Metagenomic Shotgun Sequencing in the Microbiology laboratory: Routine Implementation for complex cases

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Inserm

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IMRB INSTITUT MONDOR DE RECHERCHE BIOMÉDICALE

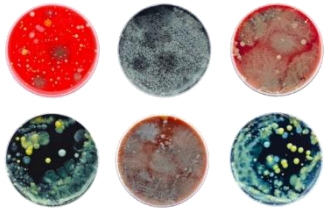
Background

- Infectious disease diagnosis

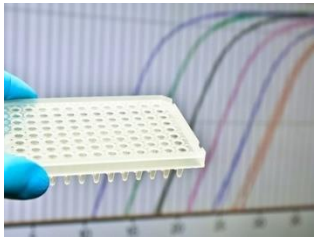
Symptoms of infection ± medical imaging

Encephalitis, Endocarditis, Skin infections…

Routine diagnosis in microbiology



Culture



Molecular biology

Positive results

⇒ Complete ?

⇒ incomplete ?

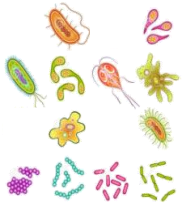
Negative results

⇒ No infection : Cancers, autoimmune diseases…

⇒ Unadapted approaches

Microbiological approaches

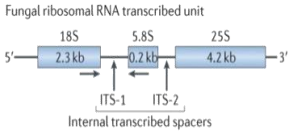
Organisms	Culture	PCR Panels	Sanger Targeted Metagenomic	NGS Targeted Metagenomic	DNA+RNA Shotgun Metagenomic
Bacteria	Partial	Targeted	16S	16S	Yes
Fungi	Partial	Targeted	ITS	ITS	Yes
Virus	Not used	Targeted	No	No	Yes
Plurimicrobial	partial	Targeted	No	Yes	Yes
Resistance	Yes (bacteria/Fungi)	Targeted	No	No	Yes
Limitations	Prior antibiotherapy, unadapted media	Biased approaches	No virus detected	No virus detected	?



Alive Pathogens

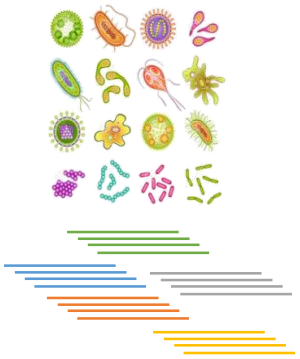
PCR panels

Syndromic approaches



Fungi, ribosomal ITS domains

Bacteria, ribosomal 16S loop (V1-V2/V3-V4…)



Whole Genome sequencing

Yarza et al., Nature reviews Microbiology, 2014
Underhill et al., Nature reviews immunology, 2014

Objectives of the study

- Develop an infection Unbiased Screening Method :
  - Pan pathogens
    - Bacteria (ECCMID 2017, ECCMID 2018 P0640)
    - Fungi (oral communication RICAI 2017)
    - Virus (Oral Communication ECCMID 2018)
  - Pan biological matrix, low volume, only one extraction

- Organize the Mg laboratory for routine diagnosis (ISO EN NF 15189)
  - Prescription
  - Preanalytical-process
  - Analytical process

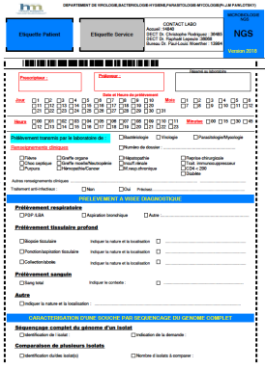
- Evaluate the benefit of this technic for patients with clinical infectious disease syndrome not or poorly documented by conventional microbiological approaches

Patients and methods (I)

