Clinical metagenomics in immunocompromised patients

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INTRODUCTION : DIAGNOSTIC OF INFECTIOUS DISEASES

- **PCR has become the routine method for diagnostic of viral infections**
- **Culture remains the reference standard for bacterial and fungal infection**
- **Culture suffers some limitations : fastidious or uncultivable microorganisms, ongoing antibiotic treatment, inconstant time to results (from hours to days)**
- **Molecular techniques have been developed:**
  - Based on specific (sequence-dependent) amplification : Singleplex or multiplex (from low to very broad range) PCRs followed by sequencing, mass spectrometry…
  - Based on random (sequence-independent) amplification : Whole-Genome Next Generation Sequencing : breadth of agents interrogated is no more limited by the capacity of multiplexing.

Analytical sensitivity

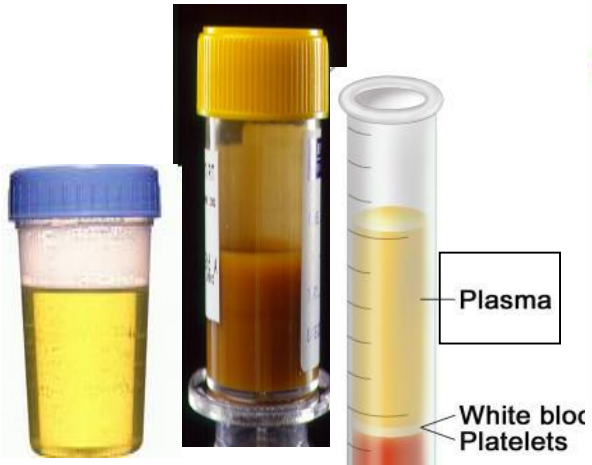
Clinical cases : patients with negative results with conventional testing

Clinical trial in a cohort of immunocompromized patients

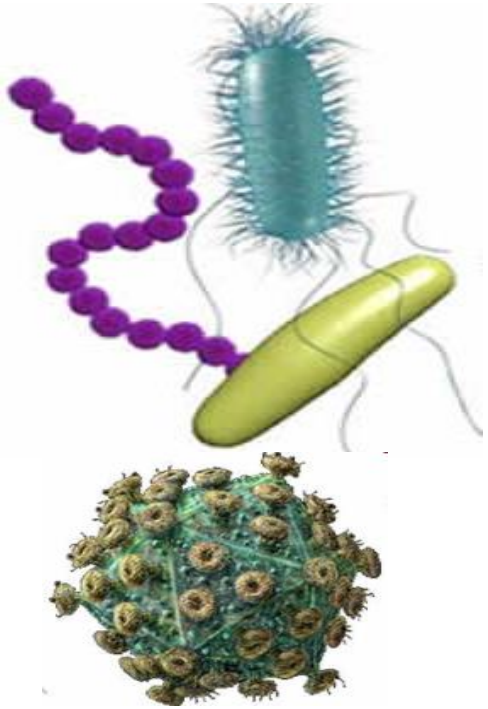
Towards a CE-IVD test

Description of the technology

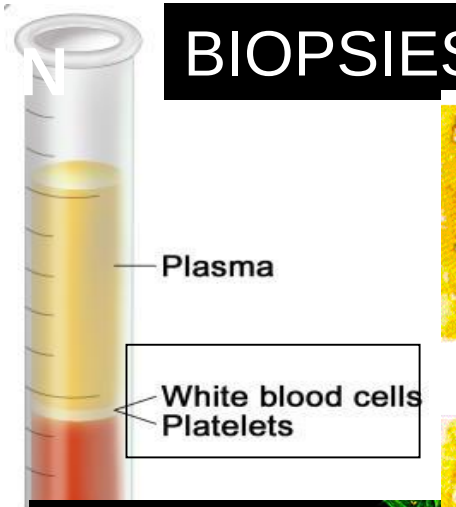
BIOLOGICAL FLUIDS

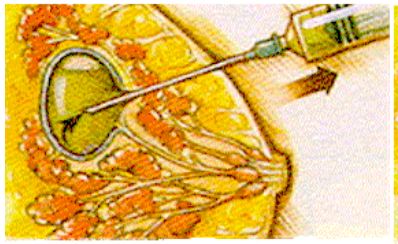


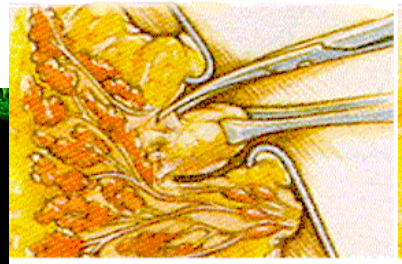
150 µL sample

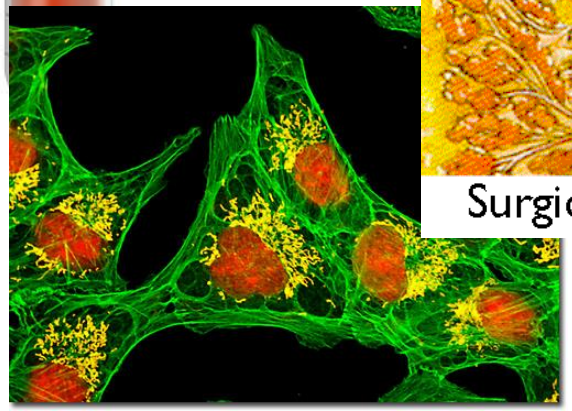


BIOPSIES, CELLS









Hydrolysis free NAs
Extraction DNA/RNA

12 µL sample

cDNAs Random amplification
(phi 29 polymerase)

Elimination DNA
Extraction RNA

DNA

BioIT workflow

MiSeq

Ion Proton

HiSeq 2500

DISCOVERY
Comprehensive taxonomic assignation

singletons

BLAST e-value = 10^{e}-3

GBVRL

BLAST e-value = 10^{e}-3

NT

NCBI Taxonomic Tree

Taxonomic Assignation

reads

Duplication removal

Quality filtering

Host sequences removal

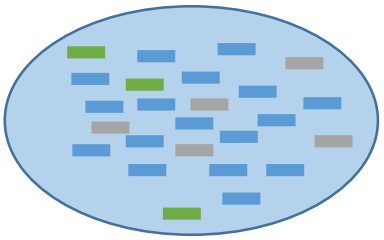
reads

***de novo* assembly**

contigs

contigs

Massive sequence alignments against full genomes



Bacteria: 799 sp.
Viruses: 405 sp.
Fungi: 49 sp.
Protozoans: 21 sp.

