Syndromic approach for microbiology testing in critical care

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cnrs

Critical care testing
Antibes, June 21-22 2018

Laboratory of Toulouse University Hospital

3,000 beds
820,000 outpatients

Connexion with research centers
- Immunology & infectious diseases
- Cardiovascular & metabolic diseases
- Oncology

24h a day & 7 days a week

122 Medical Biologists
450 Ingineers & Technicians
7 millions exams per year
Accreditations ISO 15189, ISO 17025, ISO 22870

1 Preanalytical Platform

3 Multidisciplinary Analytical Platforms
- ✓ Automated
- ✓ Specialized
- ✓ Infectiology
  - Serology
  - Molecular diagnosis
  - Morphology & Culture

The local environment

Rangueil/Larrey

Cardiology/Pneumology
Gastroenterology
Urology/Nephrology

Purpan

Children

Women

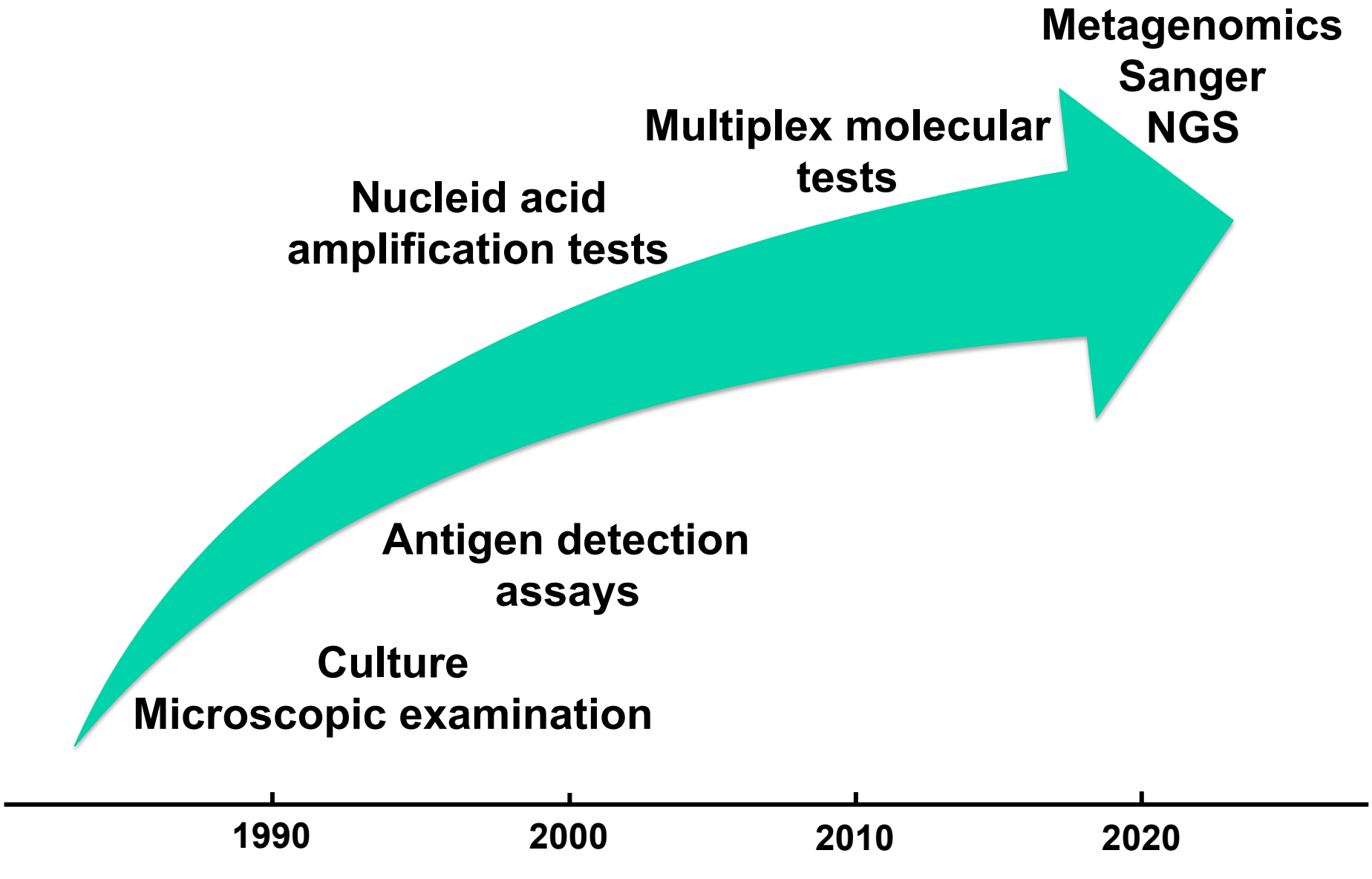
Neurosciences

Emergency & Medicine

Langlade

Oncology

Major technological evolutions in microbiology



Major advances in molecular diagnostics

- ✓ **Core lab with high level of automation → combined nucleic acid extraction / amplification / detection-quantification**

- ✓ **Miniaturization → microfluidics & nanotechnology**

- ✓ **Connected objects → cloud-connected technologies & smartphone-integrated electronic readers**