Henri Mondor,
University hospital

3000 beds, 1000 acute patients

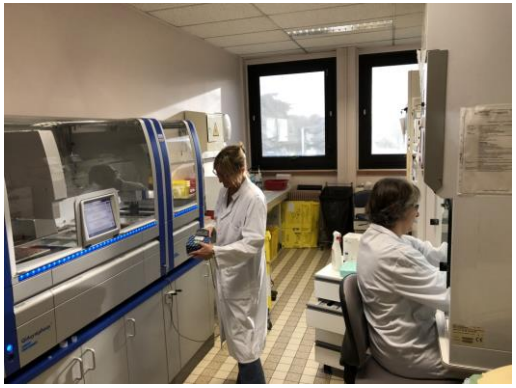
Microbiology Dpt



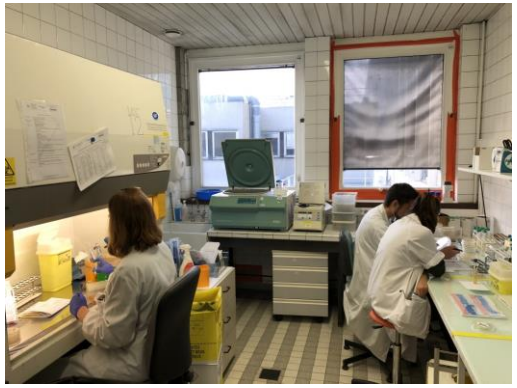
Clinicians

Bacteriology

Virology



Mycology

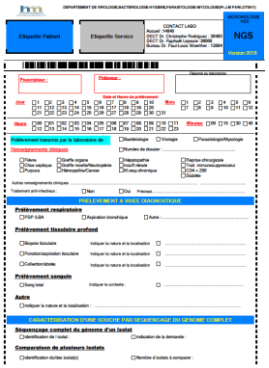


Patients and methods (II)

Microbiology Dpt

All results are negative, must be confirmed or completed

Clinicians



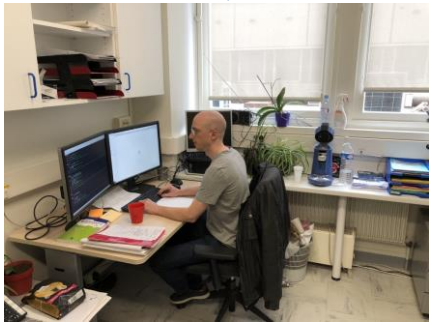
Mg prescription and sample collection

NGS platform

Pre-PCR extraction

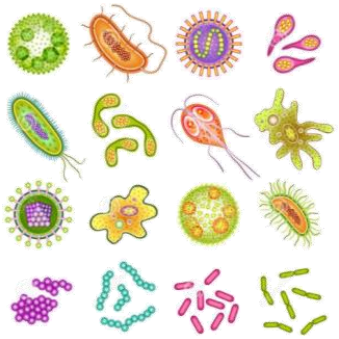
Library prep

Sequencing



Analysis

Unbiased MetaMIC Method



Various samples

DNA/RNA extraction

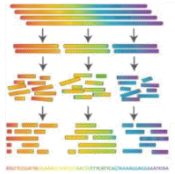
DNA/RNA Special protocols for metagenomic

Tissue

Cellular fluids

Acellular fluids

Library preparation



DNA Lib prep (adapted from Nextera XT)
+
RNA Lib prep (adapted from TruSeq RNA)

