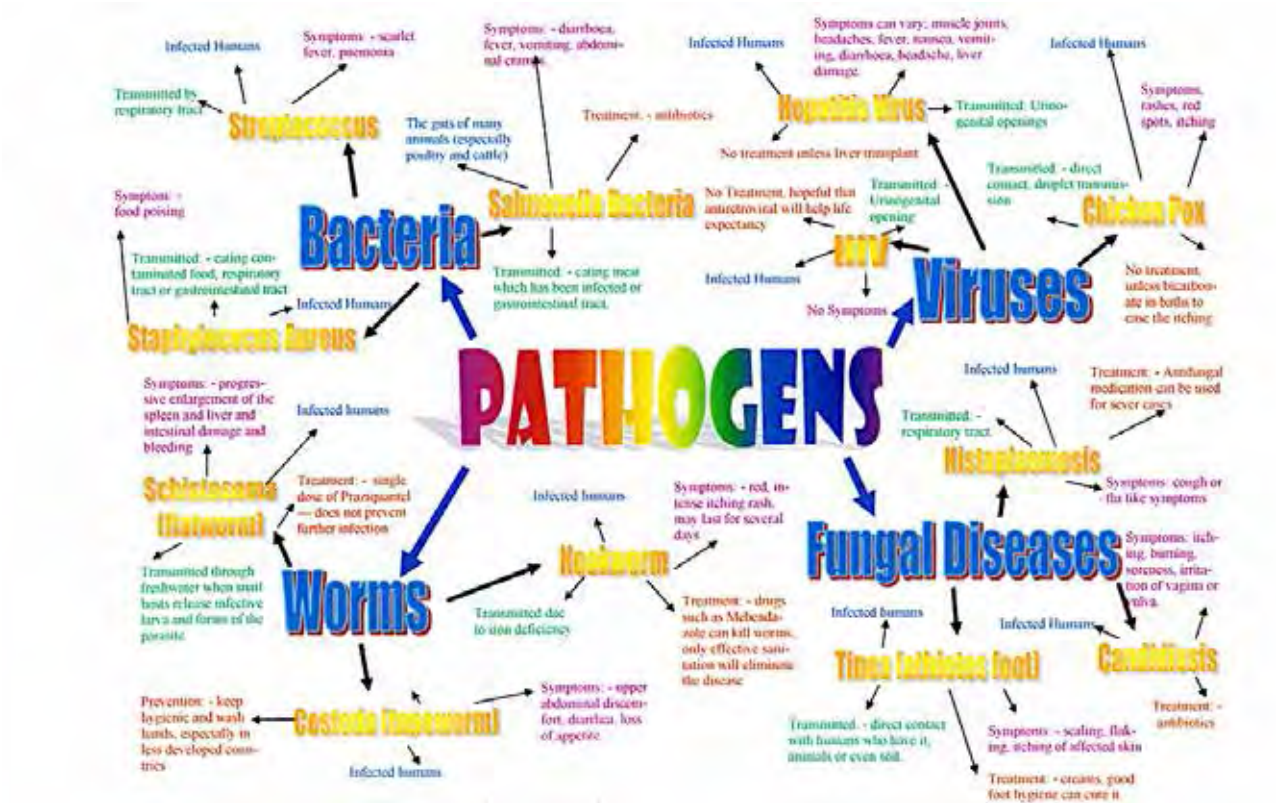
1801 Pts

Conclusions. **T2MR is the first fully automated technology that directly analyzes whole blood specimens to identify species without the need for prior isolation of Candida species, and represents a breakthrough shift into a new era of molecular diagnostics.**

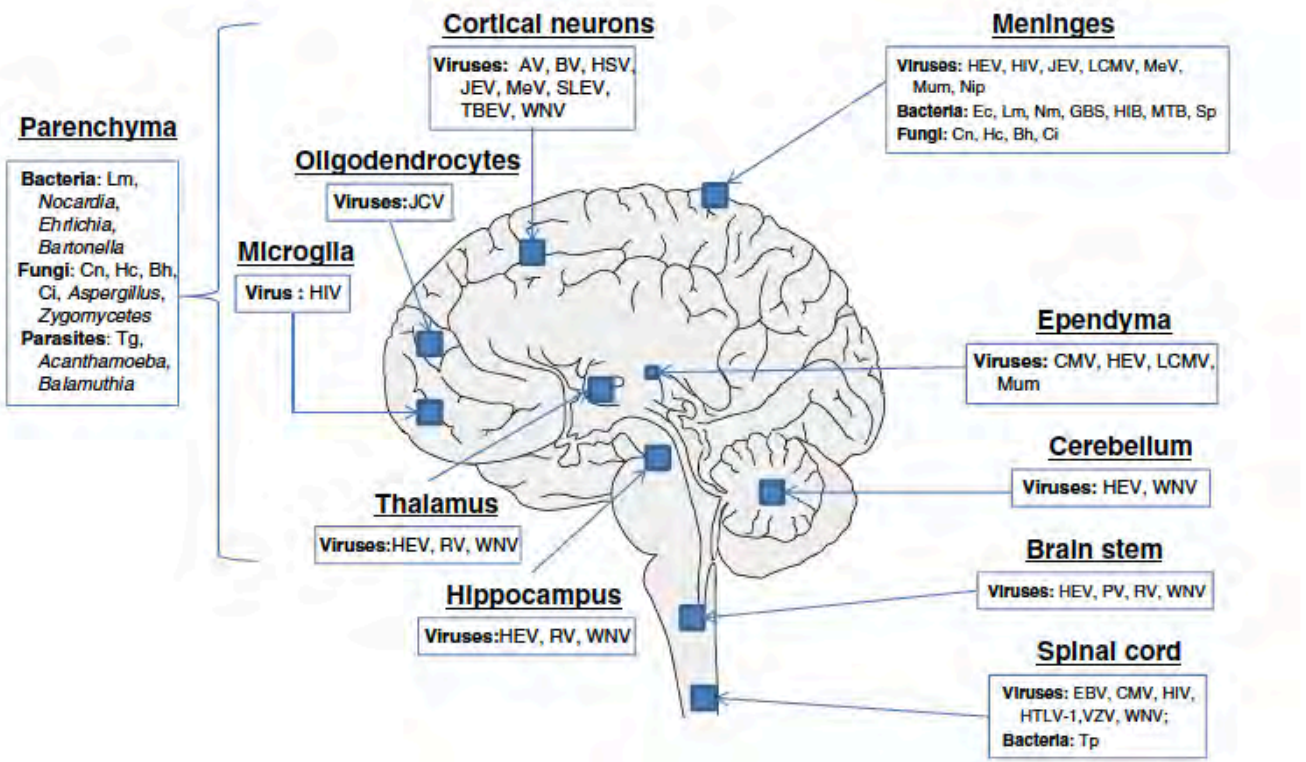
Specificity			
Overall per patient[a]	1516/1545	98.1	97.3–98.7
Overall per assay[a]	5114/5146	99.4	99.1–99.6
Per species[a]			
C. albicans/tropicalis	1679/1697	98.9	98.3–99.4
C. parapsilosis	1736/1749	99.3	98.7–99.6
C. krusei/glabrata	1699/1700	99.9	99.7–100.0