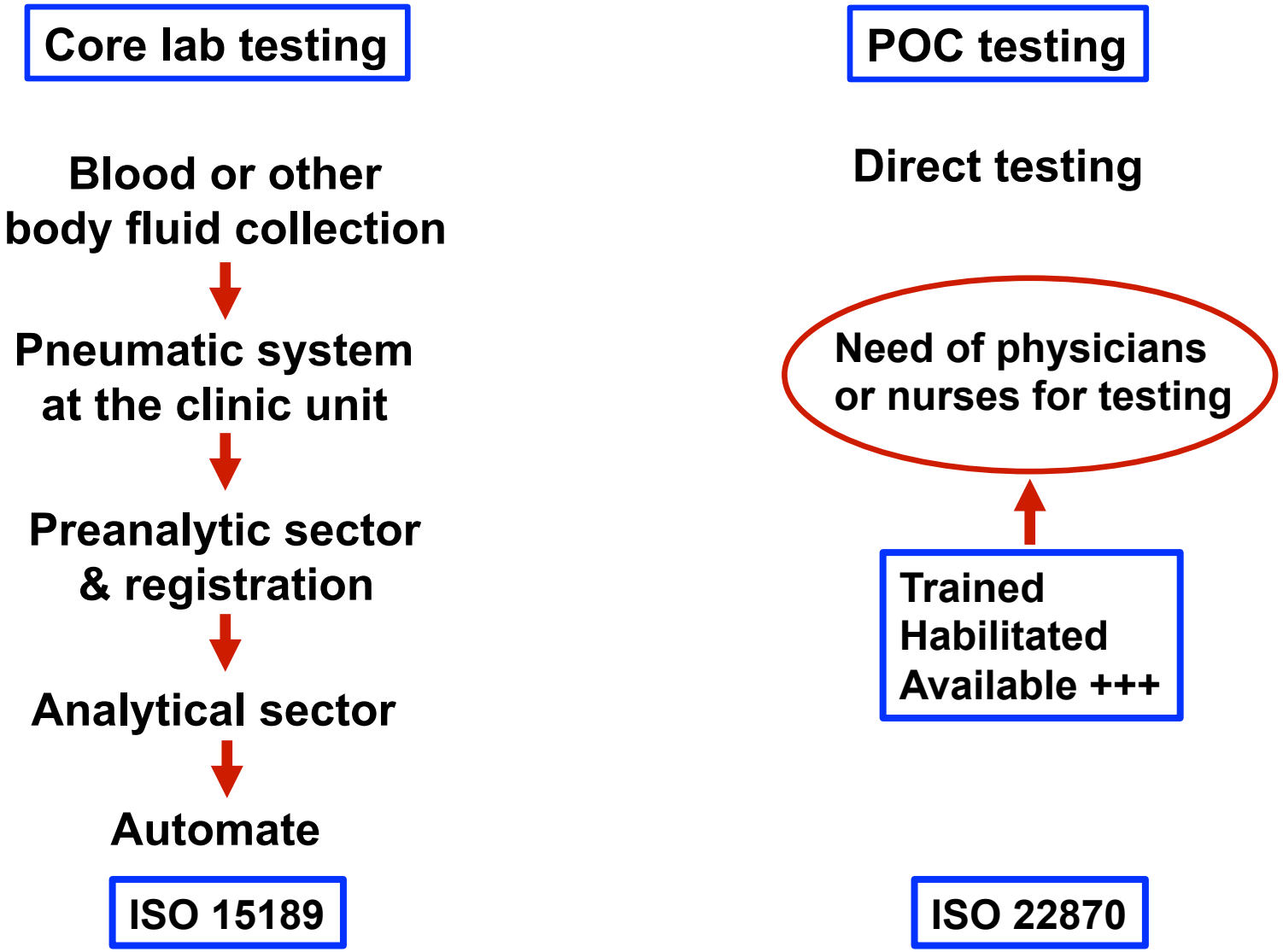
POC diagnostic tools

Rapid diagnoses of infectious diseases

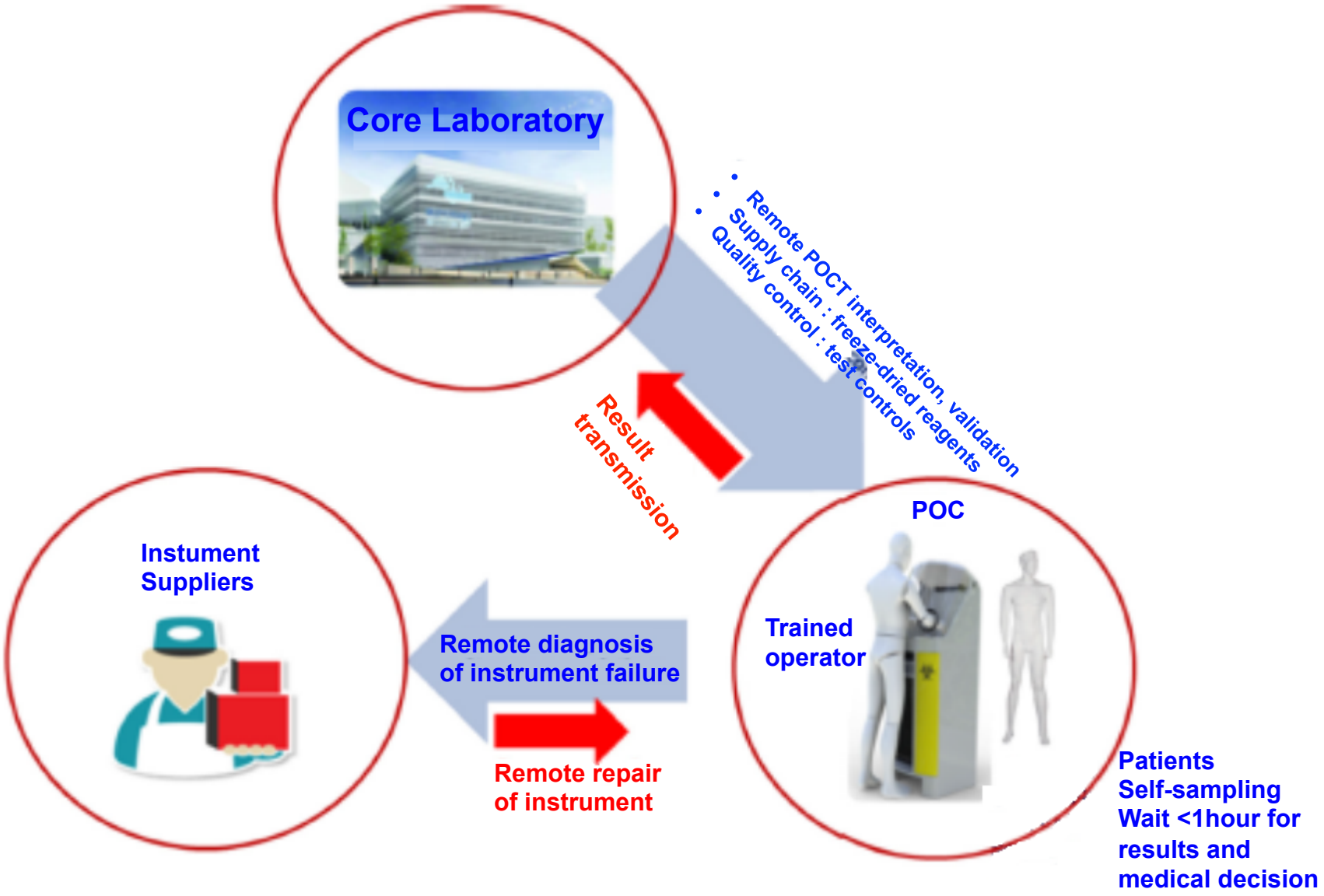
- ✓ **Medical diagnostic testing at or near the point of care**
  - → **rapid clinical decisions**

- ✓ **POC laboratories set up in remote regions**
  - → **facilitate access to testing**
    - **low-income area**
    - **high-income area due to logistics limitations, particularly transport time**