Sequencing

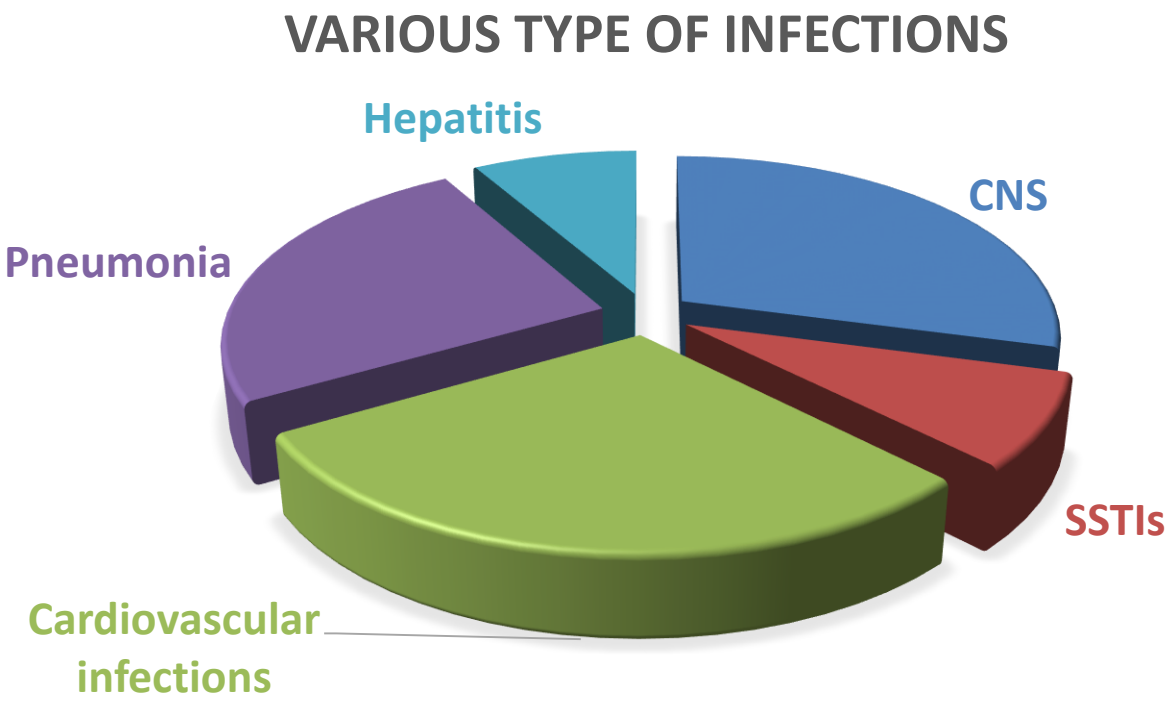
NextSeq Sequencing
*2*150bp*
(Illumina)

MetaMIC® Analysis

MetaMIC® (experimental and software are patented)

