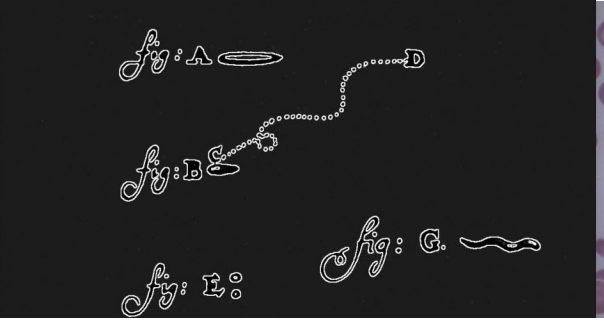
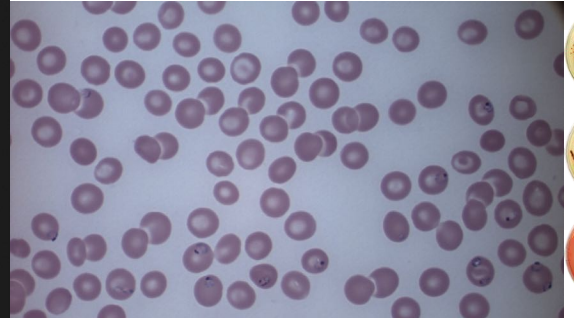
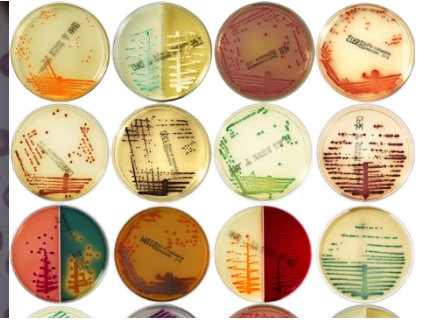
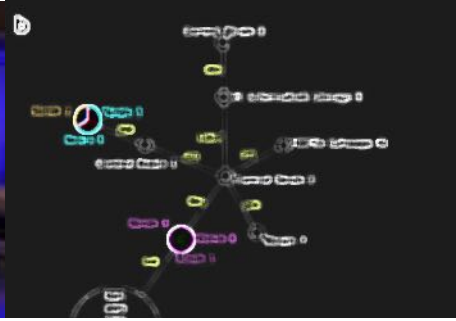






From the clinic: The changing profession of the clinical microbiologist

Dr Onya Opota, PhD., CM.

Diagnostic department, Institute of Microbiology
Lausanne hospital university, Switzerland

ESCMID Postgraduate Technical Workshop
Clinical bioinformatics for microbial genomics and metagenomics
Lausanne, 9 September 2019

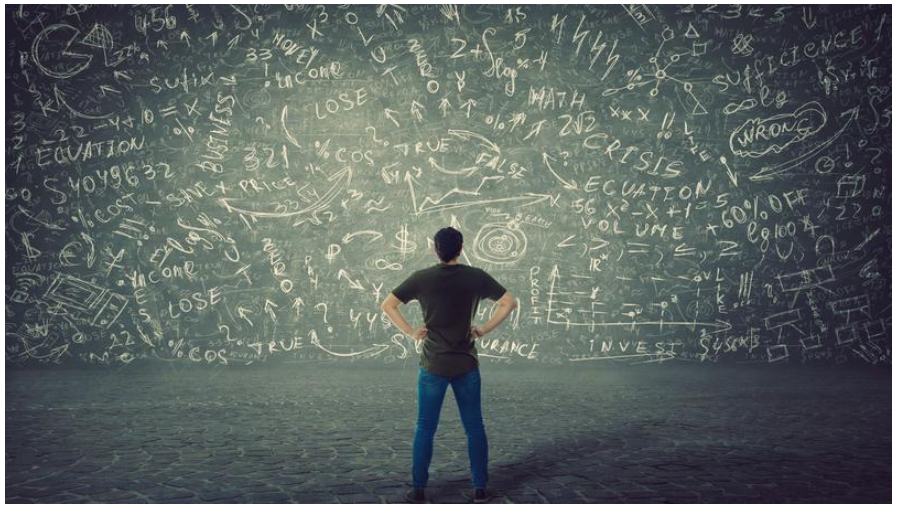
Disclosure

- Research project and InnoSwiss grant with Resistell AG (Switzerland)

Acknowledgements

Including content from

- Dre Katia Jaton
- Prof. Gilbert Greub
- Dre Aitana Lebrand
- Dre Alix Coste
- Dr Antony Croxatto
- Prof. Eric Juengst



Backgrounds and Purpose

Is the profession of clinical microbiologist changing?

❖Backgrounds:

- Clinical microbiology is a field in constant evolution
- Increasing technological opportunities
- Growing emphasis on societal issues

❖Purpose:

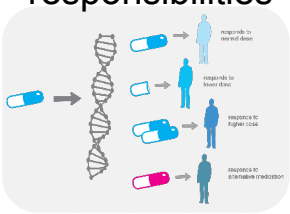
- Reflection on changes in the profession? Changes in the approaches? Changes in the perimeter of CM

Plan

1. **Brief history**
2. **New technologies**
3. **Positioning of the microbiologist**
4. **Phenotypic microbiology: ongoing improvements**
5. **Epidemiology**
6. **Ethical challenges in genomic approaches to infectious disease**
7. **Molecular & NGS: opportunity for new approaches or new applications?**
8. **Conclusions**

Brief history

Societal changes
Big data, personalized medicine, equity, parity, responsibilities



"Microbes"

Word "Microbiology", Louis Pasteur: the study of living organisms of microscopic size

Koch bacillus
Koch postulate

Improvement of phenotypic methods

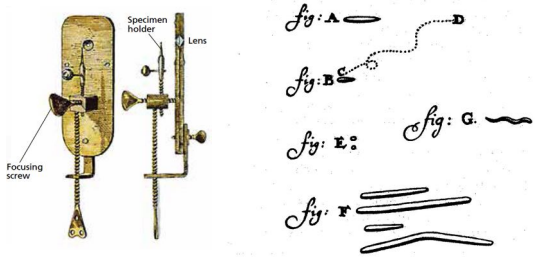
1860 **1878** **1884-1890**

?