Workflow analysis



POC testing

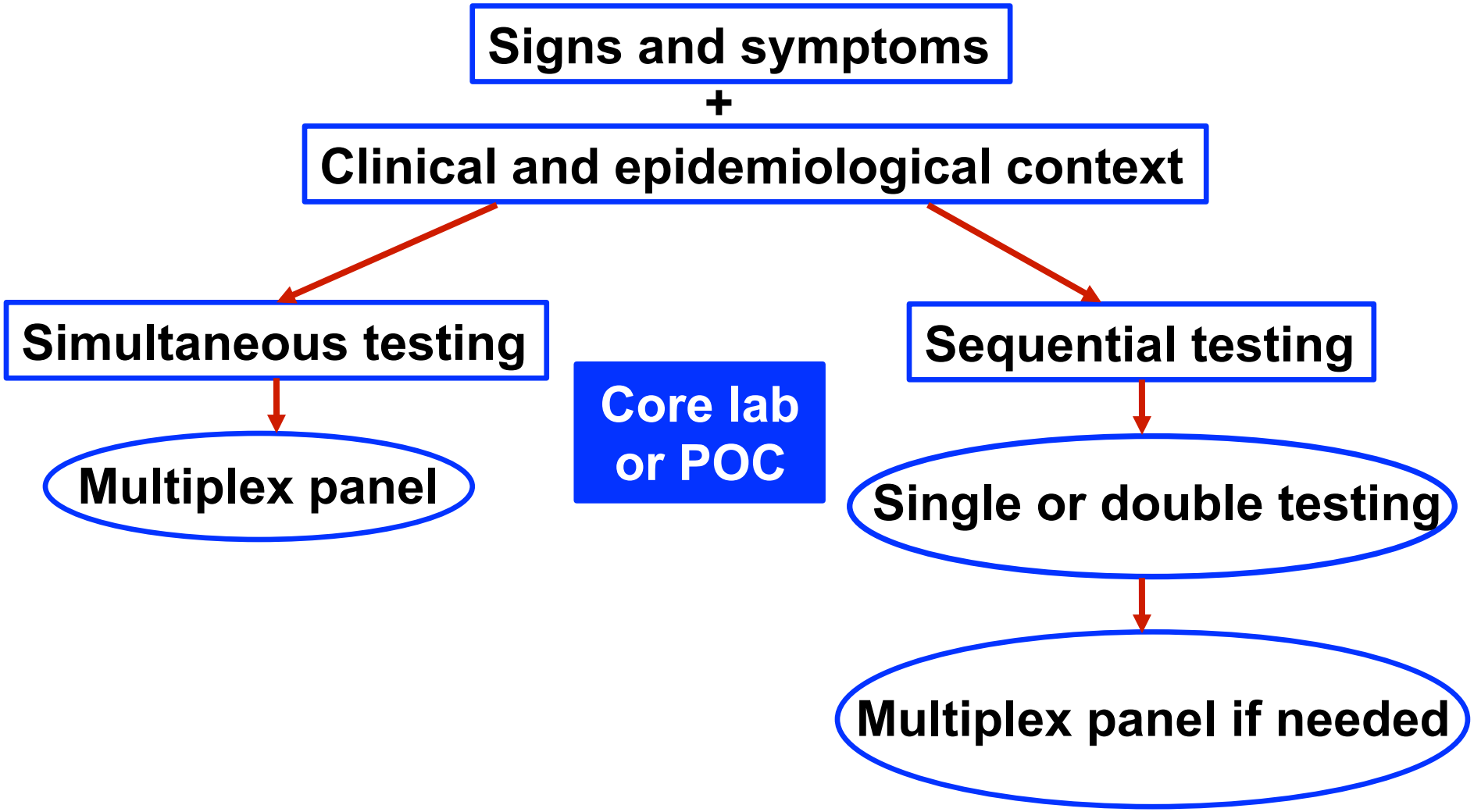


Drancourt, *Clin Microbiol Rev* 2016

Syndromic approach

- ✓ **Meaning : simultaneous testing for the presence of different pathogens that may cause a group of signs and symptoms**
  - **digestive tract infection**
  - **respiratory tract infection**
  - **meningitis / encephalitis**
  - **sepsis, fever with eruption,…**
- ✓ **Main advantages : rapidity & simplicity vs conventional methods**

Syndromic approach



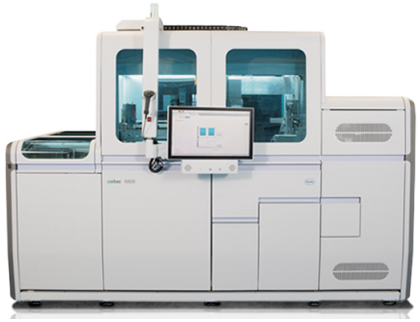
Key questions

1/ Is it a rapid, deadly infection requiring particular medical support, including hospitalization?

2/ Is it a contagious infection requiring patient isolation?