Mylonakis E. Clin Infect Dis 2015; 60: 892

Apports des nouvelles techniques d'identification des agents infectieux



Laboratory Diagnosis of Central Nervous System Infection



He T. Curr Infect Dis Rep 2016; 18:35

Laboratory Diagnosis of Central Nervous System Infection

Test	Ease of performance	Turnaround time	Result interpretation	Advantages	Disadvantages
Microscopic examination	Could be performed in routine clinical lab	0.5–1 h	Direct if correlated with symptoms	Rapid	Poor sensitivity and specificity; special skills are needed for interpretation
Rapid antigen	Could be performed in routine clinical lab	15–30 min	Direct if correlated with symptoms	Rapid	False positive results
Culture	Could be performed in sophisticated clinical lab	2–14 days	Definite	For phenotypic drug susceptibility testing	Time consuming and poor sensitivity; limited microorganisms are culturable
Serology	Could be performed in routine clinical lab	2–8 h	Indirect	Automation	Results are generally retrospective; cross-reactions; immunosuppressed host may be unable to mount a response
Molecular diagnostic	Could be performed in routine clinical lab with trend in point of care	2–8 h	Direct without knowing microbial viability	High sensitivity and specificity	Not the test of cure; clinical relevance need to be determined

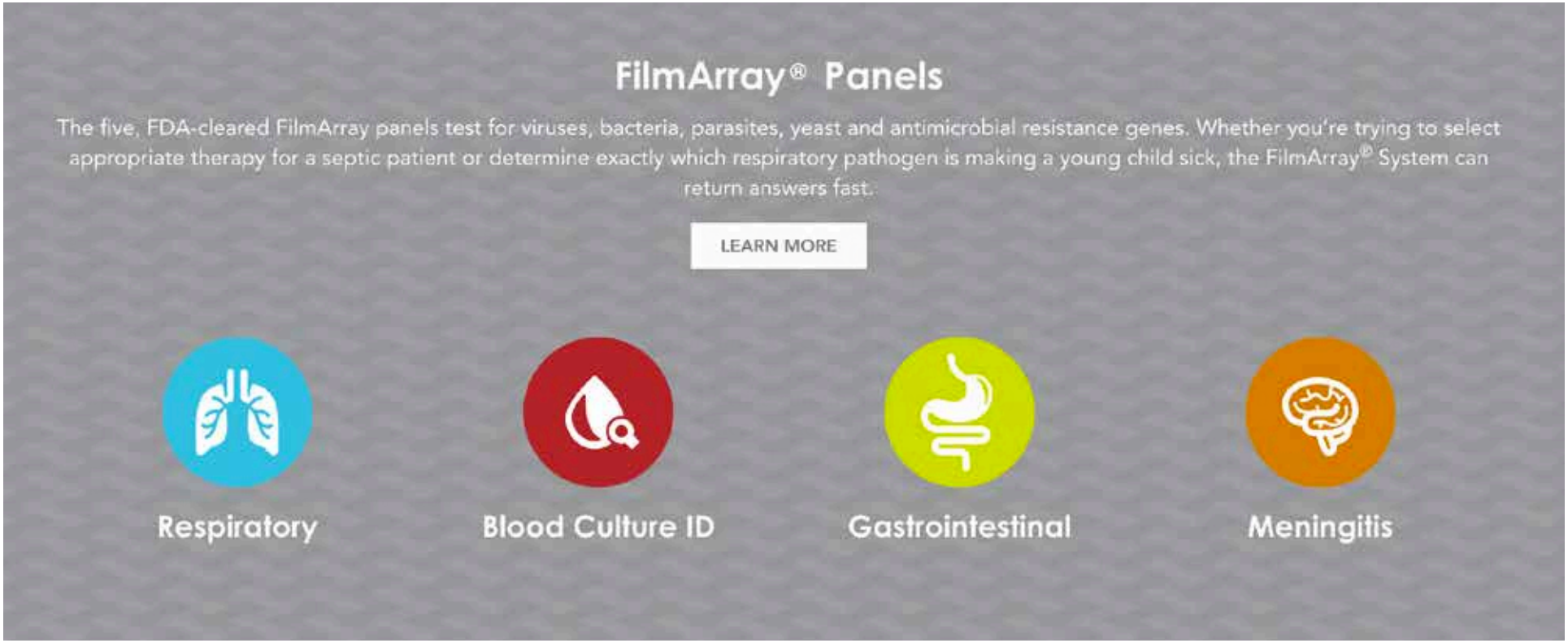
He T. Curr Infect Dis Rep 2016; 18:35

Laboratory Diagnosis of Central Nervous System Infection

Manufacturer	Assay name	Organism	Technology	Specimen type	Comments
Cepheid, Sunnyvale, CA	Xpert EV Assay	Enteroviruses	Real-time PCR	CSF	Target is the 5′ untranslated region; fully integrated and random access; 2-h 30′ TAT
bioMeriuex, Durham, NC	NucliSENS EasyQ Enterovirus assay	Enteroviruses	NASBA	CSF	Target is the 5′ untranslated region; separate nucleic acid and amplification/detection steps, automated; 5-h TAT
Focus Diagnostics, Cypress, CA	Simplexa HSV-1&2 Direct	HSV-1, HSV-2	Real-time PCR	CSF; genital lesions	Target is the DNA polymerase; semi-automated; no extraction; 1-h TAT
BioFire Diagnostics, Salt Lake City, UT	FilmArray Meningitis/Encephalitis panel	EcK1, Hi, Lm, Nm, Sa, Sp, CMV, enterovirus, HSV-1, HSV-2, HHV-6	Multiplex PCR followed by solid array	CSF	Fully integrated and random access; approximately 1-h TAT

He T. Curr Infect Dis Rep 2016; 18:35

Comparative evaluation of the FilmArray meningitis/encephalitis molecular panel in a pediatric population



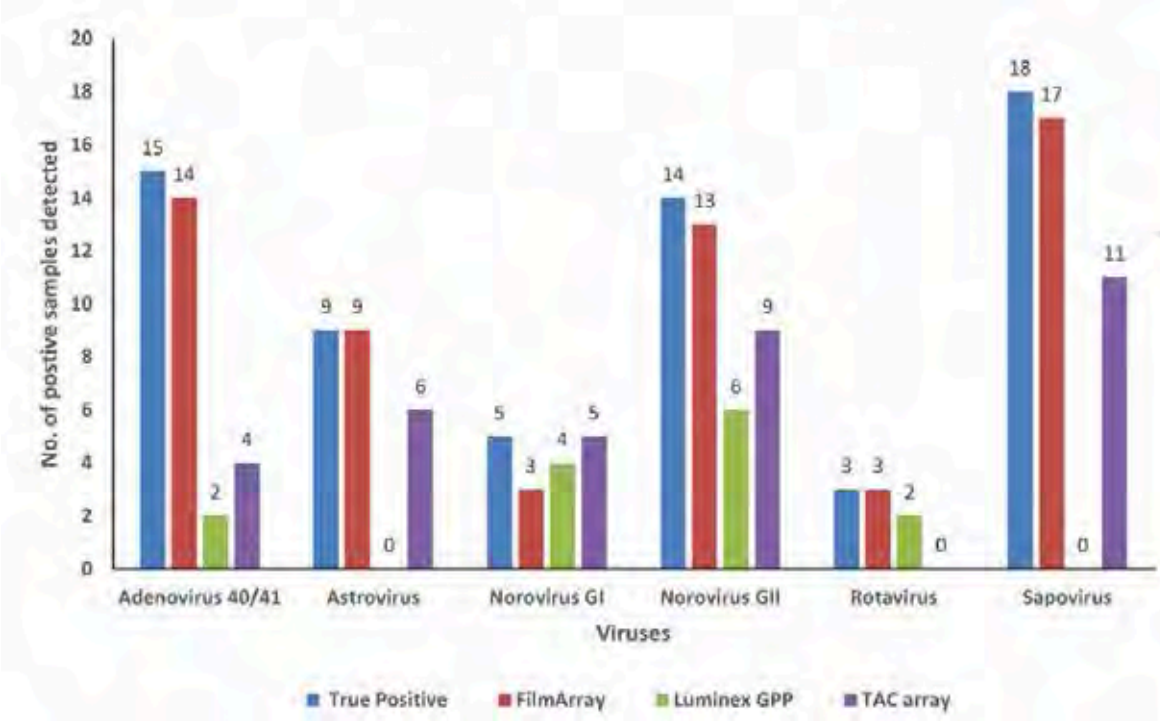
Graf EH. Diag Microb Infect Dis 2017; 87: 92