1663-1685

Microscopy
Spermatozoids
Infusoria
Animalcules

A. van Leeuwenhoek



1850

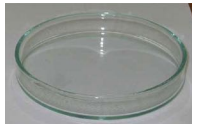
Broth

1880

Potato

1890

Petri dish



1990

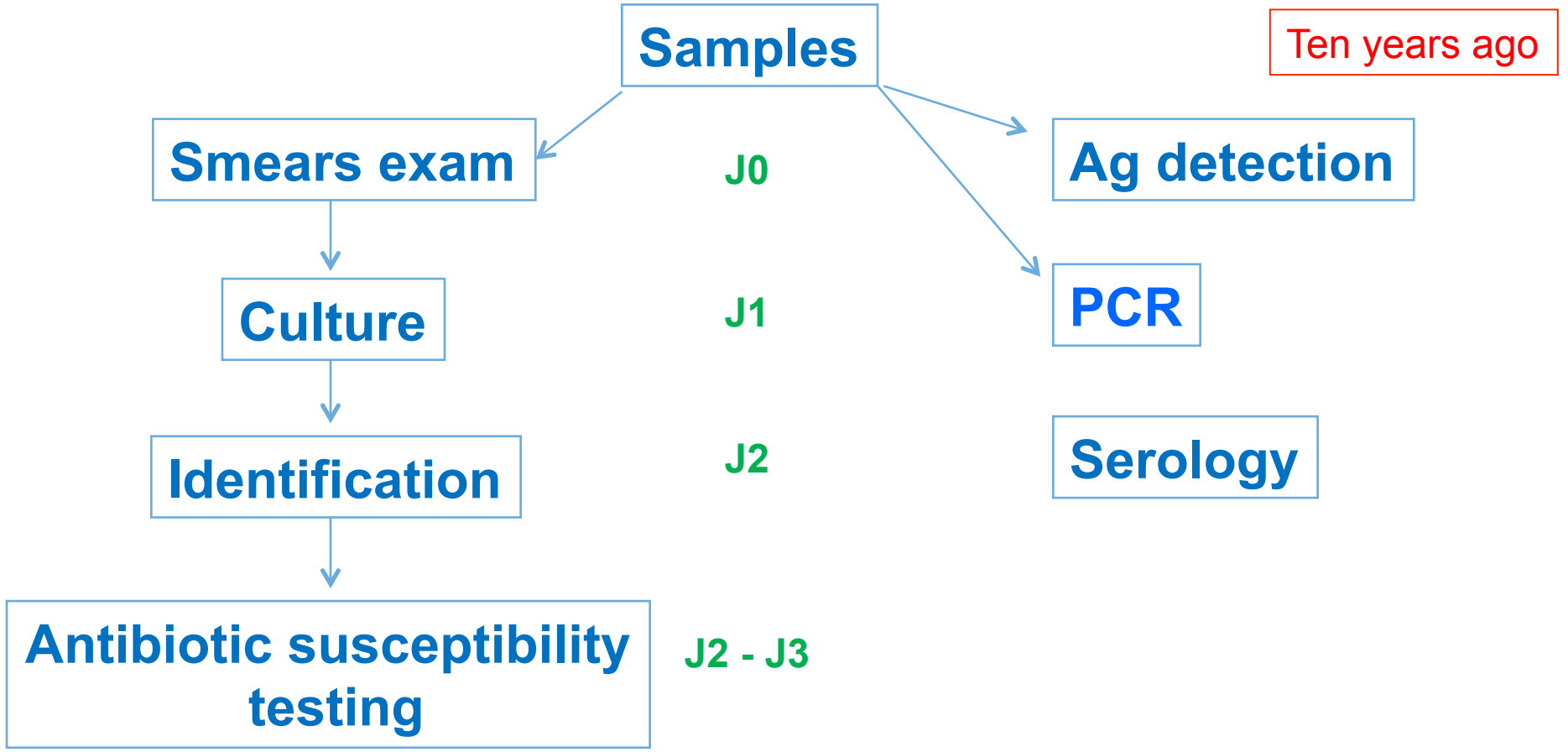
Automates
AST
Blood culture

2000-2019

Molecular diagnostic
MALDI-TOF MS
Automation
NGS

Approach in clinical microbiology

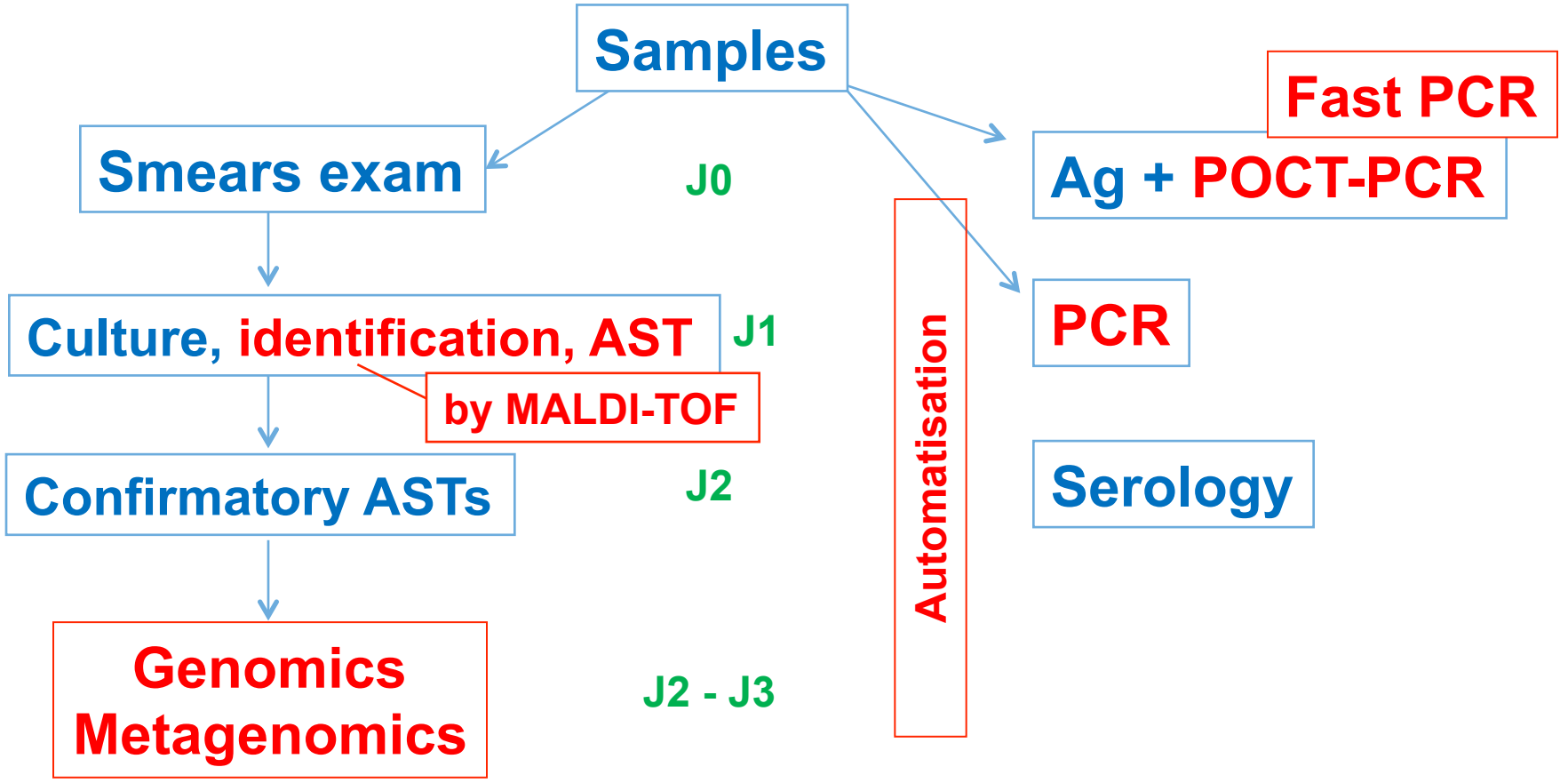
Technological opportunities of the past 10 years



Courtesy of Dre Jaton and Prof. Greub

Approach in clinical microbiology

Technological opportunities of the past 10 years



More information, more rapidly

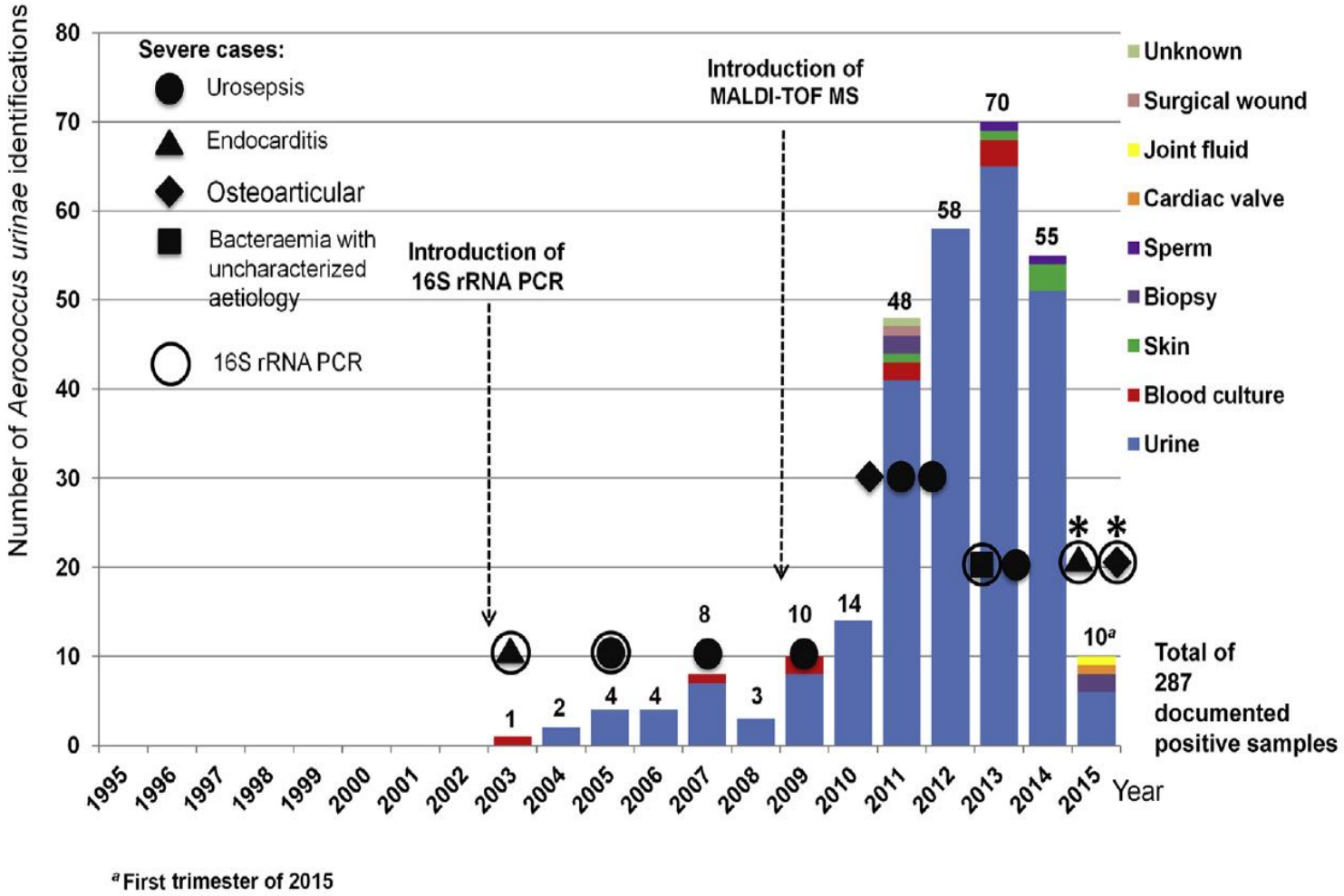
Courtesy of Dre Jaton and Prof. Greub

Pathogens identification

Impact of new technologies

- *Aerococcus urinae*
  - Gram positive cocci, pairs and clusters
  - α-haemolytic
- Identification hampered by morphotype ambiguity with streptococci or coagulase-negative staphylococci.
- Rarely identified before 2009.
- Identification improved by 16S rRNA PCR and MALDI-TOF MS

Significant pathogenic potential that should not be neglected (bloodstream infections, osteoarticular Infections etc …)