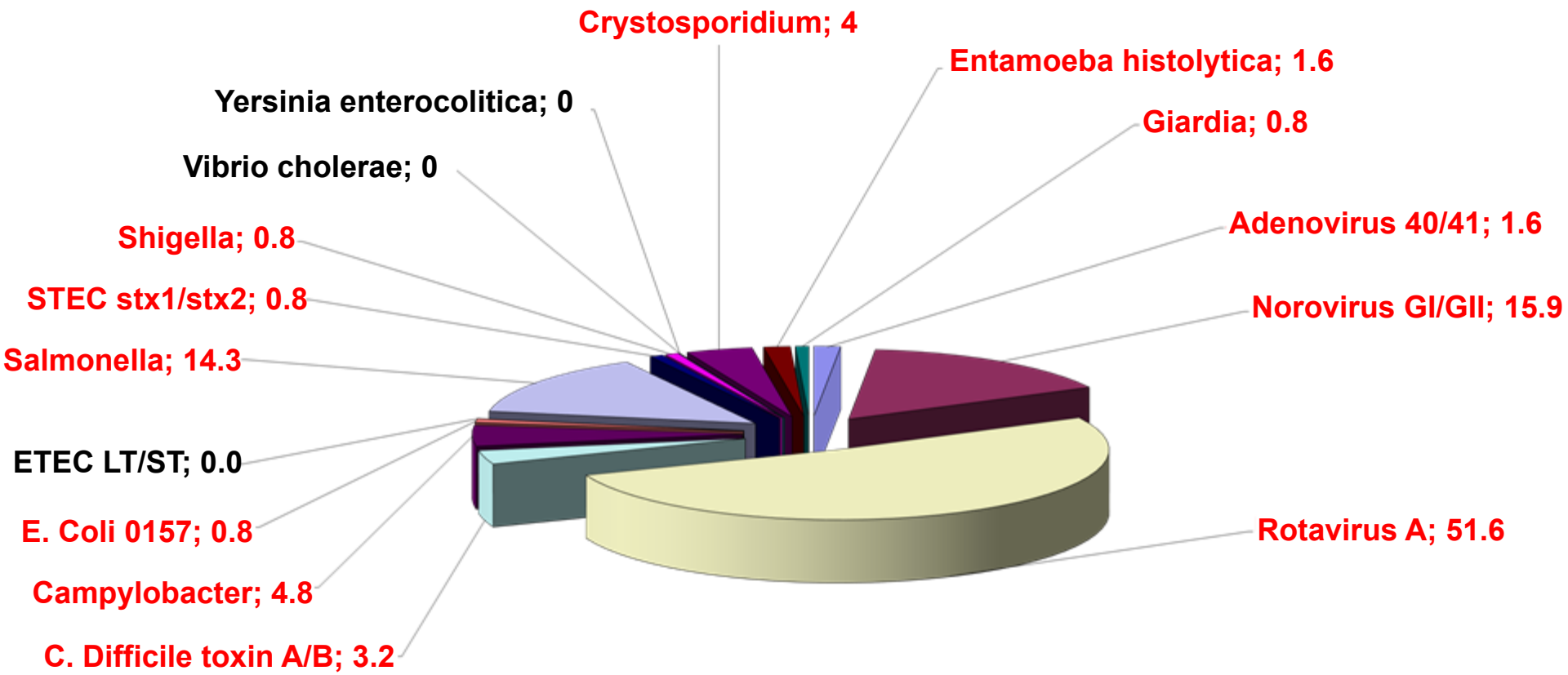
3/ Is it an infection requiring any specific antiinfectious treatment?

Multiple molecular instruments



Gastrointestinal pathogens

Luminex-based molecular assay – Core lab 4h

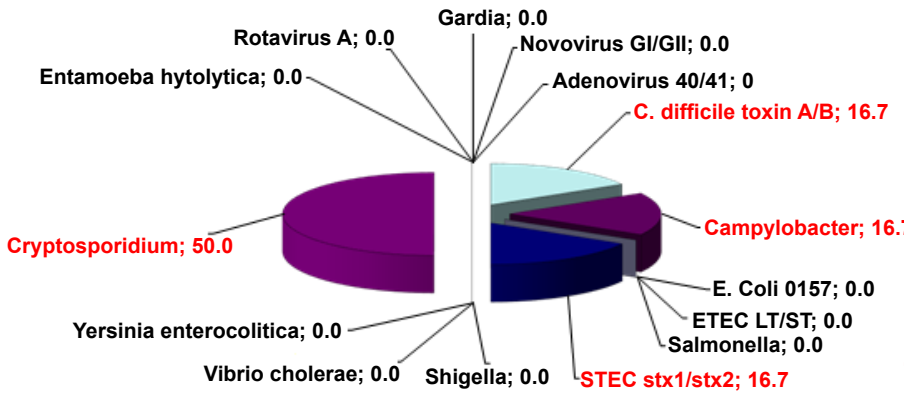


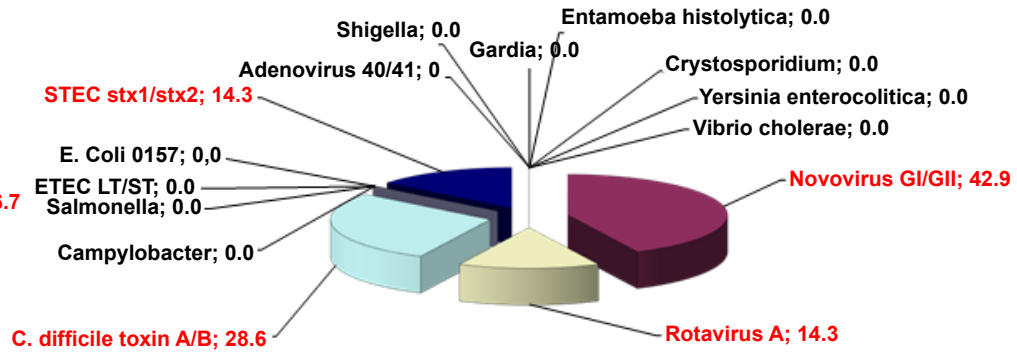
Diarrheic children – Emergency unit
Detected pathogens 93/119 (78 %)
Co-infections (34 %)

Mengelle, *Clin Microbiol Infect* 2013

Gastrointestinal pathogens

Luminex-based molecular assay – Core lab 4h





Diarrheic children – Neonatology unit
Detected pathogens 8/60 (13 %)
Co-infections (0 %)

Diarrheic children – Hematology unit
Detected pathogens 9/53 (17 %)
Co-infections (0 %)

Mengelle, *Clin Microbiol Infect* 2013

Gastrointestinal pathogens

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology® March 2018

Low Yield of FilmArray GI Panel in Hospitalized Patients with Diarrhea: an Opportunity for Diagnostic Stewardship Intervention

Matthew M. Hitchcock,[a] Carlos A. Gomez,[a,b] Niaz Banaei[a,b,c]

Hospitalization adult patients with diarrhea
Film Array 22 pathogens: 13 bacteria/5 viruses/4 parasites – Core lab 1h
Pathogens detected 29/481 (6 %)

Gastrointestinal pathogens

Seegene vs Mobidiag – Core lab 4h

	Seegene	Mobidiag	Concordance (%)
	Allplex GI panel	Amplidiag Viral GE	
Rotavirus	42	42	100
Norovirus 1	3	3	100
Norovirus 2	17	19	89.5
Sapovirus	3	2	66.7
Astrovirus	4	4	100
Adenovirus 40/41	5	5	100

Gastrointestinal pathogens

	Luminex GPP	Verigene	BioFire GIP	Seegene	Mobidiag
Platform	Magpix or Luminex 100/200	Verigene System	FilmArray or Torch	Allplex Gi panel	Cfx
Specimen type	Fresh stool or Cary-Blair	Cary-Blair	Cary-Blair	Fresh stool or Cary-Blair	Fresh stool or Cary-Blair
No of targets	14	9 (no parasites)	22	26	19
No of samples	24	1-32	1-12	24	12
Time to results (h)	~5	< 2	~1	~3	6