BLAST e-value = 10^{e}-3

NT

BLAST e-value = 10

NRPROT

BLAST e-value = 10^{e}-3

WGS

NCBI Taxonomic Tree

Taxonomic Assignation

DIAGNOSTIC
Identification among a database of known pathogens

Min. ← **RESULTS** → Day.

Analytical sensitivity

Sensitivity : NIBSC* candidate viral reference sample for NGS (1)

- Pool of 25 human viruses produced in cells, eggs, or from clinical specimens
- Diluted and pooled to have equivalent Ct values
- No absolute quantification
- Post formulation qPCR suggested variable concentrations : 6 viruses were undetectable by qPCR

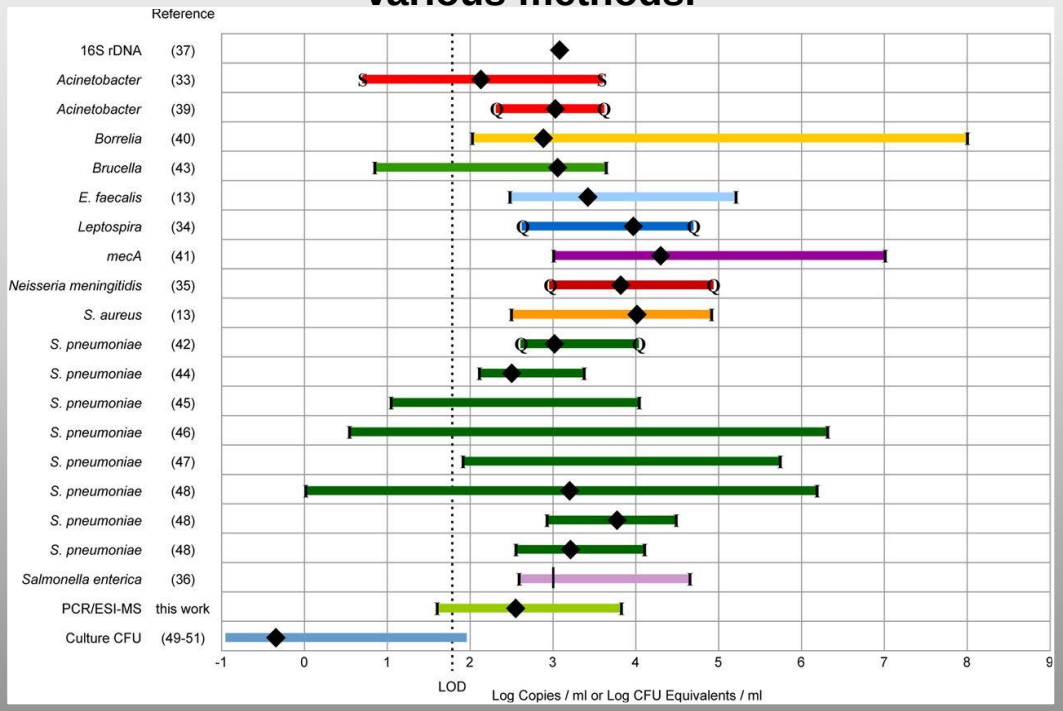
*National Institute for Biological Standards and Control, UK

Sensitivity : NIBSC viral candidate reference sample for NGS (2)

Group	Family	Envelope	Species/Type	NGS	Genome coverage (%)	qPCR(CT)
dsDNA	Adenoviridae	No	Adenovirus 2	Positive	4	29,7
			Adenovirus 41	Not Det.		Not Det.
	Herpesviridae	Yes	Human herpesvirus 1/ 2 HSV	Positive	2	32,5
			Human herpesvirus 3 VZV	Positive	93	29
			Human herpesvirus 5 CMV	Positive	33	28,9
dsRNA	Reoviridae	No	Rotavirus A	Positive	96	24,5
ssRNA(+)	Astroviridae	No	Astrovirus	Positive	95	30,5
	Caliciviridae	No	Norovirus GI	Positive	37	Not Det.
			Norovirus GII	Not Det.		Not Det.
			Sapovirus C12	Positive	98	33,4
	Coronaviridae	Yes	Coronavirus 229E	Not Det.	(free nucleic acids)	36,5
	Picornaviridae	No	Parechovirus 3	Positive	97	29,3
			Rhinovirus A39	Positive	94	31,2
			Coxsackievirus B4	Positive	35	30,7
ssRNA(-)	Orthomyxoviridae	Yes	Influenza B virus	Not Det.		Not Det.
			Influenza A virus H1N1	Positive	34	32
			Influenza A virus H3N2	Not Det.		Not Det.
	Paramyxoviridae	Yes	Metapneumovirus A	Positive	92	31,9
			Respiratory syncytial virus	Positive	20	34,3
			Parainfluenzavirus 1	Positive	96	34,4
			Parainfluenzavirus 2	Positive	56	33,4
			Parainfluenzavirus 3	Positive	21	Not Det.
			Parainfluenzavirus 4	Positive	87	31,9

Sensitivity : what is the target LOD for bacteria in bloodstream infections ?