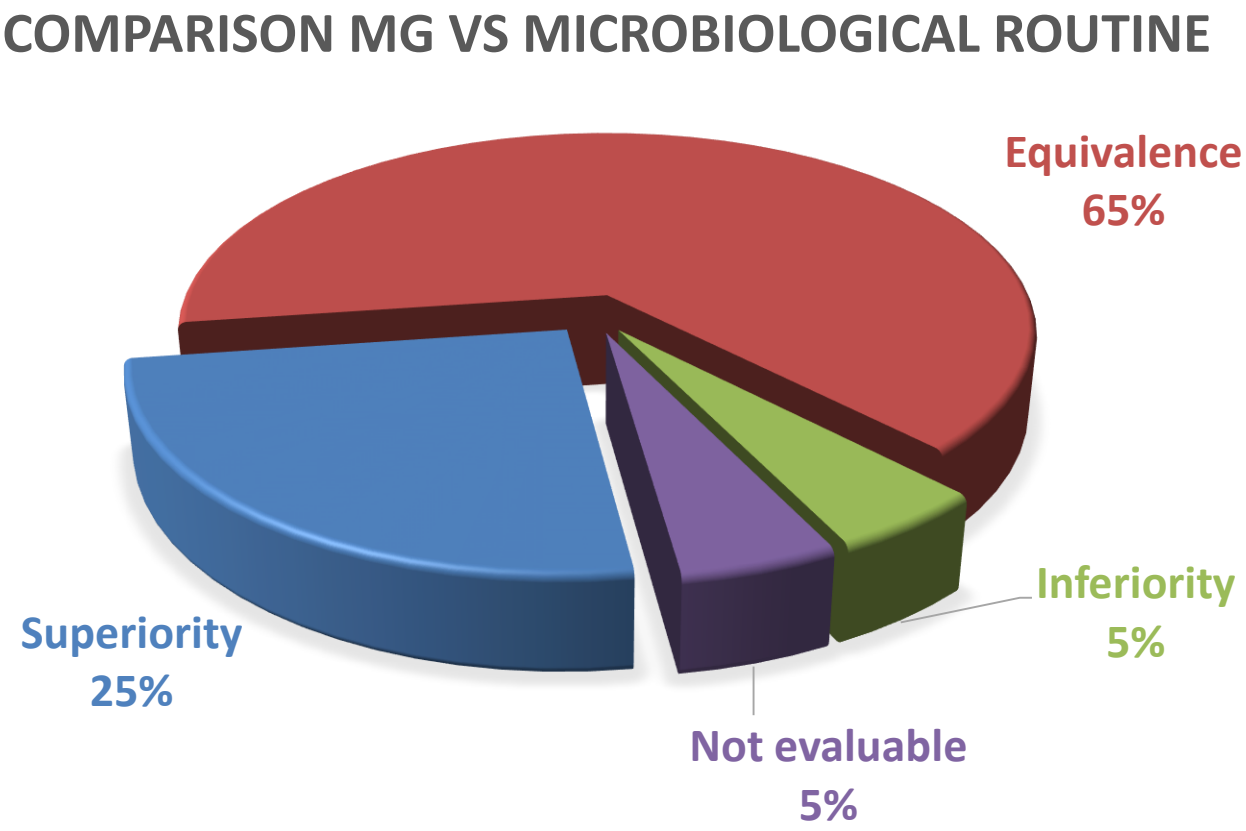
Results 1 : type of infection

- 29 patients included from June 2017 to Dec 2017
- High suspicion of infection without documentation using conventional tools



Results 2 : detection

- 25% of superiority vs Routine diagnosis



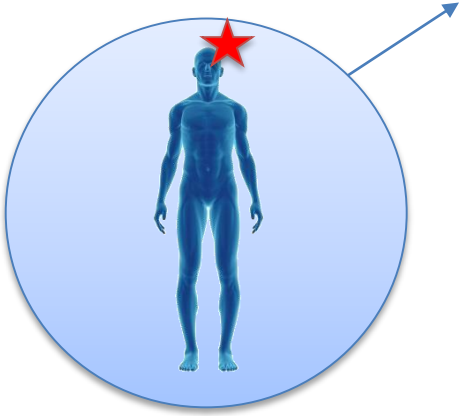
Results 3 : Inferiority (1 case)

Patient	Underlying condition	Nature of the sample	Results of Microbiology							Mg Results	Conclusion
			Bacteriology		Virology	Parasitology/Mycology					
			Culture	16S PCR	PCR*	Direct examination	Fungal culture	Antigen detection	PCR		
9	pulmonary tuberculosis, abdominal pain, alteration of general status	peritoneal punction	*S. anginosus,* + *M. tuberculosis*	NP	NP	NP	NP	NP	NP	*Prevotella melaninogenica* + *Fusobacterium nucleatum* + *Streptococcus anginosus*	Inferiority

Results 4 : Superiority (5 cases)