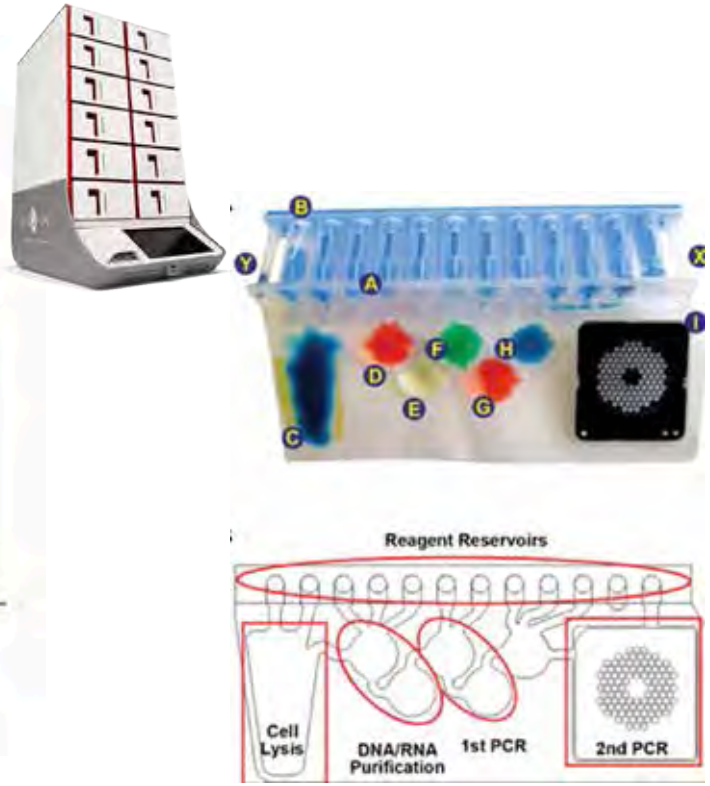
Comparison of three multiplex gastrointestinal platforms for the detection of gastroenteritis viruses

Parameters	FilmArray	Luminex GPP (IVD)*	TAC system	Reference testing (real-time PCR + sequencing
Number of targets	22	15	36 (Max. of 40)	1 (max 2)
Hand-on processing time (minutes)	5	60	60	60
Total turn-around time (hours)	1	7	4	48
No. of clinical specimens per assay	1	96	6	96
Type of system	closed	open	Open extraction, closed system thereafter	open
Technology	Nested PCR and melting curve	Fluorescent bead-based detection	fluorescent (TaqMan) detection	fluorescent (TaqMan) detection

Chabra P. J Clin Virol 2017; 95: 66

Comparison of three multiplex gastrointestinal platforms for the detection of gastroenteritis viruses