Quantitative bacterial loads in whole blood determined by various methods.



Andrea Bacconi et al. J. Clin. Microbiol. 2014;52:3164-3174

- Bacteriema average loads in bloodstream infection are typically 0.1 to 1 cfu/mL = 1,000 to 10,000 genome copies/mL
- Minimum loads are generally in the range of 1-10 genome copies/mL

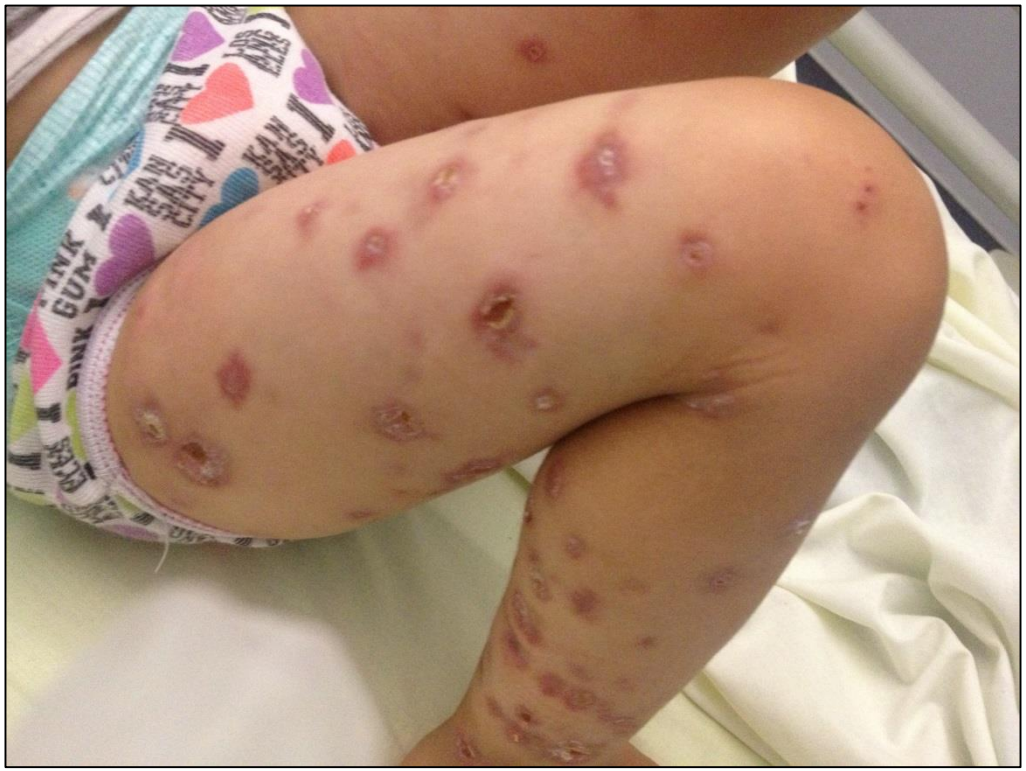
Sensitivity : whole blood spiked with bacteria and viruses (2)

Family	Species	Genome copies /mL whole blood	NGS	Genome coverage (%)	PCR
Moraxellaceae (Gram -)	Acinetobacter baumannii	150 12	Positive Positive	15,9 3,2	Not detected
Enterobacteriacae (Gram -)	Morganella morganii	900 160	Positive Positive	6,0 0,7	Not detected
Enterococcaceae (Gram +)	Enterococcus faecalis	1600 270	Positive Positive	10,8 0,8	Not detected
Streptococcaceae (Gram +)	Streptococcus agalactiae	280	Positive	21,2	Not detected
Herpesviridae (DNA ds, enveloped)	Human Herpes Virus 3 (VZV)	50	Positive	37	Positive
Parvoviridae (DNA ss, non-enveloped)	Human parvovirus B19	50	Positive	70	Positive
Reoviridae (RNA ds, non-enveloped)	Rotavirus A	40	Positive	87	Positive