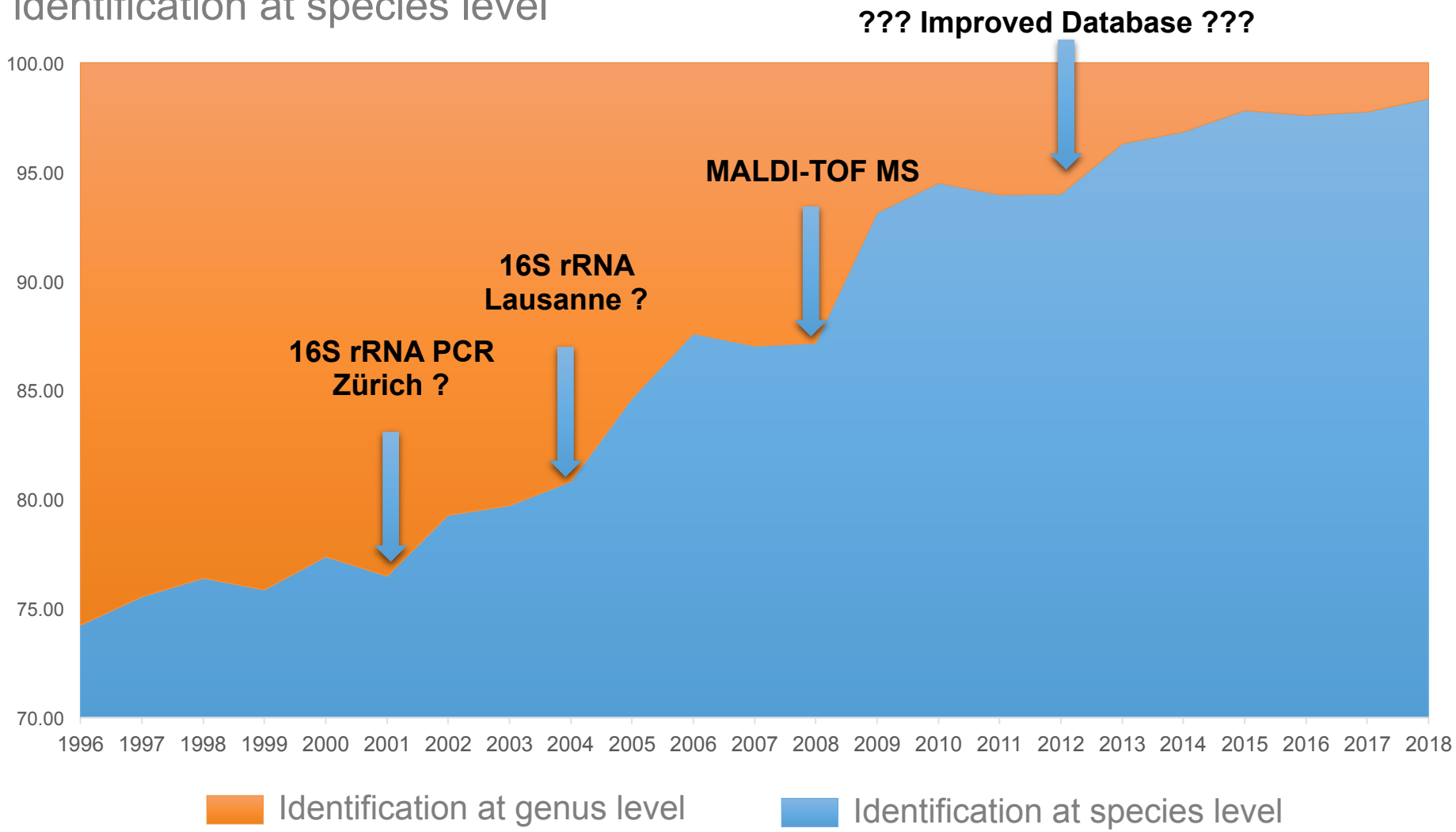


Opota, O., et al. 2016. Clin Microbiol Infect **22**(1): e1-2.

Pathogens identification

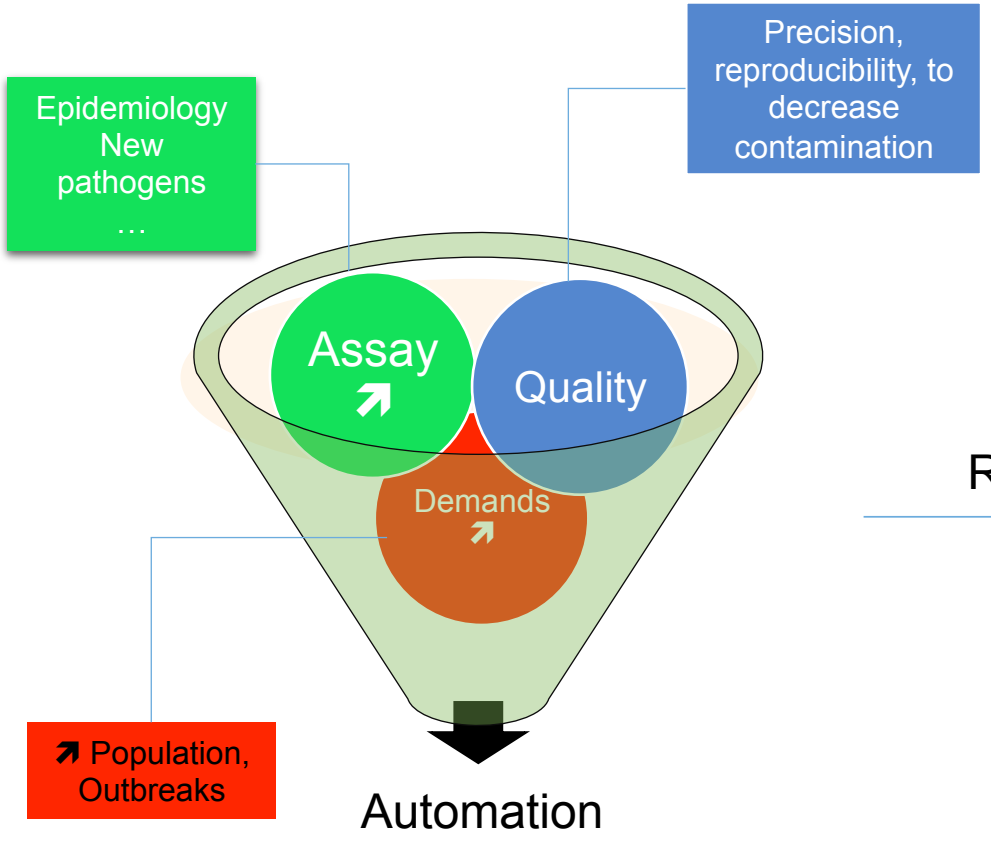
Impact of new technologies

Successful identification at species level

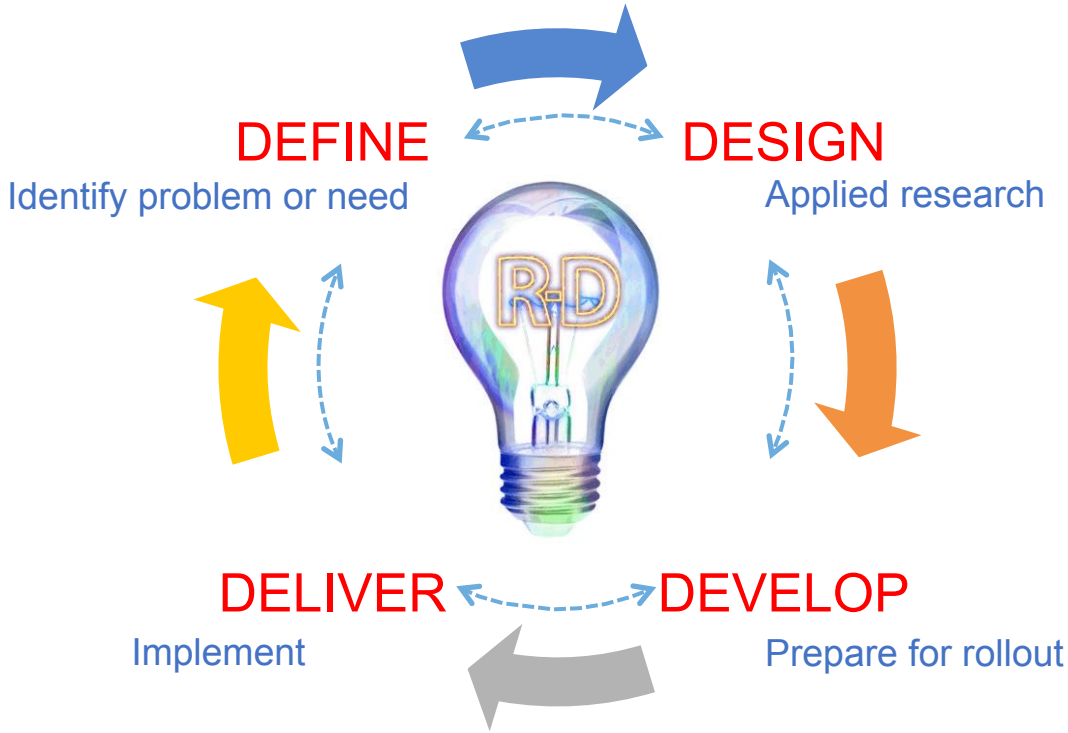


Courtesy of Dre Alix Coste

Importance of Research and Development



R&D



Importance of Research and Development

In-house automated molecular diagnostic plateform

M. pneumoniae
C. pneumoniae
L. pneumophila
M. tuberculosis complex
B. pertussis
B. parapertussis
N. meningitidis
L. moncytogenes
H. influenzae
C. trachomatis
N. gonorrhoeae
B. henselae
B. quintana
M. hominis
A. fumigatus
Bacillus anthracis
Plasmodium sp
Toxoplasma gondii
eubactérienne
panmycobactérienne
panfongique
Inluflenza A/B
RSV A/B
HSV/VZV
HHV6
HPV
Parvovirus B19
CMV
EBV
Candida sp.
Rickettsia sp.