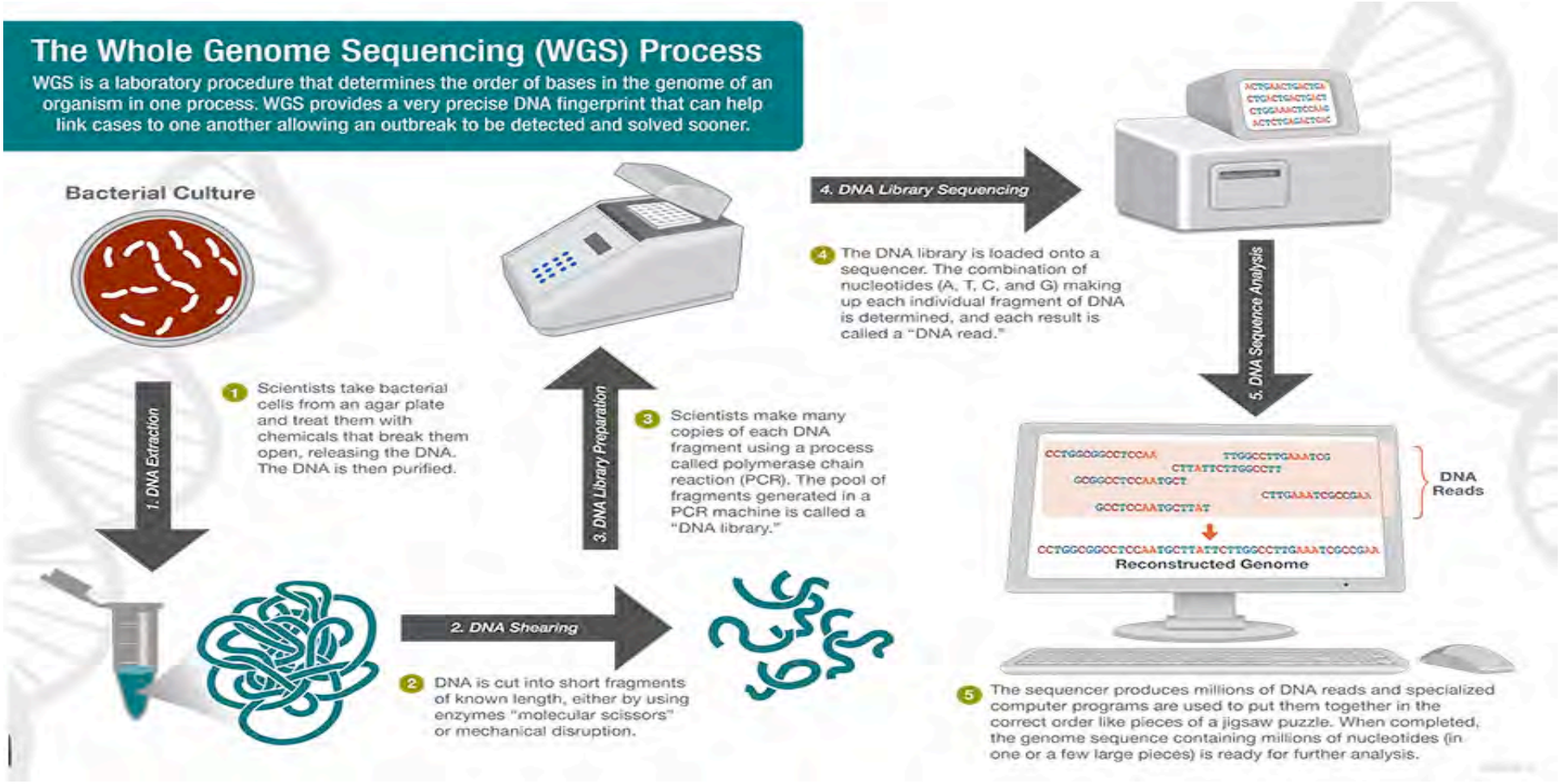
Chabra P. J Clin Virol 2017; 95: 66

Apports des nouvelles techniques d'identification des agents infectieux

Apports des nouvelles techniques d'identification des agents infectieux

The Whole Genome Sequencing (WGS) Process

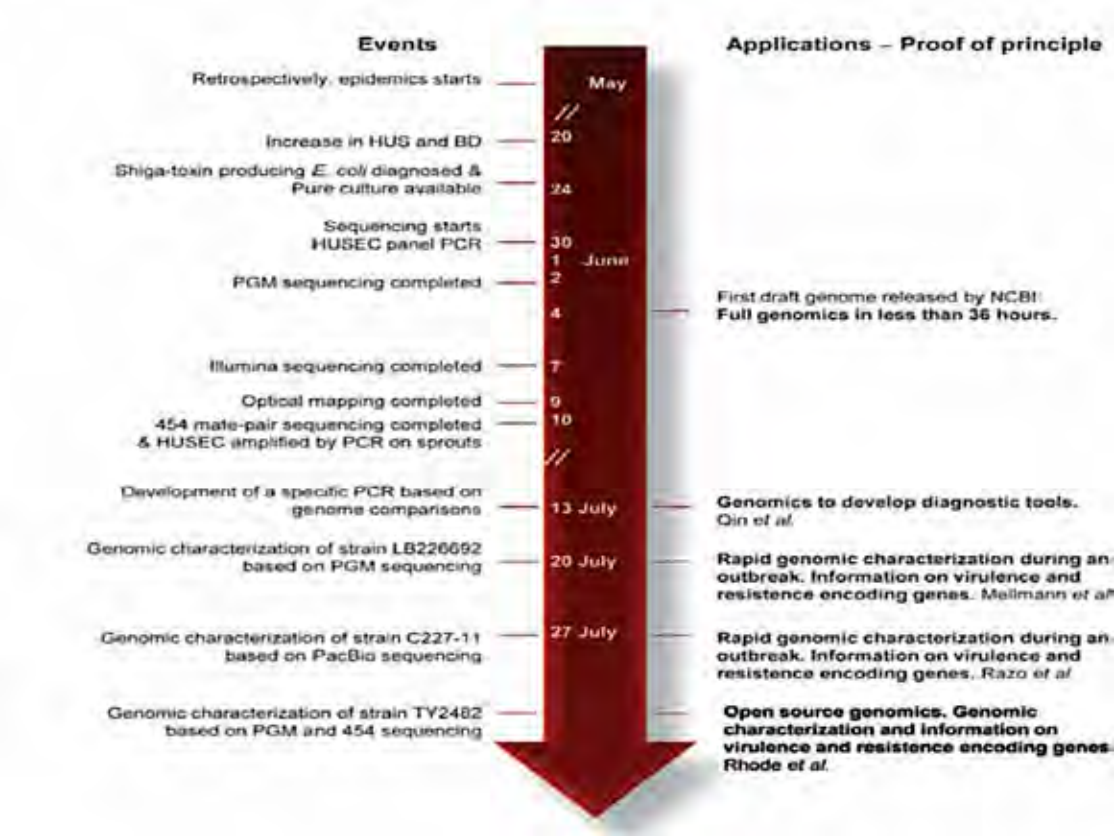
WGS is a laboratory procedure that determines the order of bases in the genome of an organism in one process. WGS provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.



Whole genome sequencing: clinical applications

1. **Strain identification for epidemiological typing and pathogen monitoring during outbreaks**
2. Identification of target DNA sequences and antigens to rapidly develop diagnostic tools
3. Investigation of strain properties, such as the presence of antibiotic resistance or virulence factor
4. Potential of rapid diagnostics to improve both treatment and antibiotic stewardship

The Escherichia coli O104:H4 epidemics



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Whole genome sequencing: clinical applications