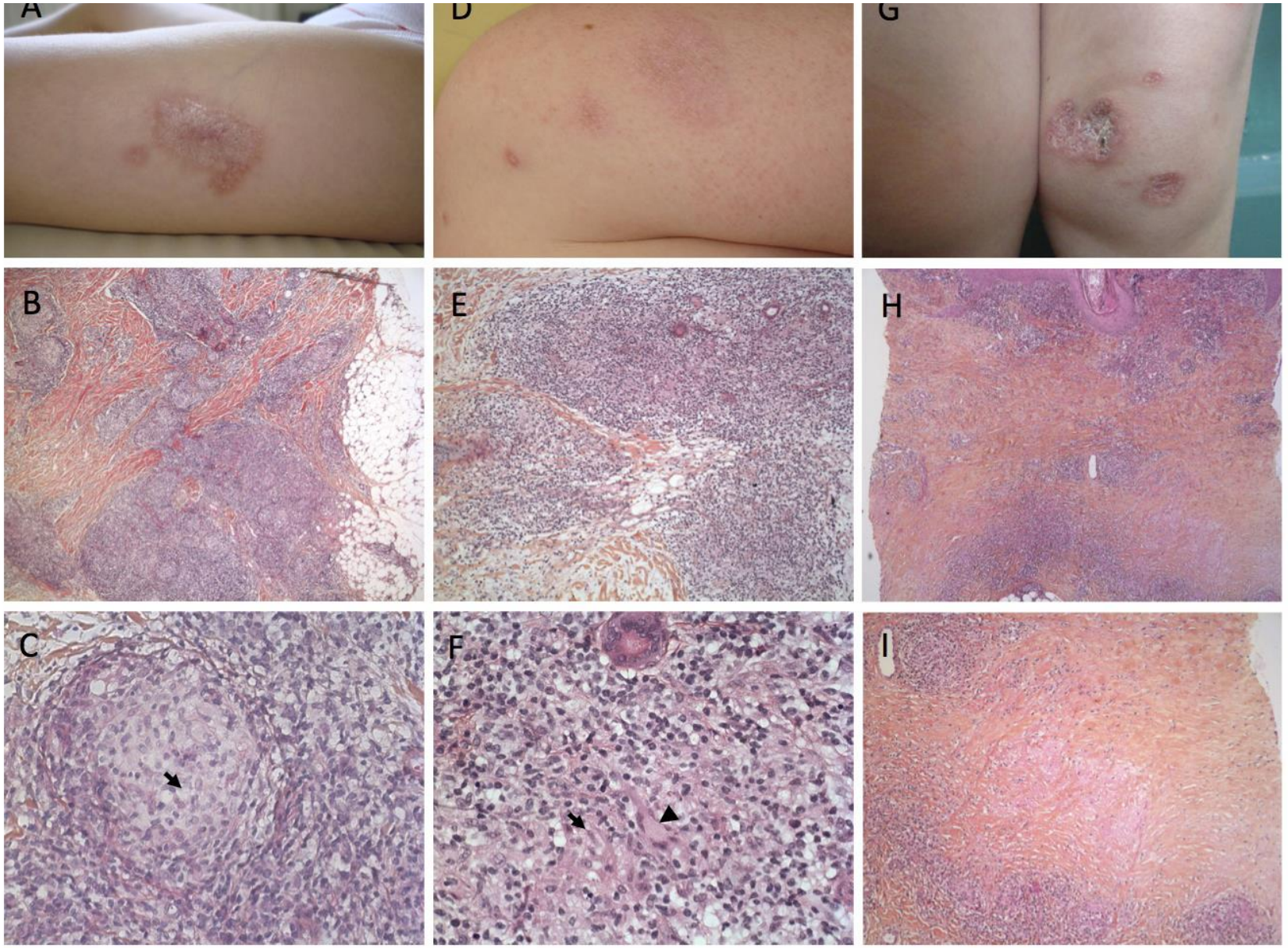
Clinical cases : patients with negative results with conventional testing

Clinical serie of cases 1 : skin granuloma in immunocompromized children (breadth of detection)

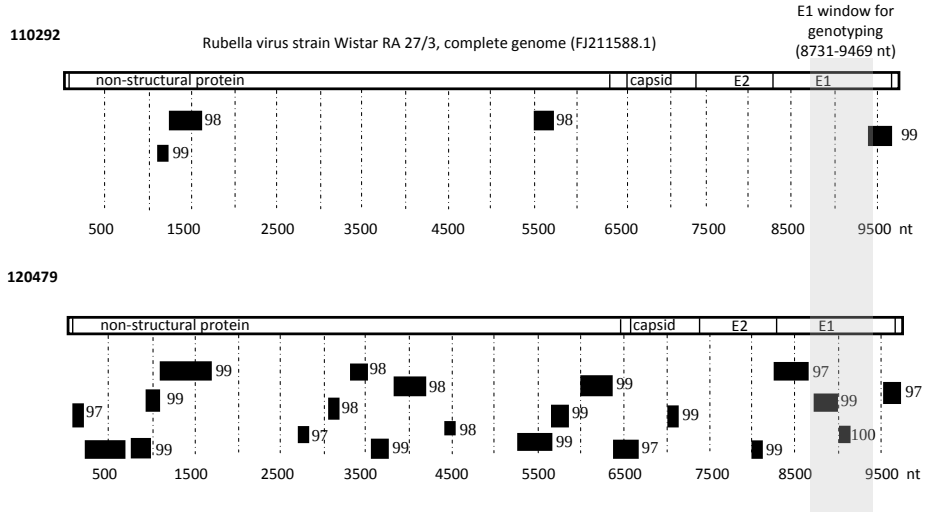
- Etiology of pediatric cutaneous granuloma in the context of primitive immune deficiency unknown
- All classical microbiological investigations have failed
- Attempt to identify a causative agent by NGS of cutaneous granuloma tissue biopsies.