TAT 48-72 hours

TAT 24-48 jours

6-24 hours

ACTIVITY

2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
S. pneumoniae JC virus BK virus Norovirus HMPV	*C. burnetii* *Acantamoeba* *Haemophilus sp.* *Pneumocystis* Coronavirus Parainfluenza Picornavirus	HDV HBV Influenza H1N1 GeneXpert EV	*Brucella sp.*	*T. whipplei* *Leishmania* Pan chlamydia HHV8 HEV GX Mtbc/Rif GX Flu A/H1N1/B	L28B Diminution TAT	*S. aureus PVL* *S. aureus mecC* *F. tularensis* *Yersina pestis* *Anaplasma sp*	*C. psitacci* *C. abortus* *N. gonorrhoeae* GX Flu A/B/RSV GX Noro	C extraction Fast PCR HM	*Aspergillus sp.* *A. terreus* *A. flavus* *A. niger*	*Kingella kingae* *Zika virus* *Measle*

< 2007

MagNA Pure LC ADN

EasyMag ARN

GeneXpert

MagNA Pure 96 ADN/ARN

FAST real time PCR

2x HAMILTON

2X Quantstudio

BD-MAX

Fastfinder (informatique)

Research and developement to introduce new tests and to achieve automation

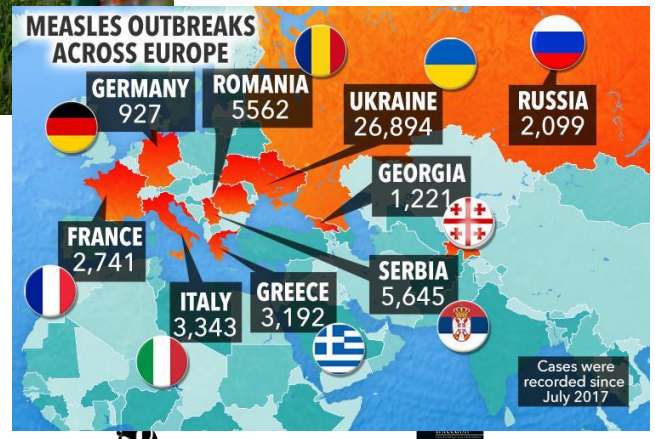
Ten years of R&D and full automation in molecular diagnosis. Gilbert Greub, Roland Sahli, René Brouillet and Katia Jaton. Future Microbiology (11), 403-425, 2016.

Courtesy of Dre Katia Jaton

Importance of Research and Development

- Reactivity and Pertinence
  - development and innovation that fit with (local) needs of the laboratories
  - Epidemiology change
  - Emerging pathogens
  - Outbreaks
- Clinical microbiologist
  - Stay up to date
  - Follow up after the introduction of new tests
    - Translational studies with physicians
    - To determine the added value of new tests
    - The needs for new tests
    - How to interpret

Ebola



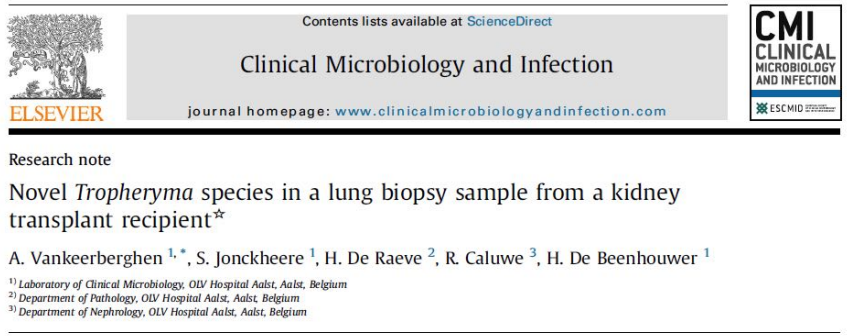
Measles

Original article

Genomics of the new species *Kingella negevensis*: diagnostic issues and identification of a locus encoding a RTX toxin

Onya Opota [a,**,1], Sacha Laurent [a,1], Trestan Pillonel [a], Marie Léger [b], Sabrina Trachsel [b], Guy Prod'hom [a], Katia Jaton [a], Gilbert Greub [a,c,*]

Kingella negevensis


Clinical Microbiology and Infection 24 (2018) 548.e5–548.e8
Contents lists available at ScienceDirect
Clinical Microbiology and Infection
journal homepage: www.clinicalmicrobiologyandinfection.com
ELSEVIER
CMI CLINICAL MICROBIOLOGY AND INFECTION
ESCMID

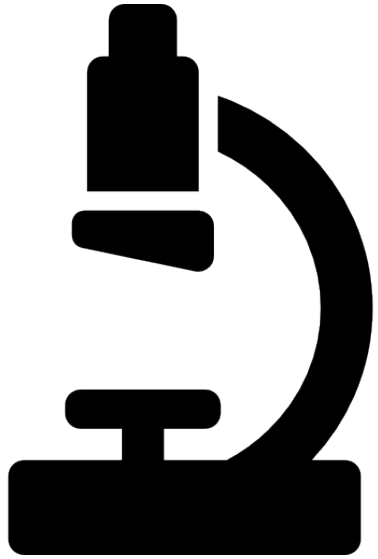
Research note

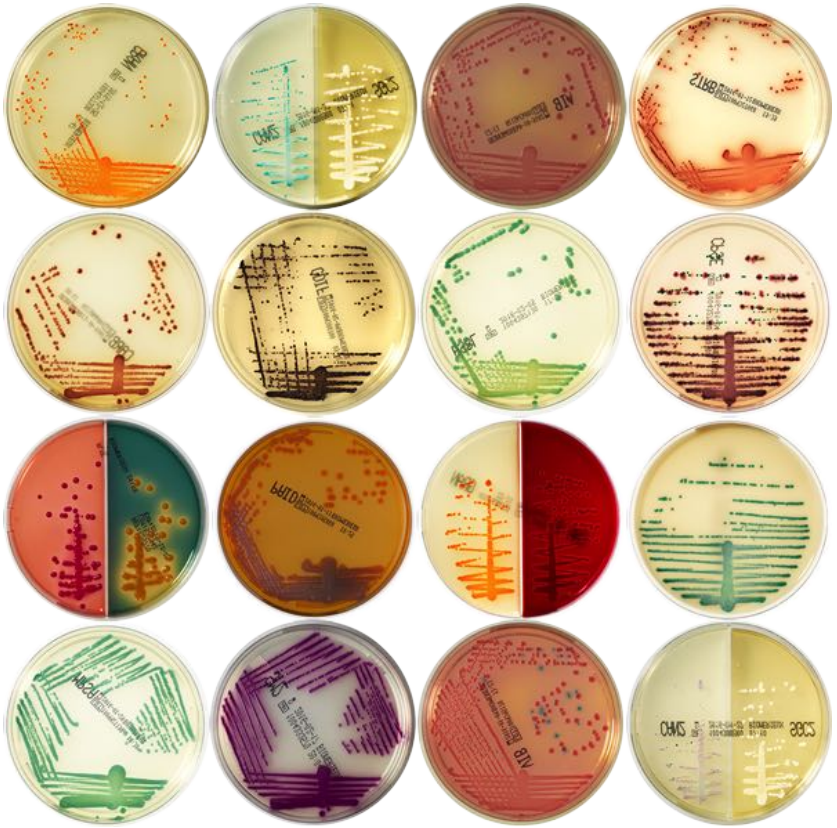
Novel *Tropheryma* species in a lung biopsy sample from a kidney transplant recipient☆

A. Vankeerberghen [1,*], S. Jonckheere [1], H. De Raeve [2], R. Caluwe [3], H. De Beenhouwer [1]
[1] Laboratory of Clinical Microbiology, OLV Hospital Aalst, Aalst, Belgium
[2] Department of Pathology, OLV Hospital Aalst, Aalst, Belgium
[3] Department of Nephrology, OLV Hospital Aalst, Aalst, Belgium

Tropheryma sp.

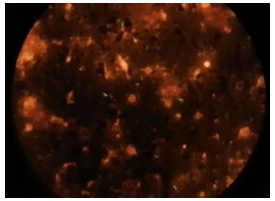
Ongoing development in phenotypic microbiology

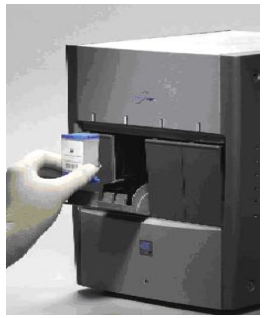


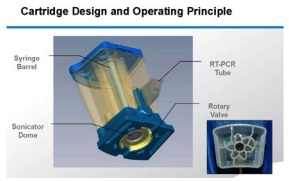


Ongoing development in phenotypic microbiology

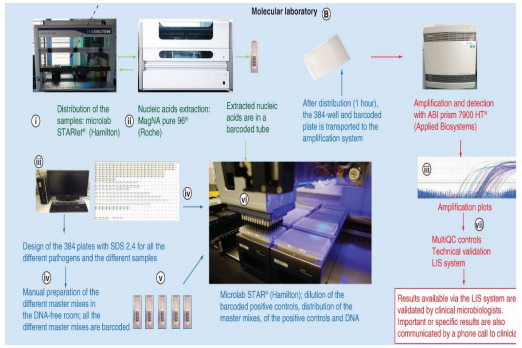
	Microscopy	Rapid PCR test	PCR	Culture (BSL3)
LIMIT OF DETECTION	5'000-10'000 CFU/ml		100-1'000 CFU/ml	10-100 CFU/ml
SENSITIVITY	-			+
TIME TO RESULT		2 hours Respiratory specimens	6-24hours Any specimens	6-8 weeks



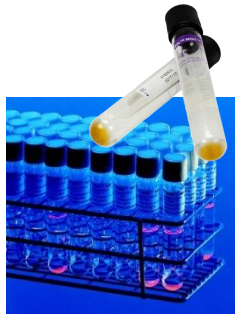




Xpert MTB/RIF
TAT 2h, emergency, 7/7
Xpert MTB/RIF (Cepheid)
Specific detection of *Mtbc*
Sensitivity +++
Detection of resistance marker (*rpo*B)



In-house developed automated Molecular Dx platform
TAT 6-24h, opening hours, 5 days/7
Specific detection of *Mtbc*
Sensitivity +++
– Batches, High throughput
– +/- Coast effective
– Specialized lab



Added value of molecular assay Xpert MTB/RIF compared to sputum smear microscopy to assess the risk of tuberculosis transmission in a low-prevalence country. Opota O, Senn L, Prod'hom G, Mazza-Stalder J, Tissot F, Greub G and Jaton K *Clin Microbiol Infect* 2016; **22**: 613-9.

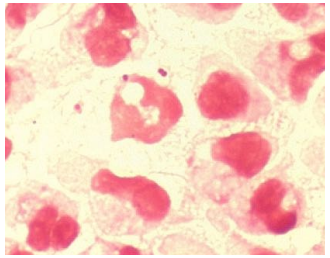
The rapid molecular test Xpert MTB/RIF ultra: Towards improved tuberculosis diagnosis and rifampicin resistance detection. Opota O, Mazza-Stalder J, Greub G, Jaton K. *Clin Microbiol Infect* 2019.