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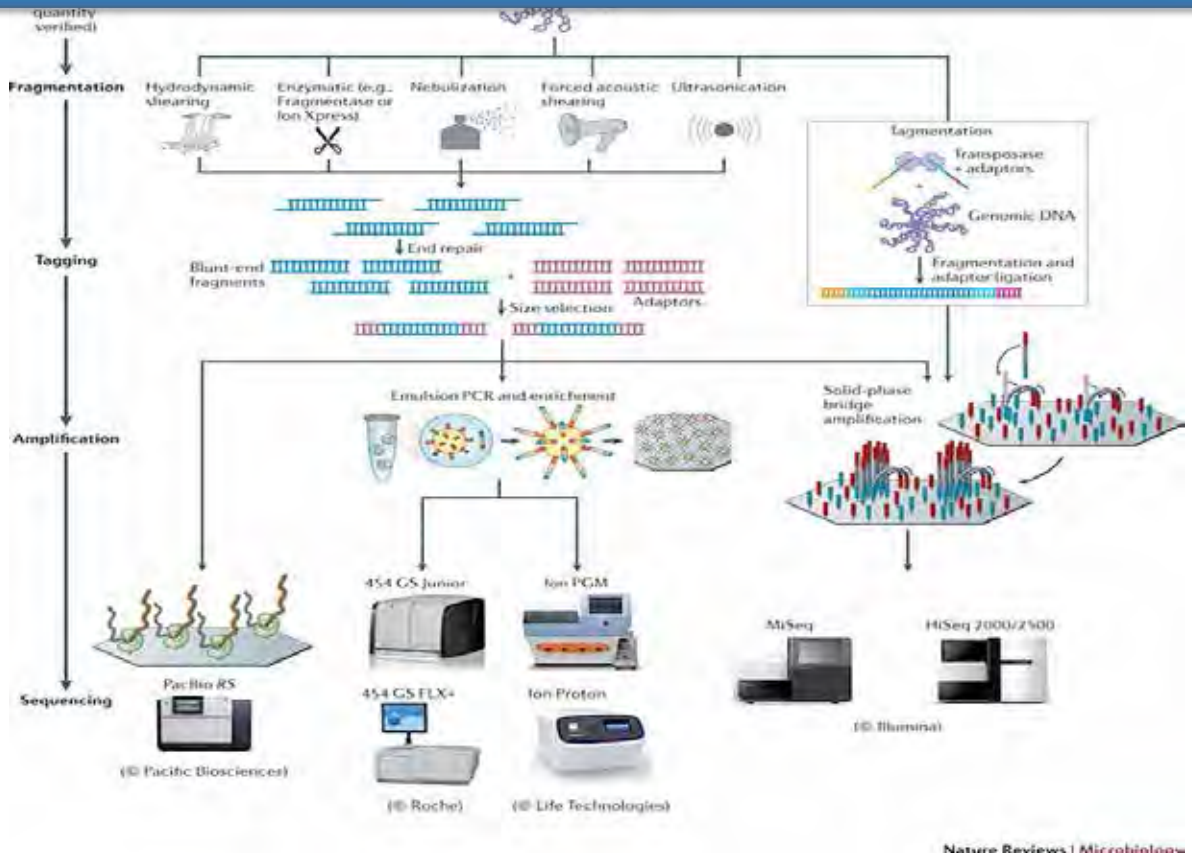
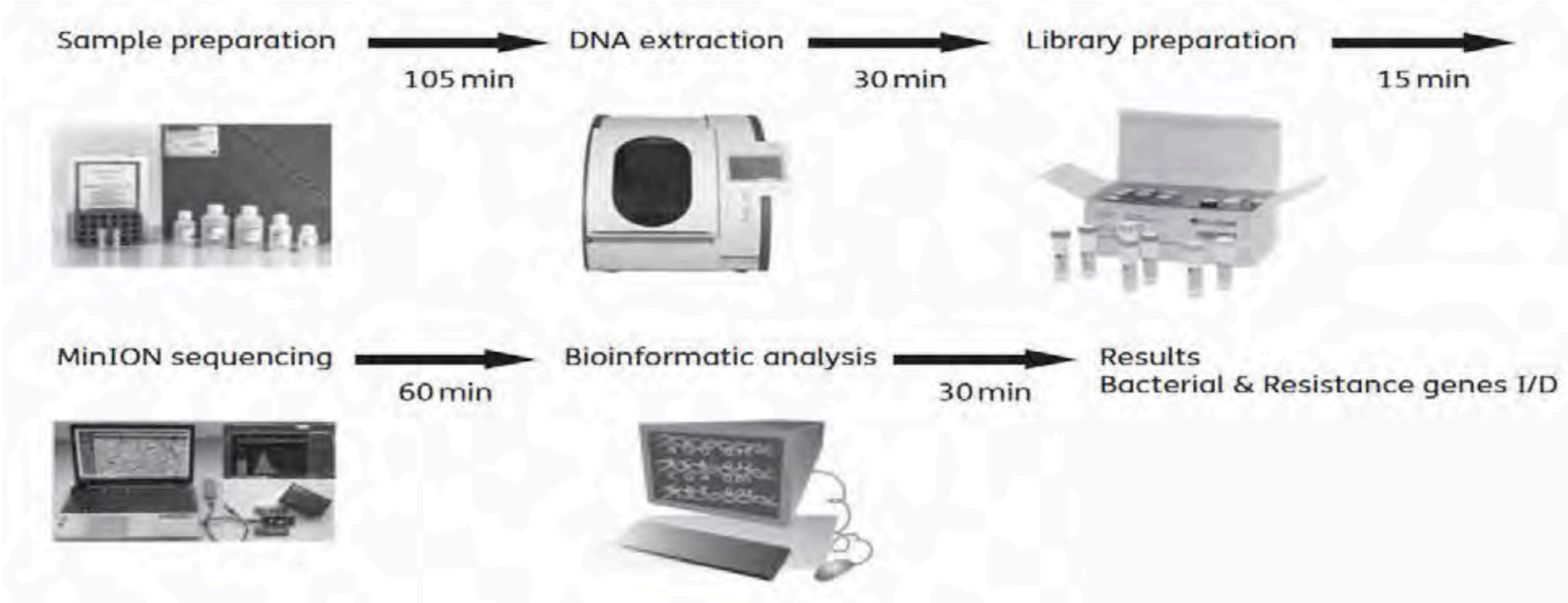
2. **Identification of target DNA sequences and antigens to rapidly develop diagnostic tools**
3. **Investigation of strain properties, such as the presence of antibiotic resistance or virulence factor**
4. **Potential of rapid diagnostics to improve both treatment and antibiotic stewardship**

Whole genome sequencing: a routine tool for large clinical microbiology laboratories?