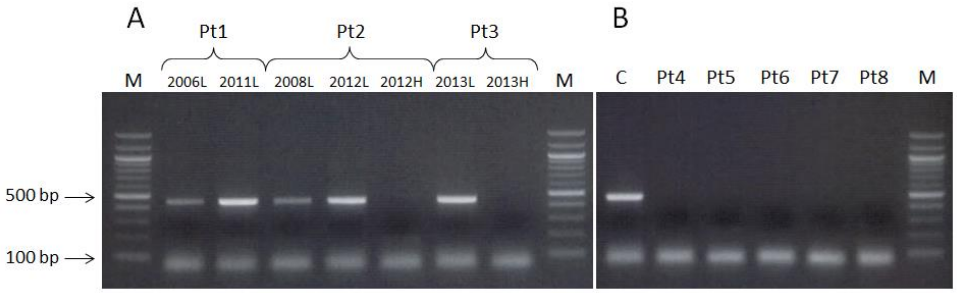
The three first cases



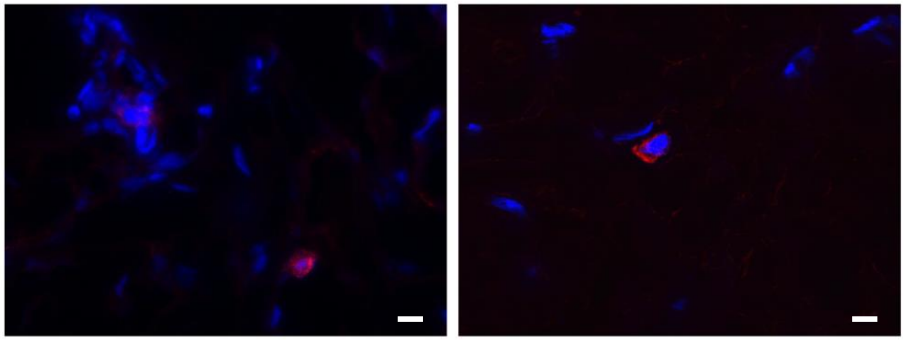
A : Deep Sequencing



B : RT-PCR



C : Immunofluorescence

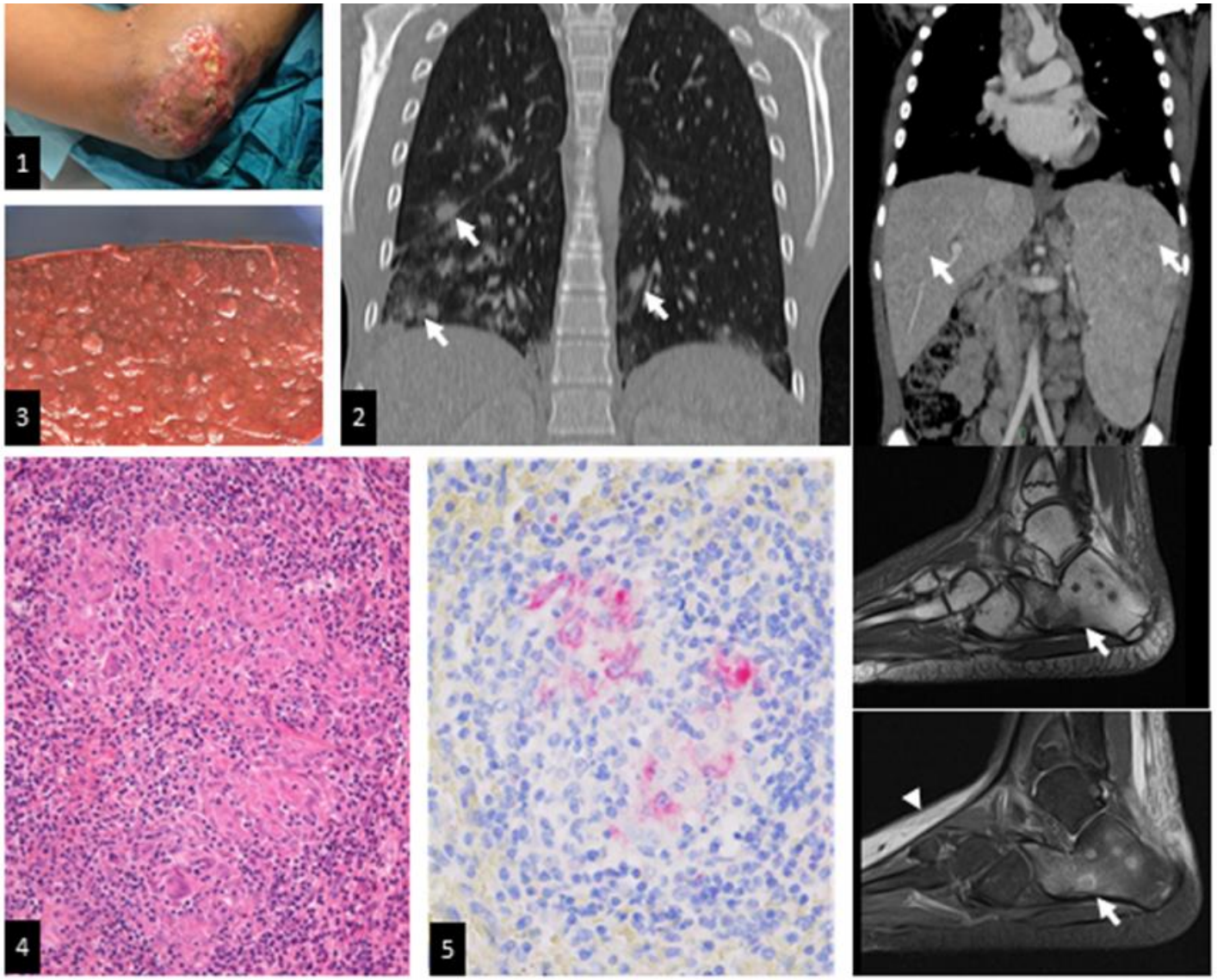


Live rubella virus vaccine long-term persistence as an antigenic trigger of cutaneous granulomas in patients with primary immunodeficiency

Article published online: 30 January 2014
Clin Microbiol Infect

C. Bodemer[1,2,3], V. Sauvage[4], N. Mahlaoui[5,6], J. Cheval[4], T. Couderc[8,9], S.Leclerc-Mercier[1,2,10], M. Debré[5], I. Pellier[11], L. Gagnieur[12], S. Fraitag[2,10], A. Fischer[3,5,6,7], S. Blanche[3,5,6], M. Lecuit[3,6,8,9,13] and M. Eloit[4,12,14]

Now 11 cases, including one child with visceral granulomatous disease



Cutaneous and visceral chronic granulomatous disease triggered by a Rubella Virus vaccine strain in children with primary immunodeficiencies, Clinical Infectious Diseases, in press