Ongoing development in phenotypic microbiology

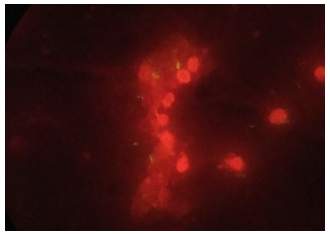
MICROSCOPY

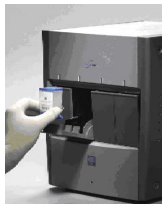
MOLECULAR BIOLOGY

GRAM for CSF



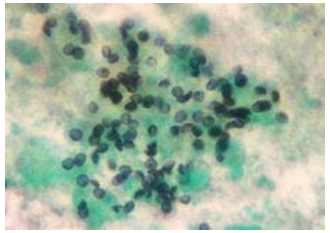
Auramine/Ziehl staining for mycobacteria

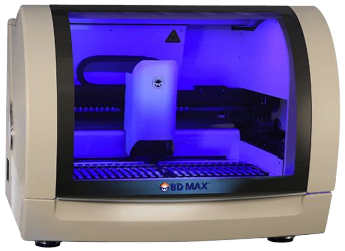




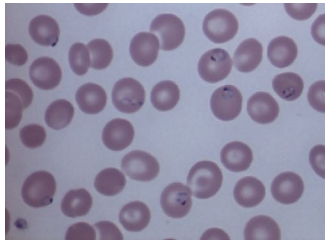
\+ AUTOMATED MICROSCOPY

Silver staining for PCP





Thin smear for malaria



Ongoing development in phenotypic microbiology

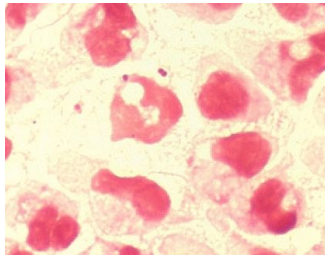
MICROSCOPY

MOLECULAR BIOLOGY

+

AUTOMATED MICROSCOPY

GRAM for CSF



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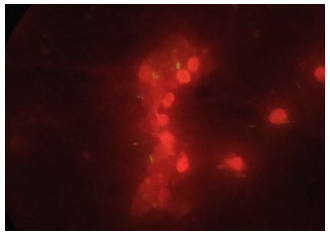
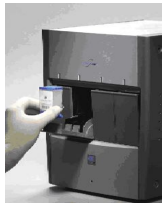
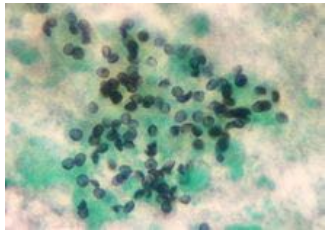
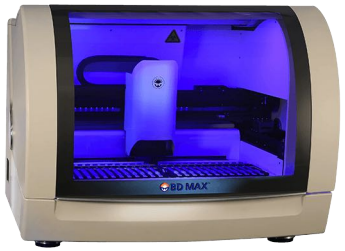


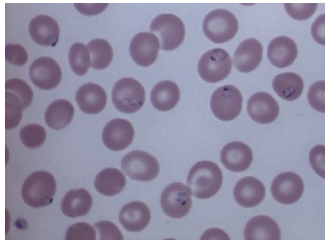


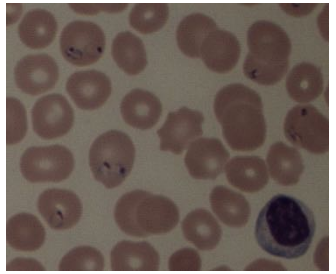
Silver staining for PCP



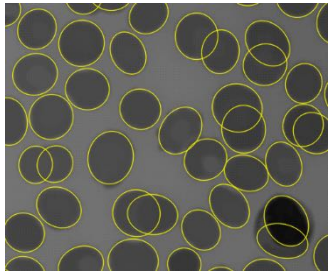


Thin smear for malaria

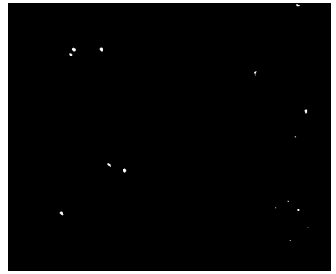




1. Original image

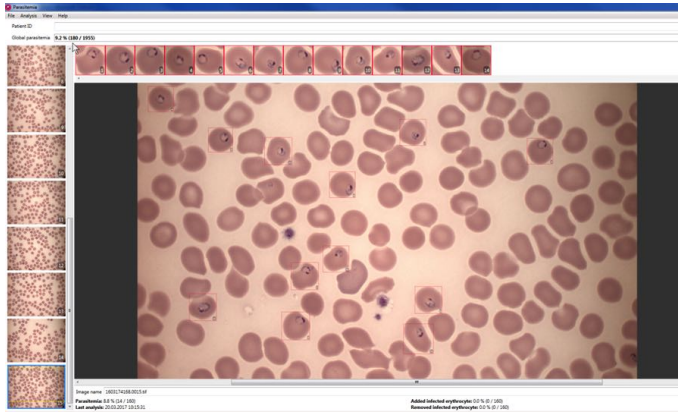


2. Finding location of cells by ellipsis matching.

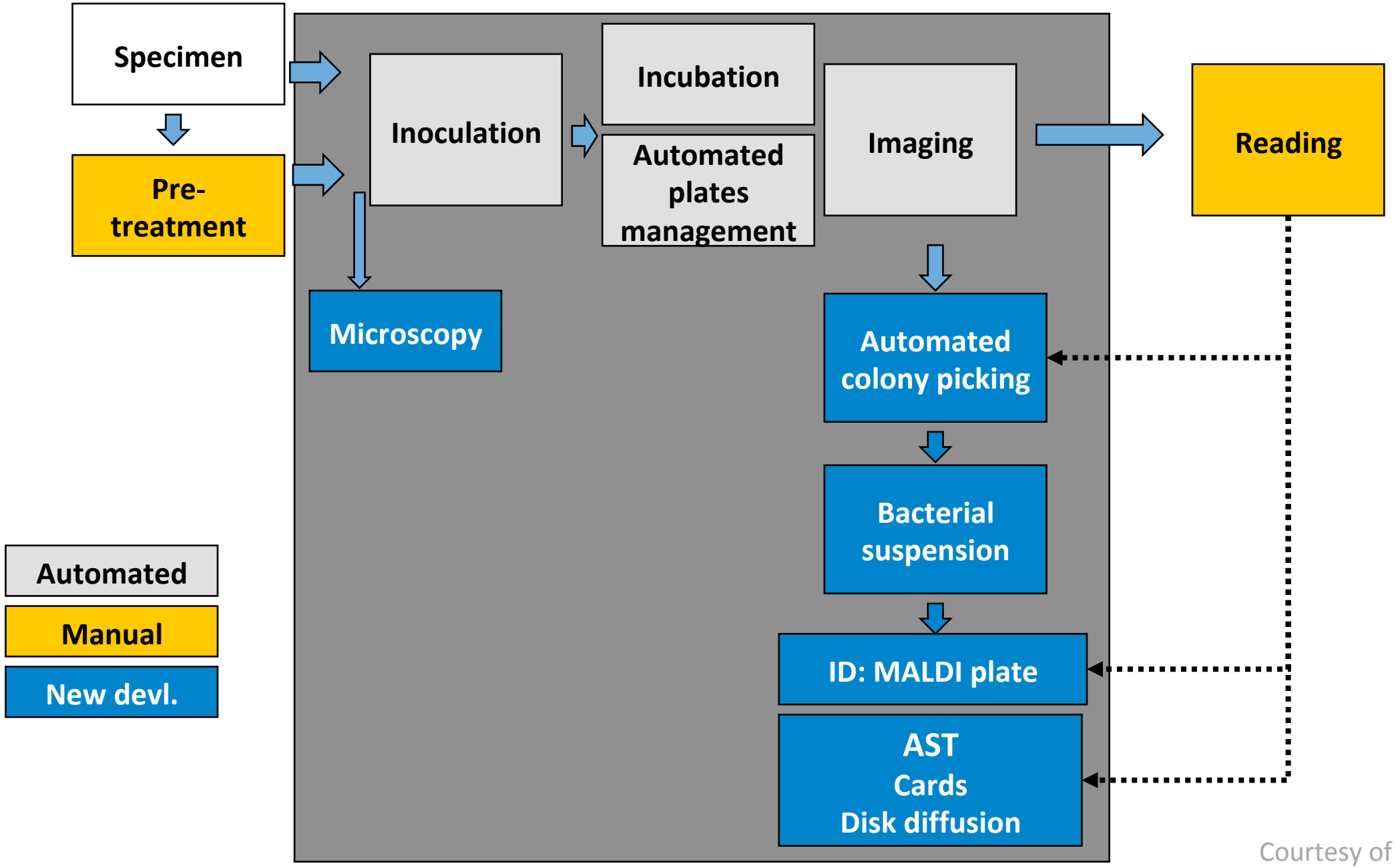


3. Segmentation of parasites and other stained elements.

4. Classification of cells and computation of the parasitemia. Blue cross: healthy cells, red cross: infected cells, black cross: other elements