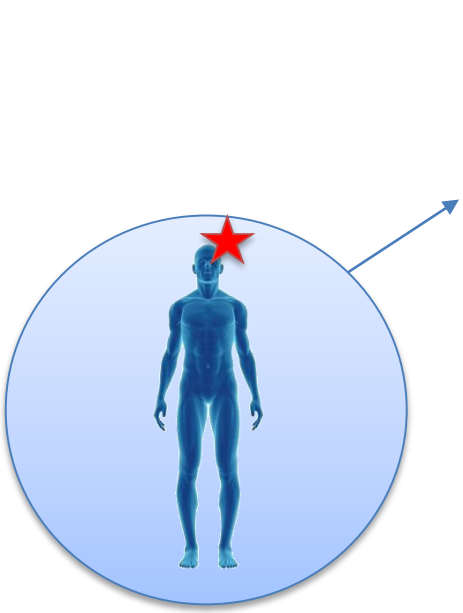
Patient	Underlying condition	Nature of the sample	Microbiology Results							NGS results	Conclusion
			Bacteriology		Virology	Parasitology/Mycology					
			Culture	16S PCR	PCR*	Direct examination	Fungal culture	Antigen detection	PCR		
2	Fasciitis	abdomino-pelvic sample	P. distasionis	NP	NP	NP	NP	NP	NP	*P. gingivalis* + *P. distasionis*	Superiority
7	Meningitidis	CSF	Negative (including Mycobacteria)	NP	neg (CMV; HSV1/2; VZV)	NP	NP	NP	NP	*N. cyriageorgica*	Superiority
14	Endocarditis	cardiac valve	S. pasteuri	NP	NP	NP	NP	NP	NP	*S. gallolyticus*	Superiority
18	Acute hepatitis	plasma	NP	NP	NP	NP	NP	NP	NP	*HHV6*	Superiority
20	ARDS in a diabetic patient	LBA	Negative (including Mycobacteria)	NP	Respiratory pannel: neg; HSV-1: pos (Ct=26);	Negative	*C. albicans*	Galactomannan Antigen: Negative	P. jirovecii: neg Aspergillus (28S, mitoch): neg	*P. aeruginosa, Candida albicans, HSV1*	Superiority

Exemple of Real life Mg



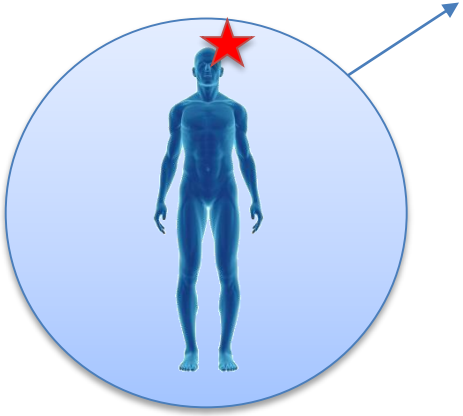
Clinic :
Man, 52yo
Febrile Coma, meningoencephalitis
MRI showed a cerebral abcess,
Pulmonary aspergillosis but patient not IC

Exemple of Real life Mg



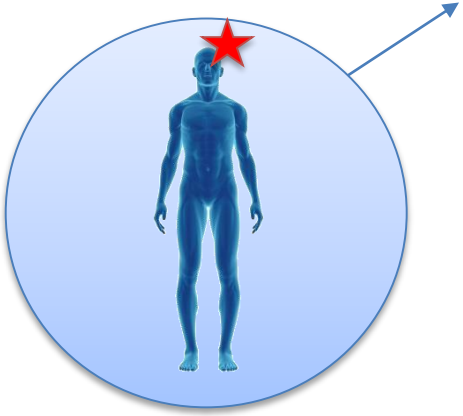
CSF Microbiology:
>1000 white blood cells (L predominance)
Culture neg
Virus qPCR neg

Exemple of Real life Mg



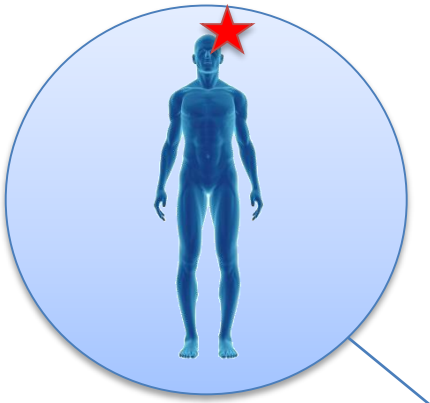
CSF sended to NRC of Nocardia:
PCR+ *Nocardia cyriageorgica*