A diversity of immune deficits

case	sexe	diagnosis	CD3 Cells (Nadir/µl) or other cellular immune deficiency markers	Hypogamma globulinemia	age vaccination (months)	age onset skin lesions (months)
1	F	Ataxia Telangiectasia	Low (1150)	+	16	18
2	M	Activated phospoinositide 3-kinase δ syndrome**	Low CD4 naïve cell	+	13 and 67	132
3	F	Ataxia Telangiectasia	Low (672)	+	17 and 28	33
4	F	Undefined Combined Immunodeficiency	Low CD4 naïve and NK cell	+	NA	NA
5	M	Rag-1 deficiency	Low (540)	+	13 and 18	30
6	F	Ataxia Telangiectasia	Low CD4 naïve cell	+	13	45
7	F	Rag-2 deficiency	Low (418)	+	18	21
8	M	Ataxia Telangiectasia	NA	+	8 and 13	24
9	M	Ataxia Telangiectasia	Low (773)	+	11 and 88	156
10		cartilage hair hypoplasia				
11		Immune deficit HLA class II	(CD3 754, CD4 427, CD8 220)	+	10	23

Clinical case 2 : breadth of detection

CLINICAL PICTURE

- 14-year boy developing severe encephalitis
- Genetic defiency XLA (X-linked agammaglobulinemia)
- Treated for epilepsy for 4 years with recurrent seizures
- Severe cognitive disorders
- Severe neurological disorders

DIAGNOSTIC PICTURE

Viral screening	CSF	Brain biopsy
Parvovirus	negative	negative
EBV	negative	negative
CMV	negative	negative
Adenovirus	negative	negative
HSV-1	negative	negative
HSV-2	negative	negative
VZV	negative	negative
HHV6	negative	negative
HHV8	negative	negative
Enterovirus	negative	negative
Astrovirus	negative	negative
Respiratory viruses	negative	negative
Measles	negative	negative
Mumps	negative	negative
JC Virus	negative	negative

- **NEGATIVE** results from conventional diagnostic pipeline

Clinical case 2 : breadth of detection

PATHOQUEST NGS-based ASSAY

ASTROVIRUS POSITIVE (variant)

CLINICAL OUTCOME

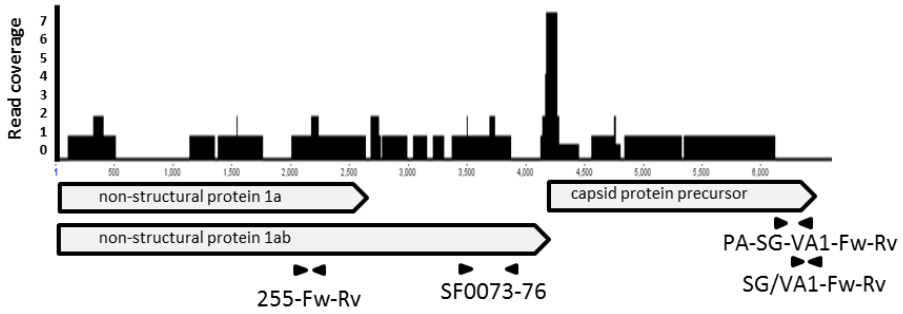
- **Initial therapy was adapted :**

Addition of IV and then oral ribavirin associated with weekly subc. injection of (PEG-IFN)
Increase of iv IG dosage

- **Initiation of recovery through progressive cognitive improvements**

Clinical case 2 : breadth of detection

A



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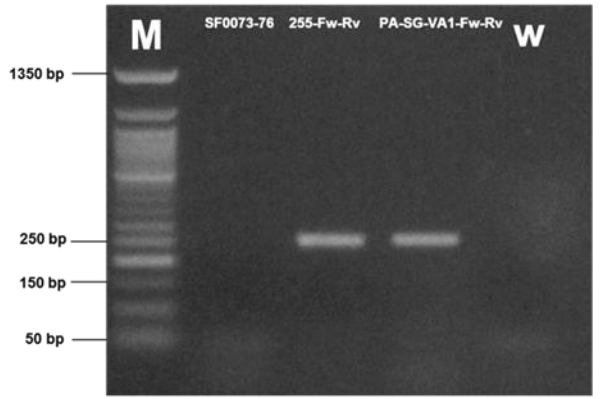

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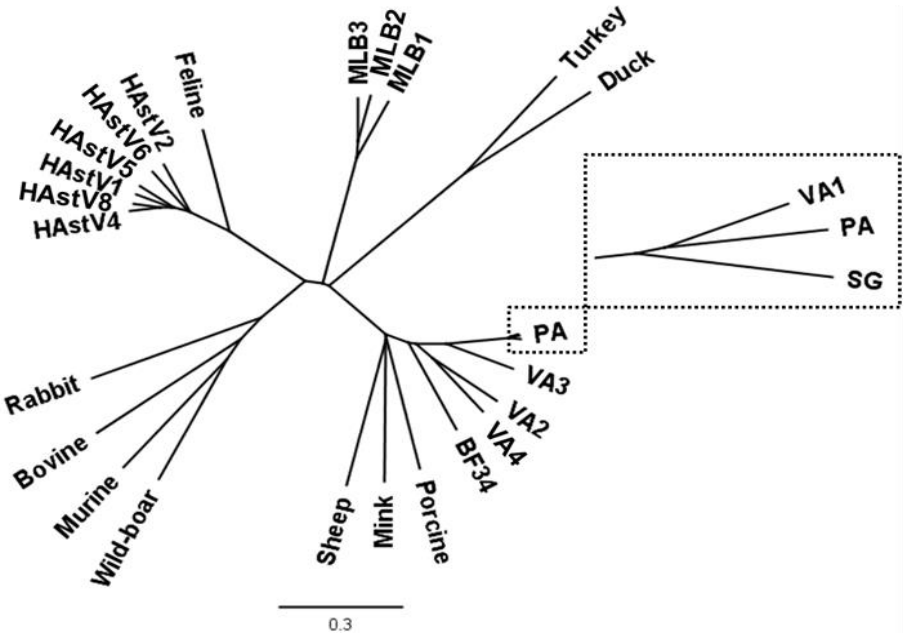


Phylogeny 27 complete genomes of astrovirus

Alignements ClustalW2 puis arbre Tamura-Nei, Neighbor-joigning, no outgroup.