Gastrointestinal pathogens Assay performance

- ✓ **More positive results than conventional testing methods**
  Mengelle, *Clin Microbiol Infect* 2013
- ✓ **Detection of co-infections, sometimes of unknown clinical significance (EAEC, EPEC, C diff)**
- ✓ **High sensitivity and specificity, with a few exceptions**
  Huang, *Diagn Microbiol Infect Dis* 2016 ; Buss, *J Clin Microbiol* 2015 ; Khone, *J Clin Microbiol* 2014 ; Wessels, *Clin Microbiol Infect* 2014 ; Spina, *Clin Microbiol Infect* 2015 ; Patel, *J Clin Microbiol* 2014 ; Ramanan, *Diagn Microbiol Infect Dis* 2017

Gastrointestinal pathogens Clinical and economic impacts

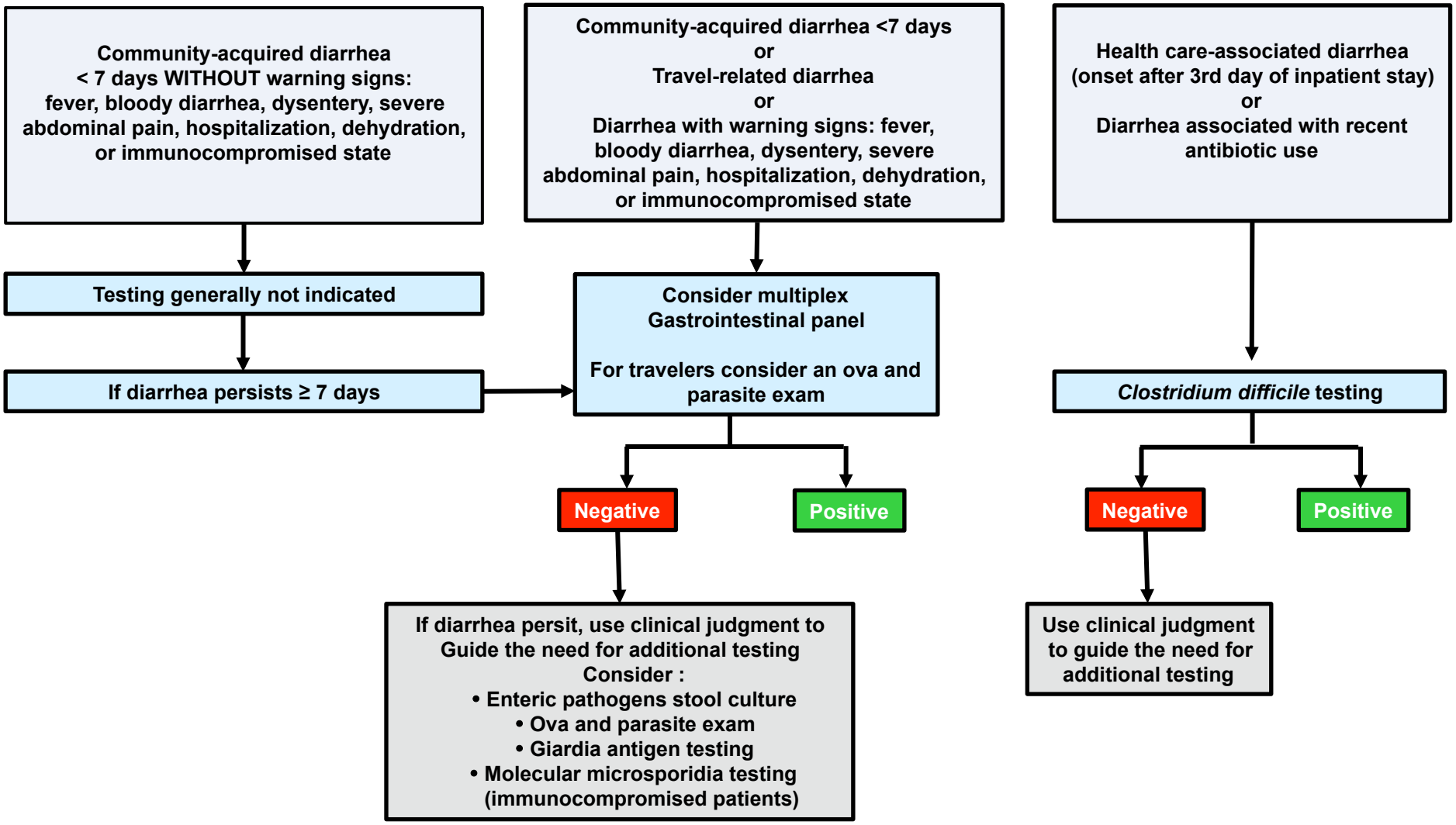
- ✓ **Increased costs to the lab (£ 22, 283) but compensation by decreasing the number of days that patients were under isolation protocols (£ 66,765 saved)**

  Goldenberg, *J Infect* 2015

- ✓ **Increased number of patients with appropriate isolation precautions and decreased number of patients under contact precautions unnecessarily**

  Rand, *Diagn Microbiol Infect Dis* 2015

Gastrointestinal pathogens
Potential testing algorithm



Riddle, *Am J Gastroenterol* 2016

Gastrointestinal pathogens Advantages and limitations

- ✓ **Broad coverage and identification of coinfections**
- ✓ **Reduced TAT**
- ✓ **Simplified workflow**
- ✓ **Conventional methods still needed:**
  - **pathogens that are not covered by the panels (eg Aeromonas, parasites,…)**
  - **antibiotic susceptibility testing… althought not recommended**

Riddle, *Am J Gastroenterol* 2016

Respiratory pathogens

Assay	FilmArray	ePlex	Verigene	Panther-Fusion	Allplex	Nx TAG
Platform	FA or TORCH	ePlex	Verigene	Hologic	Allplex	Luminex or Magpix
No of targets	22	17	16	10	22	20
No of samples	1-12	-	1-32	Random-access	-	96
Time to results	~1	~1.5	~2	~2	~4	~4

Respiratory pathogens

Viruses	Film Array 45 min	E Plex 1h40 min	Allplex 4h15 min	Luminex Nx TAG 5h	Verigene	Panther-Fusion
Adenovirus	•	•	•	•	•	•
Coronavirus HKU1	•	•		•		
Coronavirus NL63	•	•	•	•		
Coronavirus 229E	•	•	•	•		
Coronavirus OC43	•	•	•	•		
Coronavirus MERS	•	•				
Human Bocavirus		•	•	•		
Human Metapneumovirus	•	•	•	•	•	•
Influenza A Subtype H1 Subtype H3 Subtype 2009 H1N1	• • • •	• • • •	•	• • •	• • •	•
Influenza B	•	•	•	•	•	•
Parainfluenza 1	•	•	•	•	•	•
Parainfluenza 2	•	•	•	•	•	•
Parainfluenza 3	•	•	•	•	•	•
Parainfluenza 4	•	•	•	•		•
Respiratory Syncytial Virus	•			•		•
Respiratory Syncytial Virus A		•	•	•	•	
Respiratory Syncytial Virus B		•	•	•	•	
Rhinovirus/Enterovirus	•	•	•	•	•	•