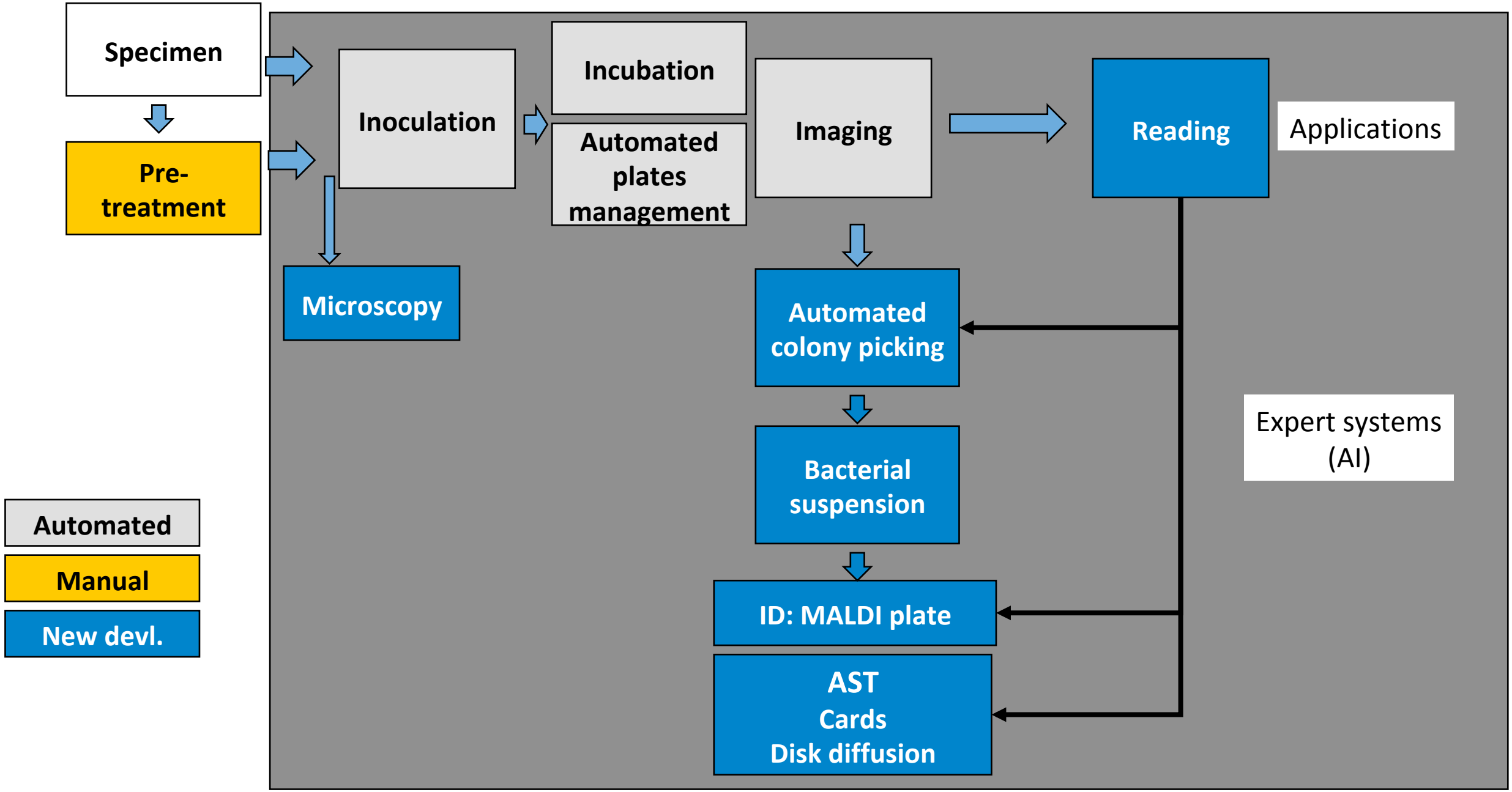


Full automation in Bacteriology



Courtesy of Dr Antony Croxatto

Full automation in Bacteriology



Ongoing development in phenotypic microbiology

Additional improvement of laboratory productivity?

Development of intelligent algorithms

Ongoing projects

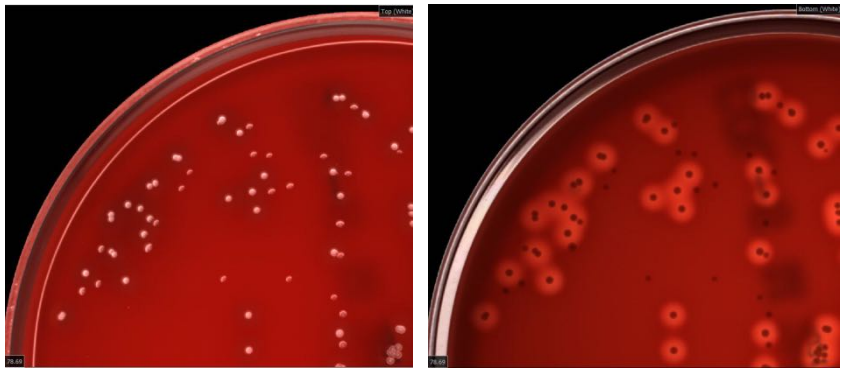
- Automated quantification
- **Automated identification (Imaging identification)**
- Automated processing
- ……

Courtesy of Dr Antony Croxatto

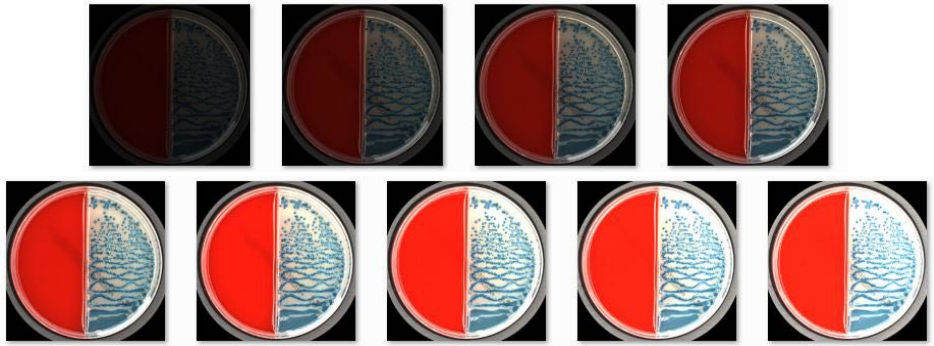
Imaging identification

Visible and invisible light characteristics

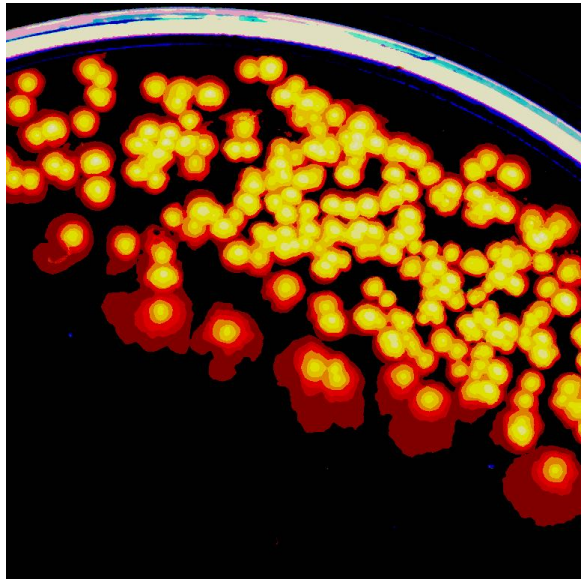
Illumination sources



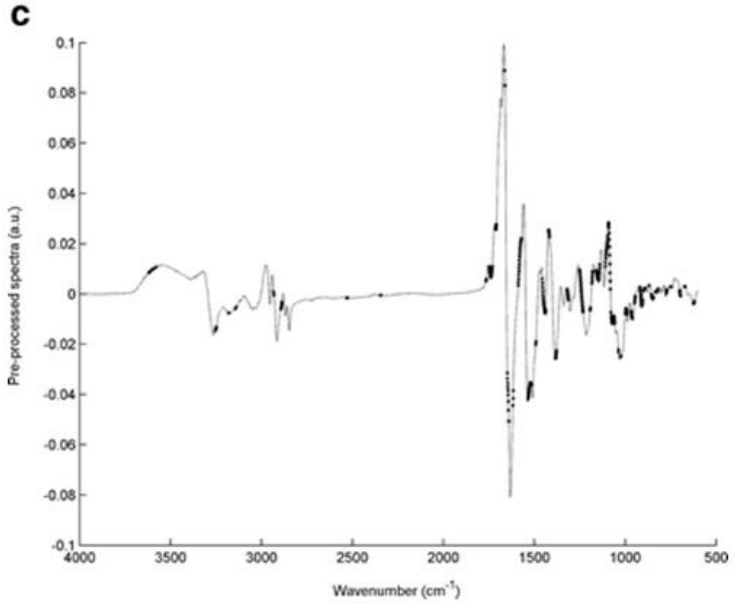
Optimal illumination (pixel, chanel)



Growth pattern



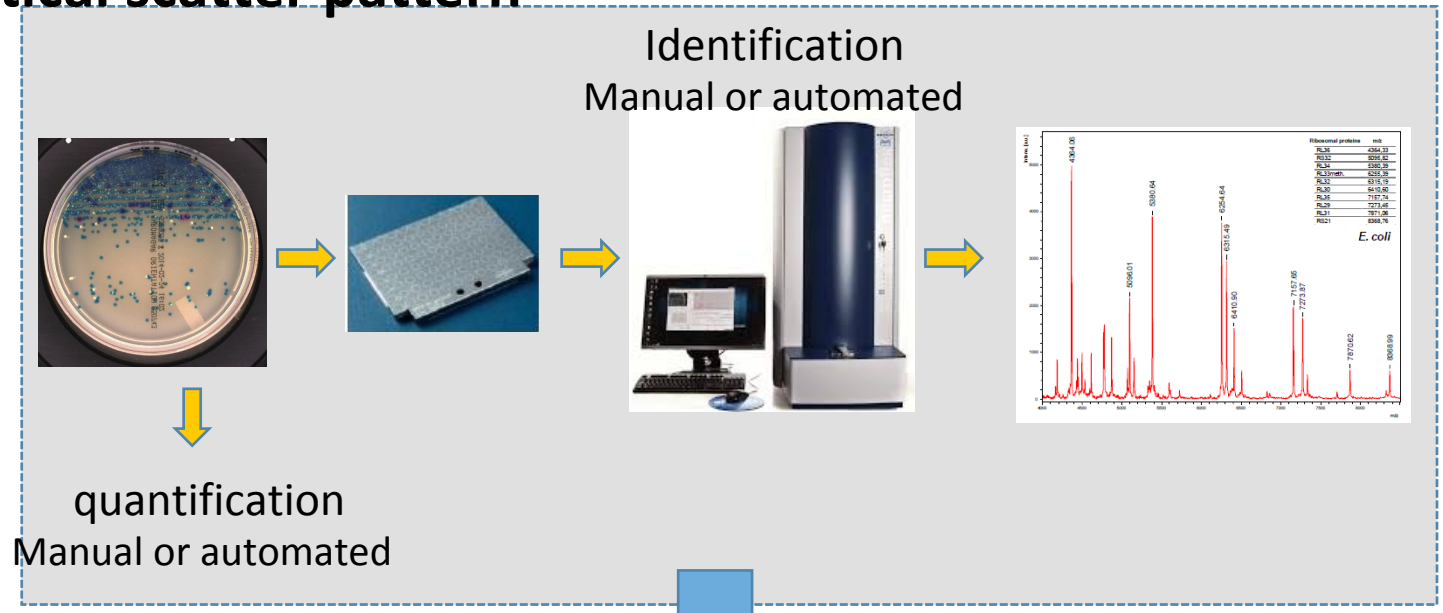
Fourier transform infrared (FT-IR) spectroscopy