A distant astrovirus clade

Finkbeiner et al, Identification of a novel astrovirus (astrovirus VA1) associated with an outbreak of acute gastroenteritis. J Virol. 2009

Quan PL Astrovirus encephalitis in boy with X-linked agammaglobulinemiaEmerg Infect Dis. 2010 Jun;16(6):918-25

.

Clinical case 3 : breadth of detection

CLINICAL PICTURE

- 16-year boy living in Guadeloupe
- developing progressive encephalopathy
- Genetic defiency XLA (X-linked agammaglobulinemia)
- Cognitive disorders since several years (reading and calculation diseability)
- Since one year : muscle pain, walking abnormalities, severe neurological disorders (Piriformis syndrome)
- Brain biospy : panencephalitis , T CD8+

DIAGNOSTIC PICTURE

Viral screening	CSF	Brain biopsy
CMV	negative	negative
EBV	negative	negative
HHV6	negative	negative
HSV1-2	negative	negative
VZV	negative	negative
enterovirus	negative	negative
astrovirus	negative	
adenovirus		negative
toxoplasma	negative	

Clinical case 3 : breadth of detection

NGS-based ASSAY

DENGUE 2*

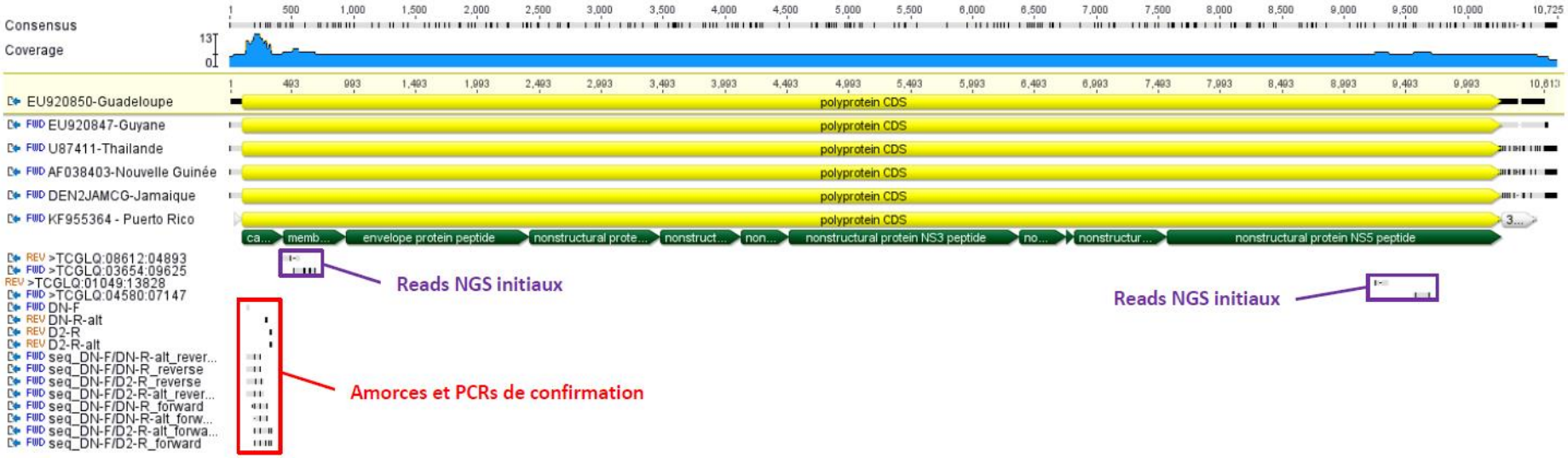
* Confirmation by PCR on brain biopsy, negative on plasma

CLINICAL OUTCOME

- **Initial therapy was adapted :**

Addition of IV and then oral ribavirin associated with weekly subc. injection of (PEG-IFN)+ increase iv IG dosage

- **Search for Iv Ig with high titers anti-DENV-2**



--- Zoom sur la zone d'amorces et PCRs de confirmation ---

Clinical case 4 : analytical sensibility

CLINICAL PICTURE

- 26-year man with X-linked agammaglobulinemia (XLA)
- Progressive decline
- Intense muscular fatigability
- MRI: abnormal meningeal enhancement
- Previous history of enterovirus-associated encephalitis

DIAGNOSTIC PICTURE

Viral screening	CSF
Enterovirus	negative

- **NEGATIVE** results from conventional diagnostic pipeline

Clinical case 4 : analytical sensitivity

PATHOQUEST NGS-based ASSAY

Enterovirus (echovirus 4)*

* Done on the same sample as PCR.
PCR became positive on a sample taken 3 weeks later