Respiratory pathogens

Bacteria	Film Array 45 min	E Plex 1h40 min	Allplex 4h 15 min	Nx TAG 5h	Verigene	Panther-Fusion
Chlamydophila pneumoniae	•	•	•	•		
Mycoplasma pneumoniae	•	•	•	•		
Bordetella pertussis	•	•	•		•	
B parapertusis	•		•		•	
Bordetella holmesii					•	
Legionella		•	•	•		
Streptococcus pneumoniae			•			
Haemophilus influenzae			•			

Respiratory pathogens
Assay performance

- ✓ **FilmAssay vs NxTAG**
  - **284 nasophyngeal Kappa = 0.92**
  - **Higher positivity rates for hMPV and PIV3 in favour of NxTAG**

Chen, *J Clin Microbiol* 2016

- ✓ **ePlex vs LDT-PCR**
  - **323 positive clinical specimens**
  - **Level of agreement Kappa = 0.97**

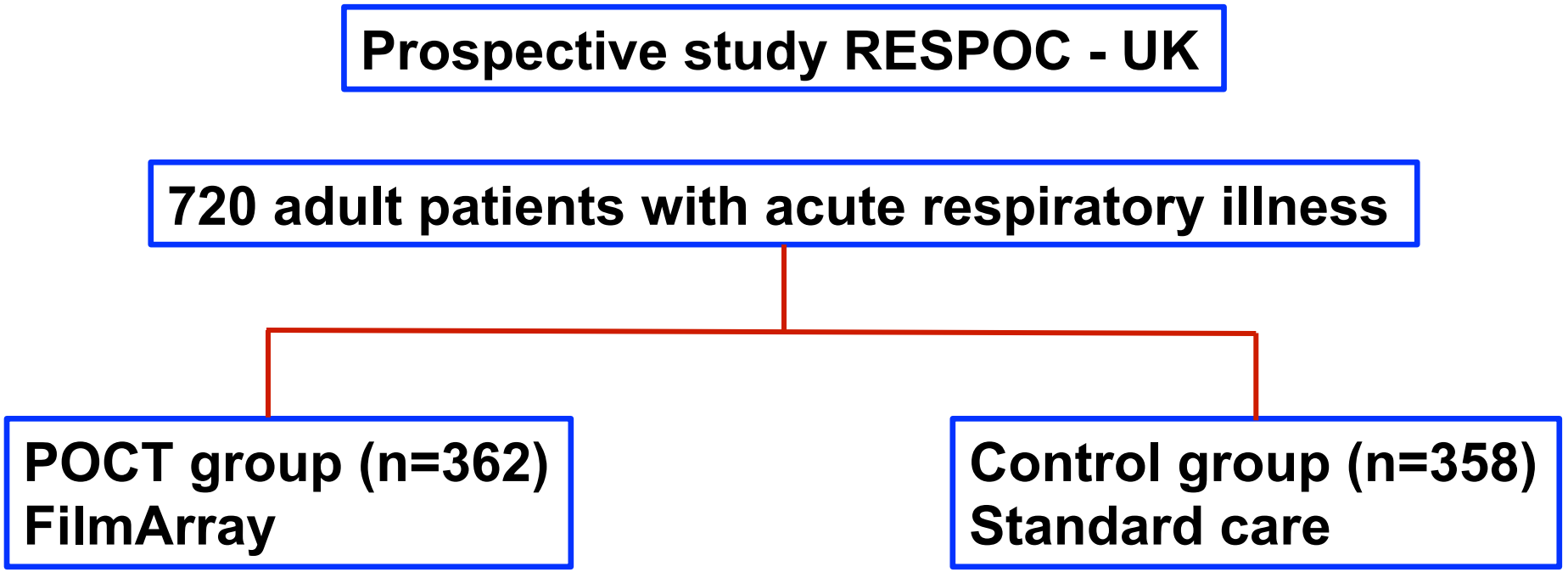
Nijhuis, *J Clin* Microbiol 2017

Respiratory pathogens
Clinical and economic impacts

Pre/Post intervention studies with FilmArray

Study	Population	Impact
Rogers, *Arch Pathol Lab Med* 2015	Children with uncomplicated acute respiratory illness	↓ duration of antibiotics ↓ length of stay* ↓ time in isolation*
Rappo, *J Clin Microbiol* 2016	Adult with acute respiratory illness	↓ time to diagnosis 1.7 vs 7.7 for Flu 1.5 vs13.5 for other V ↓ length of stay ↓ duration of antibiotics ↓ n° of chest radiographs
Subramony, *J Pediatr* 2016	Pediatric in patients	↓ duration of antibiotics ↓ n° of chest radiographs

Respiratory pathogens
Clinical and economic impacts



Brendish, *Lancet Respir Med* 2017

Respiratory pathogens Clinical and economic impacts

RESPOC study - UK

Parameter	POCT group	Control group	P
% of pts treated with antibiotics	84	83	ns
Mean duration of antibiotics	7.2	7.7	ns
% of pts with single doses or brief courses (<48h) of antibiotics	17	9	0.005
Length of stay (d)	5.7	6.8	0.04
Adequate antiviral use for Flu (%)	91	65	0.003

Brendish, *Lancet Respir Med* 2017

Respiratory pathogens
Clinical and economic impacts

Cost-effectiveness of multiplex testing

- ✓ **Versus culture and Antigen tests : favorable index if the prevalence of a respiratory viral illness was >11 %**

  Mahony, *J Clin Microbiol* 2009

- ✓ **Versus PCR and antigen tests : favorable index in an emergency department for children**