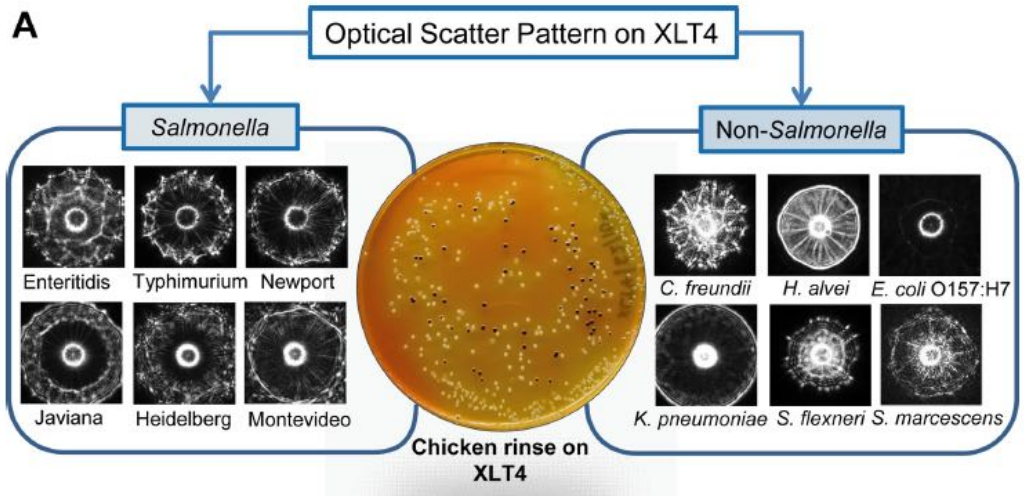


Courtesy of Dr Antony Croxatto

Imaging identification

Optical scatter pattern





Singh et al., MBIO, 2014

Courtesy of Dr Antony Croxatto

Ongoing development in phenotypic microbiology

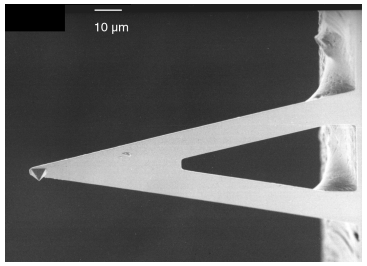
Rapid drug susceptibility test

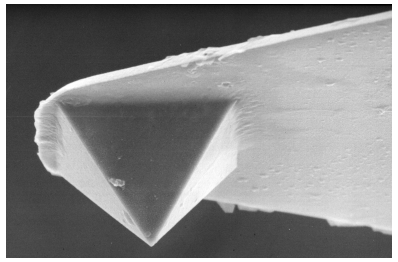
- Immunochromatographic tests
- Enzymatic, Colorimetric tests
- MALDI-TOF MS
- Imaging of bacterial growth
- Nanomotion AST

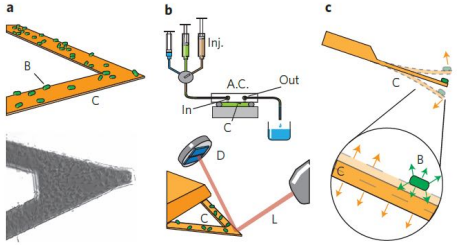
Ongoing development in phenotypic microbiology

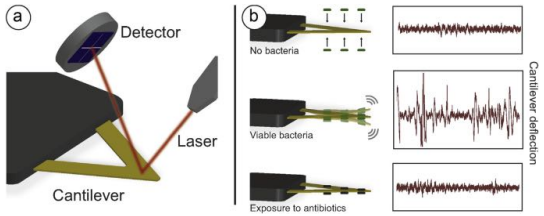
Rapid drug susceptibility test

Bacteria nanomotion: Movement as a read-out for antibiotic susceptibility test

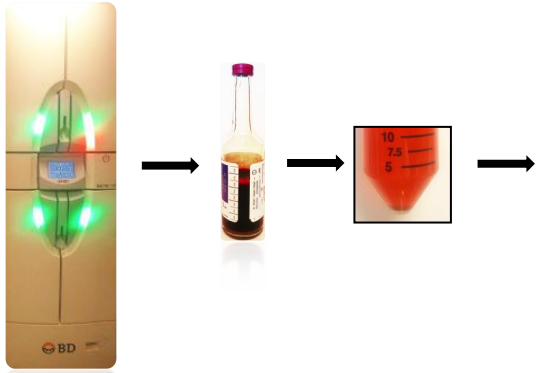


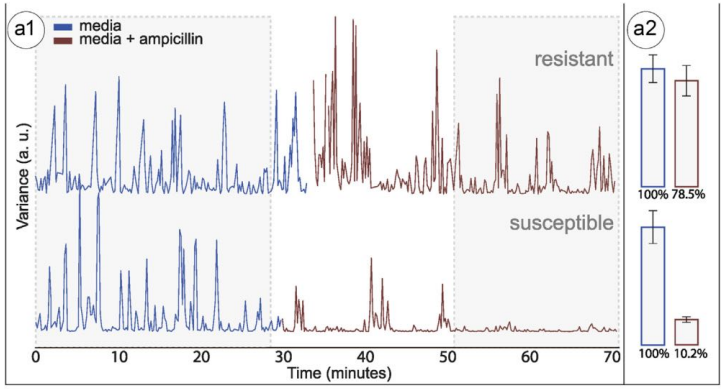


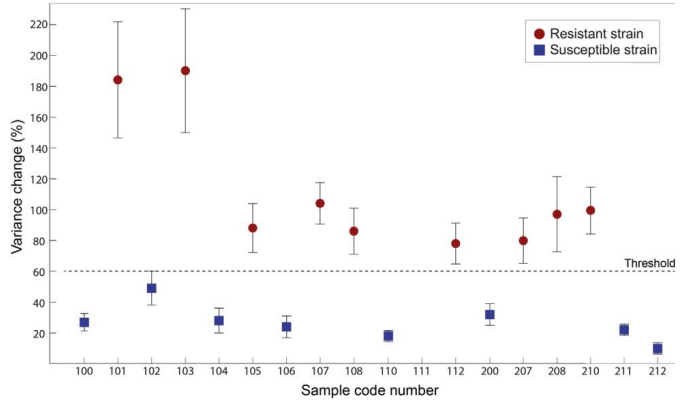




E. coli







Elapsed time < 3 hours

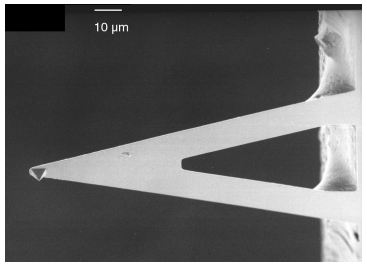
Longo, G., et al. 2013. Rapid detection of bacterial resistance to antibiotics using AFM cantilevers as nanomechanical sensors. Nat Nanotechnol **8:**522-6.

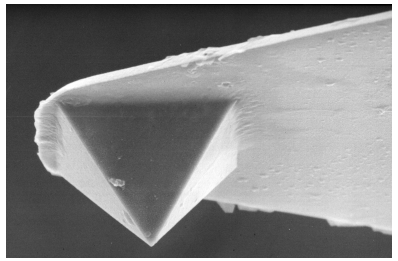
Stupar, P., O. Opota, et al. 2017. Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility

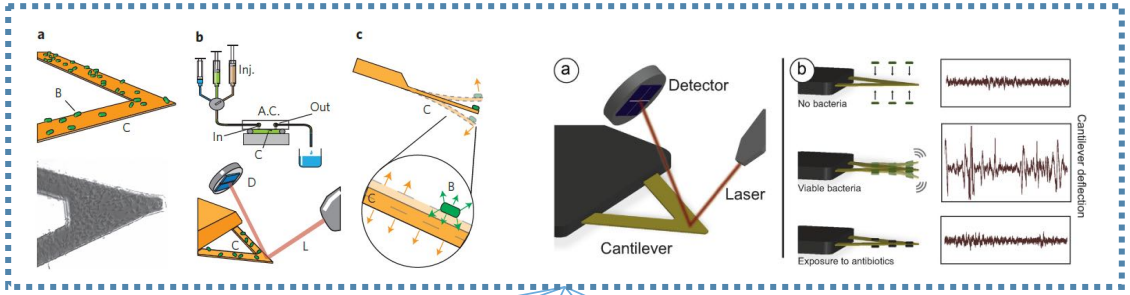
Ongoing development in phenotypic microbiology

Rapid drug susceptibility test

Bacteria nanomotion: Movement as a read-out for antibiotic susceptibility test







Eucaryotics

- *Yeast*

Fast growing

- *E. coli*
- *K. pneumoniae*
- *S. aureus*
- ...