Sample preparation
105 min
DNA extraction
30 min
Library preparation
15 min
MinION sequencing
60 min
Bioinformatic analysis
30 min
Results
Bacterial & Resistance genes I/D

Identification of bacterial pathogens and antimicrobial resistance directly from clinical urines by nanopore-based metagenomic sequencing

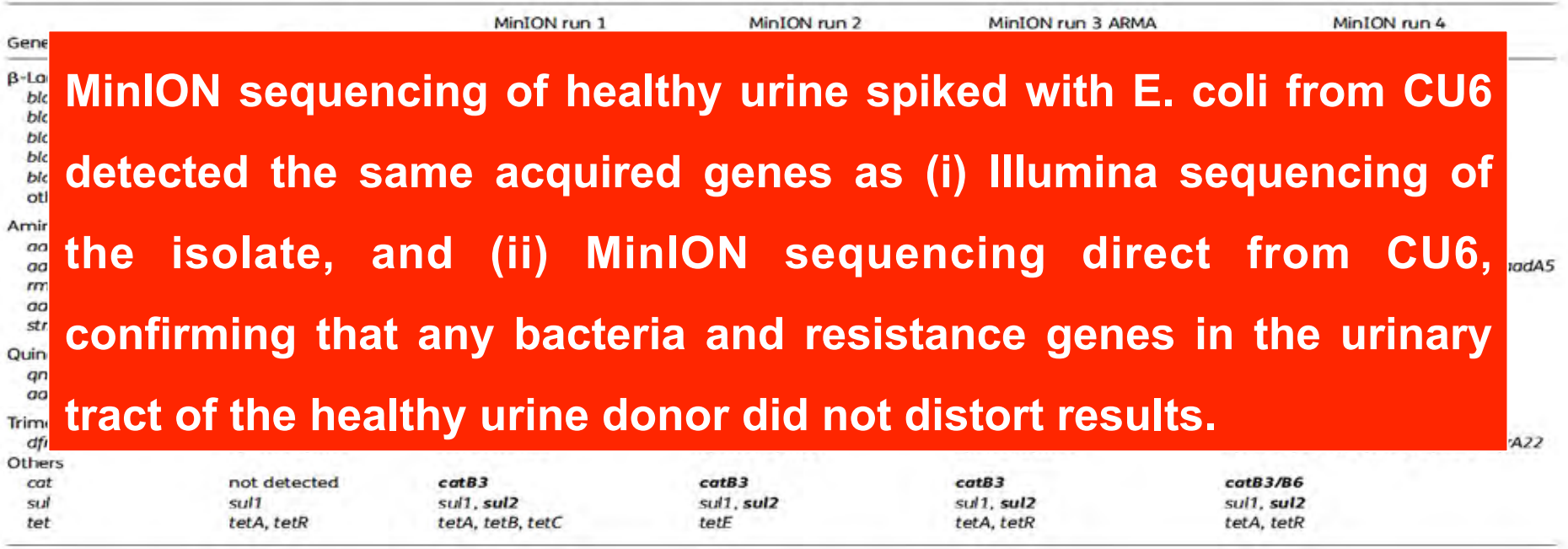
- ✓ **Ten infected clinical urines from patients**
- ✓ **Urine from a healthy volunteer spiked with 10^8 cfu/mL of the MDR *E. coli* strain H141480453 and with cultivated *E. coli* from CU6**

	CU5	CU6	Urine spiked with *E. coli* from CU 6	CU7	CU8	CU9	CU10	Urine spiked with *E. coli* H141480453 run 1	Urine spiked with *E. coli* H141480453 run 2	Urine spiked with *E. coli* H141480453 run 3
Reads used	2-D pass only	2-D pass only	2-D pass only	2-D pass only	2-D pass and fail	2-D pass and fail	2-D pass and fail	2-D pass only	2-D pass only	2-D pass only
Percentage non-human DNA reads matching Gram-negative bacteria	76%	84%	83%	84%	81%	95%	85%	98%	89%	—
Percentage DNA reads matching human	6.6%	8.5%	8.5%	8.1%	12.3%	1.7%	9.7%	1.6%	4.2%	—
Best species match to MinION sequence data	*K. pneumoniae* CG43	*E. coli* JJ1886	*E. coli* JJ1886	*E. coli* PMV-1	*E. coli* 536	*E. cloacae* NCTC 9394	*K. pneumoniae* CG43	*E. coli* APEC O78	*E. coli* K-12	*E. coli* APEC O78
Best species match to Illumina sequence data	*K. pneumoniae* MGH 78 578	*E. coli* JJ1886	*E. coli* JJ1886	*E. coli* IHE3034	*E. coli* 536	*E. cloacae* NCTC 9394	*K. pneumoniae* CG43	*E. coli* ST410	*E. coli* ST410	*E. coli* ST410
Percentage breadth of coverage to best match organism	82.57%	99.59%	100%	92.19%	99.9%	86.25%	96.70%	95.13%	96.13%	—
Average depth of coverage versus best match organism	2.71×	15.65×	10.58×	10.77×	22.84×	9.16×	17.61×	7.25×	21.55×	21.51×
Run time (h)	25.5	23	14	17.5	36	26	35	30	48	7.5