CLINICAL OUTCOME

- **Initial therapy was adapted :**

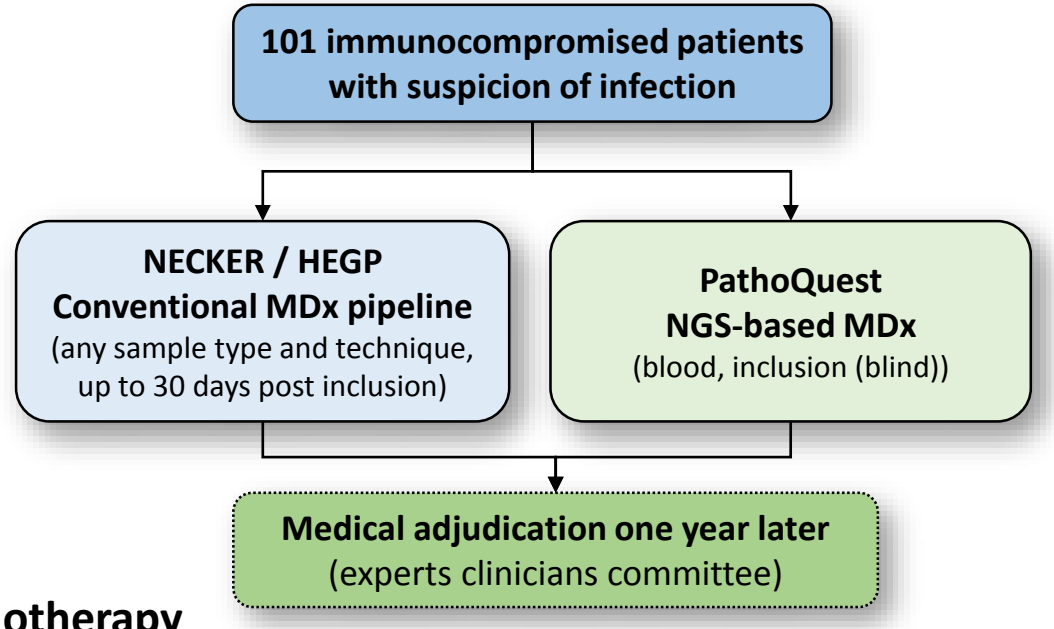
Addition of IV and then oral ribavirin associated with weekly subc. injection of (PEG-IFN)
Increase of iv IG dosage

Use as a first line in a non-interventional clinical trial test in a cohort of immunocompromized patients

- First clinical trial involving Whole Genome NGS-based diagnostic assay for infectious diseases.

Sponsor: PathoQuest

2 sites : Necker & HEGP hospitals



- Cohort (101 patients)

- **36 following anti-cancer chemotherapy**
- **22 following graft and immuno-suppressive treatment**
- **17 following Stem Cell transplant**
- 9 with primitive immunodeficiency
- 17 other causes of immuno-suppression

Key results of the clinical validity (PoC) study (1)

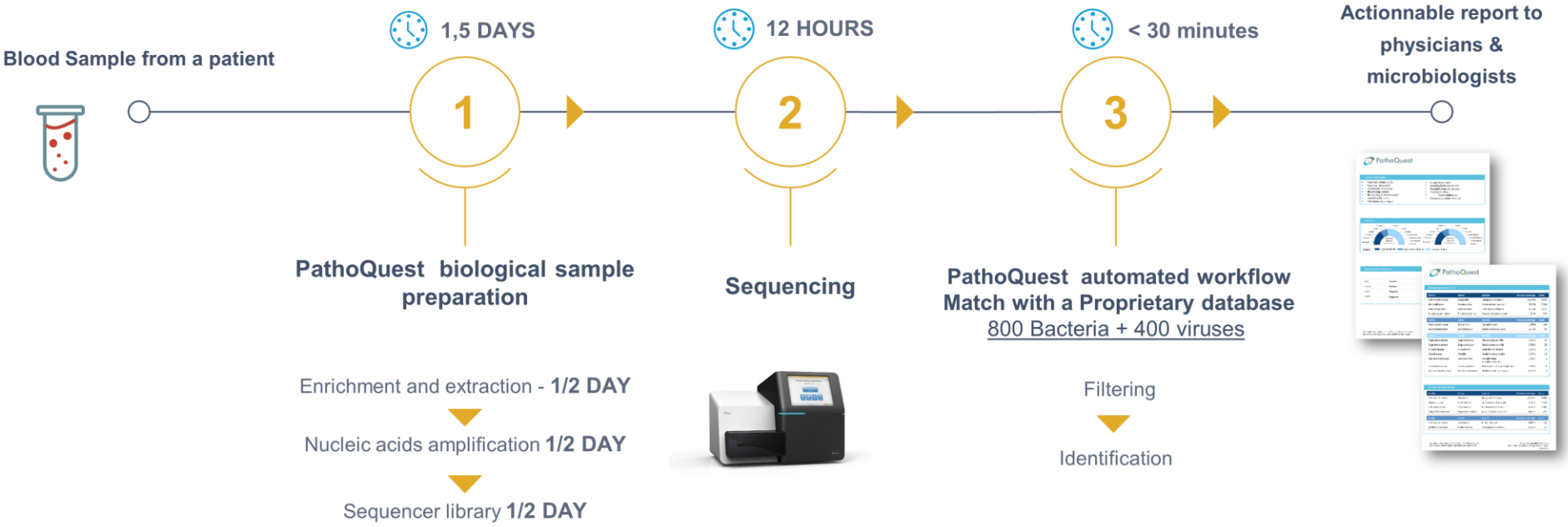
Standard methods, at inclusion

NGS	Positive	Negative	***Total***
Positive	9	27	**36 (36%)**
Negative	2	63	65
Total	**11 (11%)**	**90**	101

- Other slides not for diffusion

Towards a CE-IVD test

PathoQuest has finished the CE-marking of iDTECT™ Blood, an optimized version of the initial test for testing blood samples



First selected population : Febrile Neutropenic patients

- From hematology and oncology origins
- Adults & children
- Early Access Program in France (RIHN) and EU to evaluate the medical benefit planned in 2017
- LDT route in the US

CONCLUSIONS

- No need of specific amplification that shapes the range of detection and/or identification,
- wide range of detection
- analytical sensitivity in the range of PCR and taxonomic assignation often down to the species (or even type for viruses)
- In chronic infections of unknown etiology, often uncover pathogens in lesionnal tissues,
- In immunocompromized patients with bloodstream infections, identifies bacteria seen by culture and additionnal clinically relevant viruses and bacteria,
- Interpretation of positive results should be made by both microbiologists and clinicians,
- Interventional studies are needed to estimate the medical benefit and associated added- or saved- costs.

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