Exemple of Real life Mg



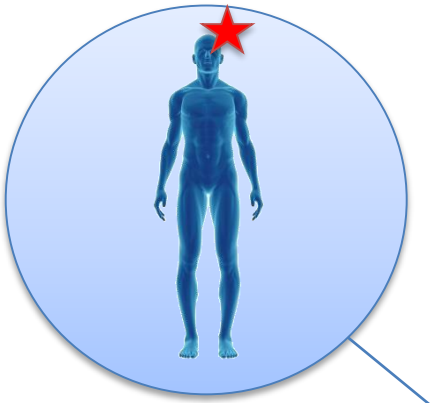
Mg Meta MIC :
Nocardia cyriageorgica, with high ratio
TMP and SMZ sensitive

Exemple of Real life Mg



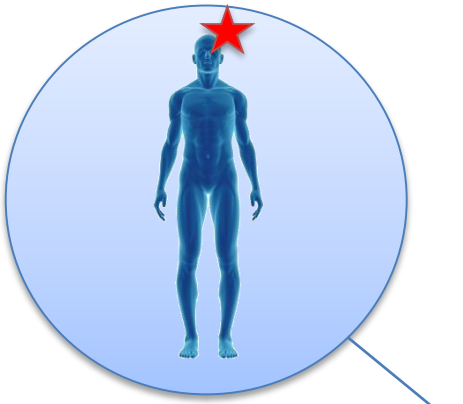
Clinic :
Tienam, Bactrim, Immuno explo : GMCSF deficiency
M1 : new febrile confusion,
Normal MRI

Exemple of Real life Mg



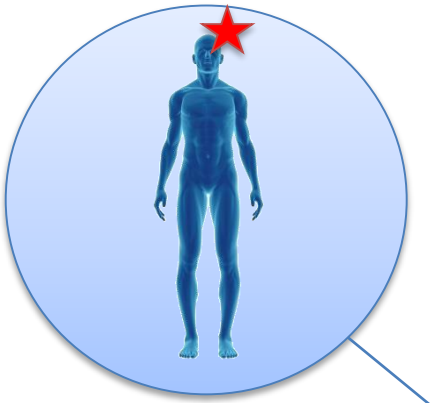
CSF microbiology:
100 white blood cells
Culture neg
Virology qPCR neg

Exemple of Real life Mg



Nocardia NRC results:
PCR Nocardia negative

Exemple of Real life Mg



Mg MetaMIC :
Negative

Conclusion

- We have developed a clinical routine Mg Diagnosis approach
- Mg could be integrated in clinical routine and reached quality norms such as ISO EN NF 15189
- Various type of infectious disease could be assessed by this approach like other Microbiological technics
- 25% of cases are (better) documented by Mg
- Ability to find not only the suspected microbial etiologies but also those that are not expected!

Many thanks…

MetaMIC Project

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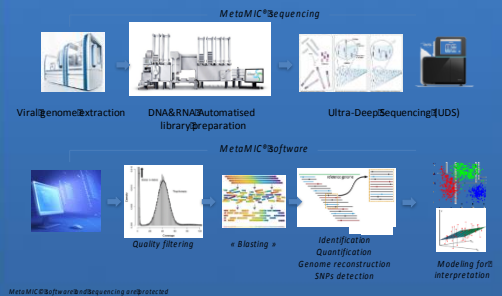
Vanessa Démontant
Emilie Sitterlé
Jean-Michel Pawlotsky
Paul Louis Woerther



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VIème EDITION DU PRIX JEUNES CHERCHEURS

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Hepatitis National Reference Center

Microbiology & Infectious disease team Mondor

Microbiology Avicennes

Microbiology Necker

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