  Nelson, *Pediatr Infect Dis* 2015

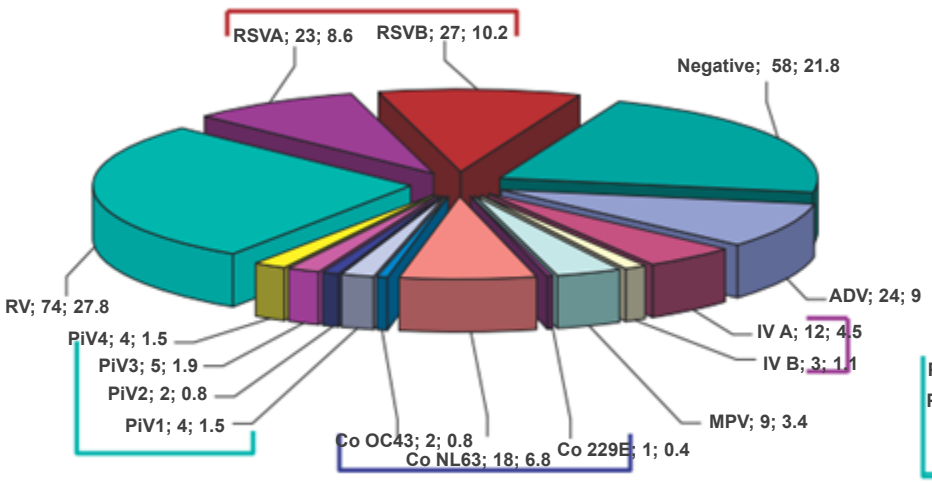
Respiratory pathogens Advantages and limitations

Cost-effectiveness of multiplex testing

- ✓ **Simplified workflow and reduced TAT**
- ✓ **Broad coverage including unexpected pathogens (eg Mycoplasma pneumoniae) but not exhaustive testing (TB, CMV, SARS, CoV, Hantavirus,…)**
- ✓ **Lack of flexibility except for Luminex tests (Verigene)**
- ✓ **Positive results may not distinguish between colonization and active infection and may miss coinfection with bacteria or fungi**
- ✓ **Better knowledge of epidemiology in different populations**

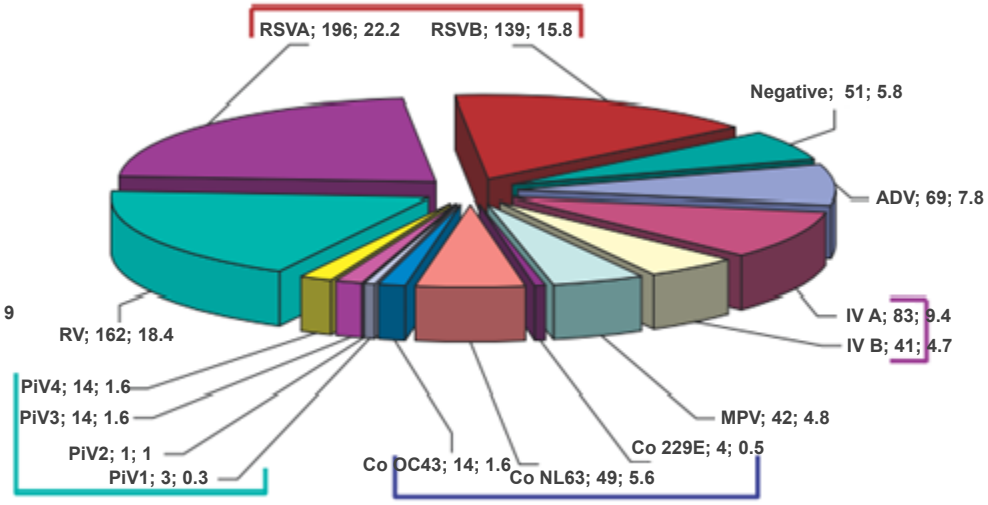
Epidemiological monitoring CHU Toulouse

Lower respiratory tract infection



Detected virus 76 %
Co-infections 10 %

Upper respiratory tract infection



Detected virus 93 %
Co-infections 20 %

Mengelle, *J Clin Virol* 2014

Epidemiological monitoring in children

- ✓ **Paris-Bichat, May 2011 – April 2014, 344 children**
  - ➔ **Detected viruses 51 %, co-infections 9 %**

  Visseaux, *Plos One* 2017

- ✓ **CHU St Etienne, Nov 2012 – April 2013, 85 CAP**
  - ➔ **Detected viruses 62 %, bacteria 5 %, mixtures 28 %**

  Cantaix, *J Clin Virol* 2014

Epidemiological monitoring in adults

- ✓ **Paris-Bichat, May 2011 – April 2016, 4958 adults**
  - ➔ **Detected viruses 29 %, co-infections 2 %**

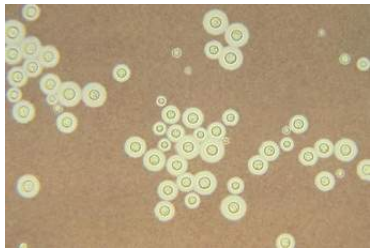
  Visseaux, *Plos One* 2017

- ✓ **Paris-Bichat, Intensive Care, Oct 2011 – June 2015, 174 CAP**
  - ➔ **Detected viruses 31 %, bacteria 26 %, mixtures 26 % (more severe outcome)**

  Voiriot, *Critical Care*

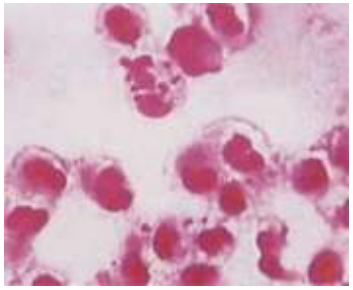
CNS pathogens
Meningitis & encephalitis

Fungi



Cryptococcus

Bacteria



Neisseria meningitidis
S pneumoniae
Listeria m
Haemophilus
S agalactiae
E coli KI

Virus

HSV-1, HSV-2
Enterovirus, VZV, CMV, HHV6,
Parechovirus

CNS pathogens

✓ **Conventional parameters**

- **CSF appearance**
- **CSF biochemical parameters (Protein, Glucose, Cl)**
- **CSF cytology (cells/µl, neutrophils, lymphocytes)**

- **Gram staining**
- **Culture of CSF**
- **Targeted molecular assays : mostly Enterovirus, HSV-1 & HSV-2**

✓ **Multiplex molecular testing**

➔ **more rapid diagnosis vs culture**
cultures may be negative for pts receiving antibiotics