K. Schmidt J Antimicrob Chemother 2016

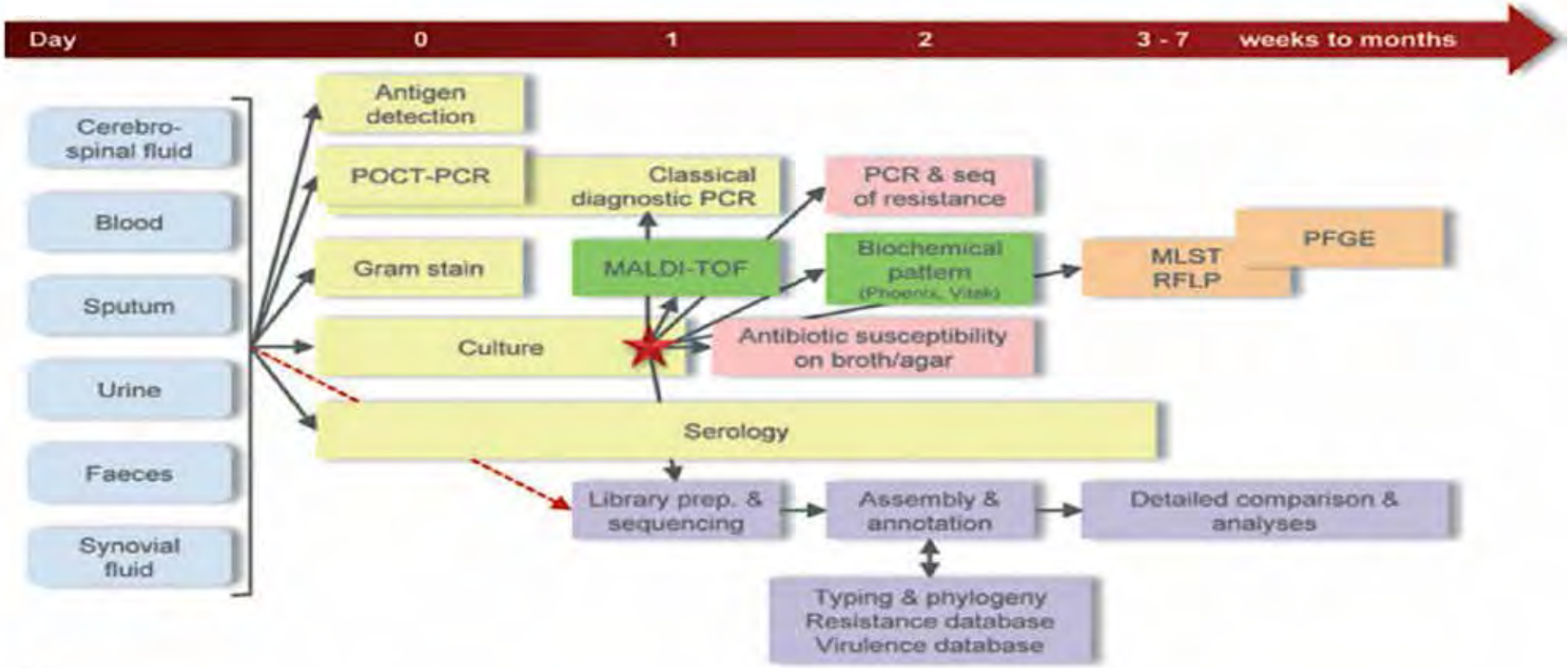
Identification of bacterial pathogens and antimicrobial resistance directly from clinical urines by nanopore-based metagenomic sequencing

E. coli H141480453, compared with Illumina sequencing of the cultivated organism



MinION sequencing of healthy urine spiked with E. coli from CU6 detected the same acquired genes as (i) Illumina sequencing of the isolate, and (ii) MinION sequencing direct from CU6, confirming that any bacteria and resistance genes in the urinary tract of the healthy urine donor did not distort results.

K. Schmidt J Antimicrob Chemother 2016


Day
0
1
2
3 - 7
weeks to months
Cerebro-spinal fluid
Blood
Sputum
Urine
Faeces
Synovial fluid
Antigen detection
POCT-PCR
Classical diagnostic PCR
PCR & seq of resistance
Gram stain
MALDI-TOF
Biochemical pattern
(Phoenix, Vitek)
MLST
RFLP
PFGE
Culture
Antibiotic susceptibility on broth/agar
Serology
Library prep. & sequencing
Assembly & annotation
Detailed comparison & analyses
Typing & phylogeny
Resistance database
Virulence database

The Role of Whole Genome Sequencing (WGS) in Antimicrobial Susceptibility Testing of Bacteria: Report from the EUCAST Subcommittee

Matthew J. Ellington, Oskar Ekelund, Frank M. Aarestrup, Rafael Canton, Michel

1. **For most bacteria, evidence for using WGS as a tool to infer antimicrobial susceptibility (i.e. to rule-in as well as to rule-out resistance) accurately is either poor or non-existent.**
2. **Most published evidence does not currently support use of WGS-inferred susceptibility to guide clinical decision making.**
3. **Only datasets passing agreed QC metrics should be used in antimicrobial susceptibility predictions, since resistance genes or mutations might be missed in sequences of poor quality**
4. **Need for a standardised, open-access database.**
5. **Time and cost**
6. **…**

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Conclusions

- ✓ **New detection methods in infectious diseases : reality**
- ✓ **Validation needed: pb of gold standard and lobbying**
- ✓ **Validation needed in fluids other than blood: specificity?**
- ✓ **Location: Microbiology department or ICU/ED?**
- ✓ **Cost-effectiveness: need or not?**


MERCI!
THANK YOU!