CNS pathogens

Prospective study, 11 US sites, n=1560 CSF samples

	Sensitivity compared to conventional testing, before discrepant analysis	Specificity compared to conventional testing before discrepant analysis	Results after discrepant analysis Concordant +/ discordant false +/ Discordant False -	Number identified by: conventional testing/ multiplex panel	Percent positive agreement, n=138
Cytomegalovirus	3/3 (100%)	1,554/1,557 (99.8%)	4/2/0		
Enterovirus	44/46 (95.7%)	1,507/1,514 (99.5%)	49/2/2	33/36	96%
Herpes simplex virus 1	2/2 (100 %)	1,556/1,558 (99.9%)	2/2/0	4/5	99%
Herpes simplex virus 2	10/10 (100%)	1,548/1,550 (99,9%)	11/1/0		
Human herpes virus 6	18/21 (85.7%)	1,532/1,536 (99.7%)	19/3/2		
Human parechovirus	9/9 (100%)	1,548/1,551 (99.8%)	12/0/0		
Varicelle zoster virus	4/4 (100%)	1,553/1,556 (99.8%)	6/1/0		
Escherichia coli K I	2/2 (100%)	1,557/1,558 (99.9%)	2/1/0	1/1	100%
Haemophilus influenzae	1/1 (100%)	1,558/1,559 (99/9%)	2/0/0	1/1	100%
Listeria monocytogenes	0/0	1,560/1,560 (100%)	0/0/0		
Neisseria meningitidis	0/0	1,560/1,560 (100%)	0/0/0		
Spretococcus agalactiae	0/1 (0%)	1,544/1,559 (99.9%)	0/1/1	3/3	100%
Spretococcus pneumoniae	4/4 (100%)	1,544/1,556 (99.2%)	9/7/0		
Crytococcus neoformans	1/1 (100%)	1,555/1,559 (99.7%)	3/2/0		

Positive agreement rate 84.4 %
Negative agreement rate > 99.9 %

Leber, *J Clin Microbiol* 2016

CNS pathogens

Preclinical study, US site, n=342 CSF samples

Organism identification	Conventional detection, no.	FA ME panel detection, no.	Baseline agreement, no.	Resolution result, no. FA+/R+	FA+/R-	FA-/R+	Sensitivity, % (95%CI)	Specificity, % (95 % CI)
Bacteria								
H. Influenza	4	5	4	1	0	0	100 (47.8-100)	100 (97.4-100)
S. Pneumoniae	3	6	3	2	1	0	100 (47.8-100)	99.3 (96.1-100)
S. Agalactiae	1	5	1	2	2	1	66.7 (9.4-99.2)	98.6 (95.0-99.8)
Escherichia coli	1	1	1	NA	NA	NA	100 (2.5-100)	100 (97.5-100)
Listeria monocytogenes	0	0	1	NA	NA	NA	NA	100 (97.5-100)
Neisseria meningitides	1	1	1	NA	NA	NA	100 (2.5-100)	100 (97.5-100)
Bacteria not in the FA ME panel	7	0	7	NA	NA	NA	NA	NA
Viruses								
EV	37	37	36	1	0	1	97.4 (86.2-100)	100 (69.2-100)
HSV-1	12	13	11	0	2	1	92.9 (66.1-99.8)	98.0 (89.1-99.9)
HSV-2	29	29	29	NA	NA	NA	100 (88.1-100)	100 (82.4-100)
HHV-6	13	18	12	6	0	1	94.7 (74.0-99.9)	100 (92.6-100)
VZV	32	32	32	NA	NA	NA	100 (89.1-100)	100 (79.4-100)
CMV	7	4	4	0	0	3	57.1 518.4-90.1)	100 (91.4-100)
EBV	13	11	11	5	9	1	94.1 (71.-99.9)	84.2 (72.1-92.5)
PV	0	1	0	1	0	0	100 (2.5-100)	100 (92.5-100)
Yeast								
C. neoformans/gatii	14	9	8	1	0	0	64.3 (35.1-87.2)	NA
Total	174	186	161	19	14	8	92.8 (88.2-96.0)	92.8 (88.2-96.0)

Hanson, *J Clin Microbiol* 2016

CNS pathogens - FilmArray

- ✓ **Positive evaluations in Spain, Pennsylvania and Uganda**
  Launes, *J Clin Microbiol* 2016 ; Messacar, *Diagn Microbiol Infect Dis* 2016 ; Rhein, *Diagn Microbiol Infect Dis* 2016

- ✓ **Delayed diagnosis of tuberculous meningitis misdiagnosid as HSV-1 in California**
  Gomez, *OFID* 2016

CNS pathogens – Advantages and limitations

- ✓ **Reduced TAT but no replacement of conventional testing methods**
- ✓ **Risk of false positive results due to latent infection (HHV-6, EBV) or lab contamination**
  - → **clinical and laboratory measures to mitigate false positive results**

Gomez, *OFID* 2016

- ✓ **Further studies are needed including cost-effectiveness analyses**

Other syndromic approaches

✓ **Sepsis :**

- **Rapid testing of positive blood culture bottles & resistance gene**

  ➔ **FilmArray, ePlex, Verigene**

  Ramanan, *Clin Microbiol Reviews* 2018

- **Direct molecular detection**

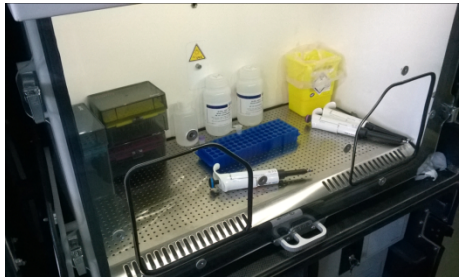
Nieman, *BMC Infectious Diseases 2016 ;* Mylonakis, *Clin Infect Dis* 2015

✓ **Fever with eruption**

➔ **Multiplex DENV, CHIKV & ZIKV**

Mansuy, *Diagn Microbiol Infect* 2018

Mobile laboratories for Ebola and other pathogens



Mansuy, *Lancet Infect Dis* 2015

Combined detection and typing by metagenomics

The Journal of Infectious Diseases
MAJOR ARTICLE

Viral Pathogen Detection by Metagenomics and Pan-Viral Group Polymerase Chain Reaction in Children With Pneumonia Lacking Identifiable Etiology

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Still complex: explosion of protocols and tools
sample-dependent
technology-dependent

SWOT of syndromic approach

Strength	Weakness
• Rapid testing • Rapid decisions for patient management : hospitalisation, isolation or treatment • Better access	• Multiple instruments : QC, HR, maintenance, reagents • Cost • Optimal strategy for tests ordering largely undefined
Opportunities	**Threats**
• Cost-effectiveness studies • Guidelines at national or international level	• No clear policy regarding tests reimbursement • Insufficient cross-talk between biologists and clinicians • Loss of quality

Acknowledgements

Infectiology Plateform

Dr Florence Abravanel
Dr Chapuy-Regaud
Dr Sébastien Lhomme
Dr Jean-Michel Mansuy
Dr Catherine Mengelle
Dr Marcel Miédougé
Dr Florence Nicot
Pr Christophe Pasquier
Dr Stéphanie Raymond
Dr Karine Sauné

Clinicans

Children Hospital

Dr Isabelle Claudet
Dr Erick Grouteau

Emergency & Medicine

Pr Pierre Delobel

Neurosciences

Dr Pascal Cintas

Transplant unit

Pr Nassim Kamar