Fastidious growing

- *Bordetella pertussis*

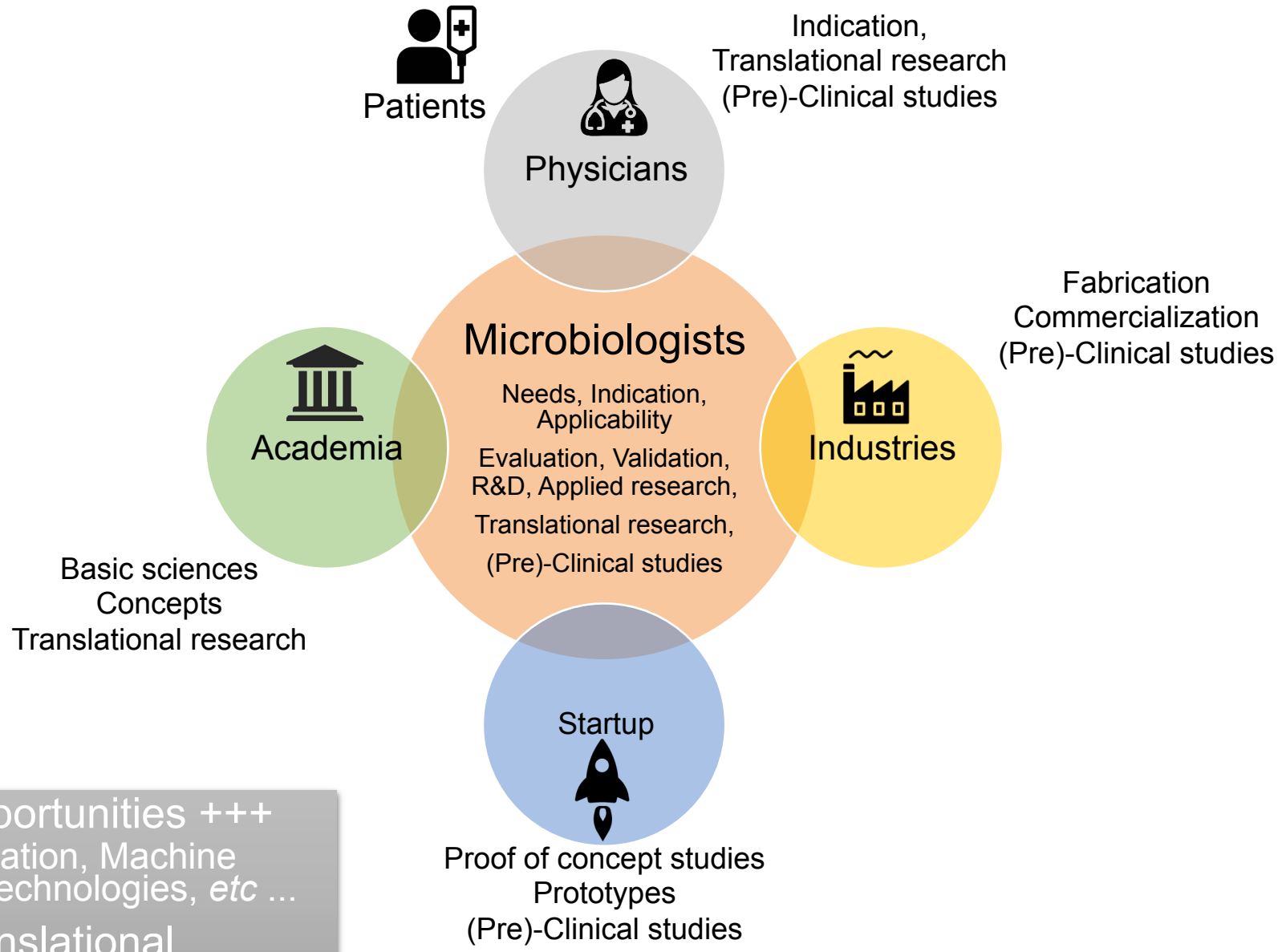
Mycobacteria

- *Mycobacterium abscessus*
- *Mycobacterium BCG* (Bacillus Calmette-Guerin)

Intracelluar?

Stupar, P., O. Opota, et al. 2017. "Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections." Clin Microbiol Infect 23(6): 400-405.
Kasas, S., et al., 2018. "AFM contribution to unveil pro- and eukaryotic cell mechanical properties." Semin Cell Dev Biol 73: 177-187.
Villalba, M. et al., 2018. "Nanomotion Detection Method for Testing Antibiotic Resistance and Susceptibility of Slow-Growing Bacteria." Small 14(4).
Mustazzolu, A., et al., 2019. "A Rapid Unraveling of the Activity and Antibiotic Susceptibility of Mycobacteria." Antimicrob Agents Chemother 63(3).

Positioning of the Microbiologist



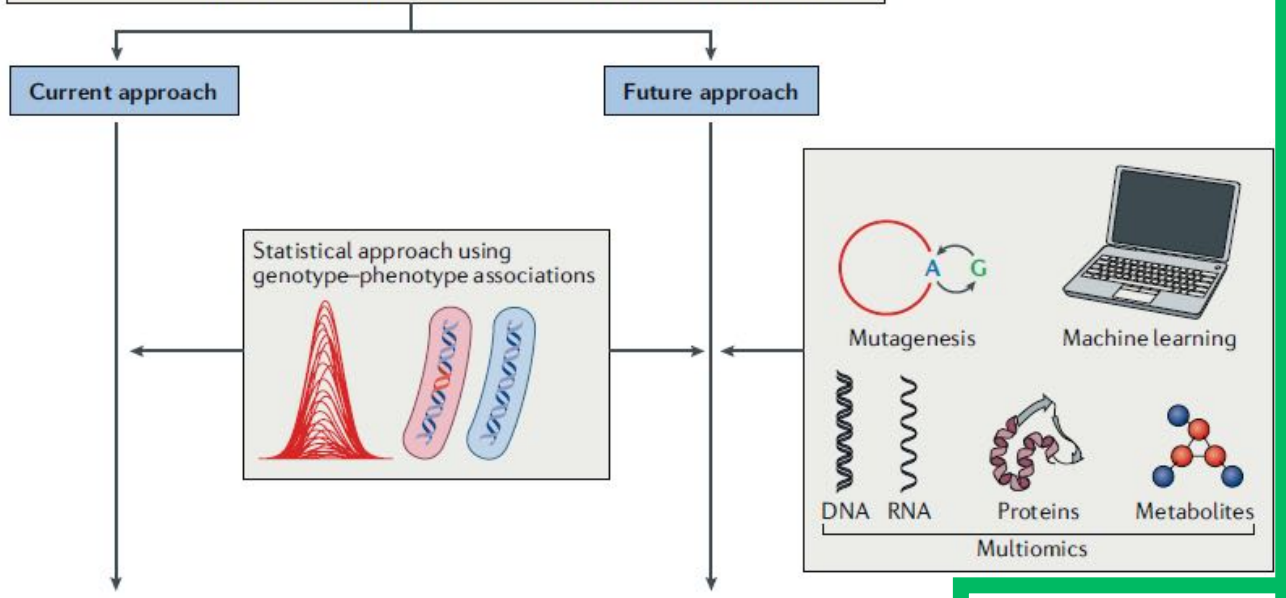
- Technological opportunities +++
  - Imaging, Automation, Machine learning, Nanotechnologies, *etc* ...
- Importance of translational research

Future of phenotypic microbiology

Current and potential approaches for determining resistance- related polymorphisms

Input whole genome sequencing data

	Strain 1	Strain 2	Strain 3	Strain 4
Lineage	4	3	1	*M. bovis*
***rpoB* mutation**	Ser450Leu	Ser450Leu	Ser450Leu	Gln429Ala
***pncA* mutation**	Val130Gly	Val130Gly	Arg123Gly	Gly108Ser
Phenotype	RIF resistant PZA resistant	RIF resistant PZA resistant	RIF resistant PZA susceptible	RIF susceptible PZA resistant



Resistance detected using a statistical approach

Gene	Mutation	Drug
rpoB	Ser450Leu	RIF
pncA	Val130Gly	PZA

Extended knowledge base

Gene	Mutation	Drug	Phenotype	Support
rpoB	Ser450Leu	RIF	Resistant	Statistical
rpoB	Gln429Ala	RIF	Susceptible	Mutagenesis
pncA	Val130Gly	PZA	Resistant	Statistical and mutagenesis
pncA	Arg123Gly	PZA	Susceptible	Machine learning
pncA	Gly108Ser	PZA	Resistant	Transcriptomics

Importance of result reporting!

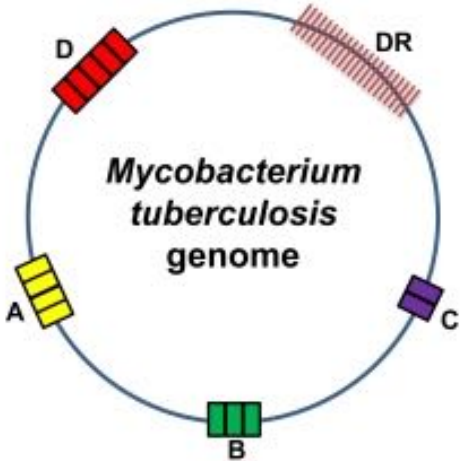
Support
Statistical
Mutagenesis
Statistical and mutagenesis
Machine learning
Transcriptomics

Meehan, C. J., et al. 2019. "Whole genome sequencing of Mycobacterium tuberculosis: current standards and open issues." Nature Reviews Microbiology **17**(9): 533-545.

Epidemiology

- Epidemiology:
  - Studies of how disease [or microbes] originates and spreads throughout a population, with the goal of preventing outbreaks and containing them when they do occur.
- Nosocomial epidemiology
- Community epidemiology
- Can rely on molecular epidemiology

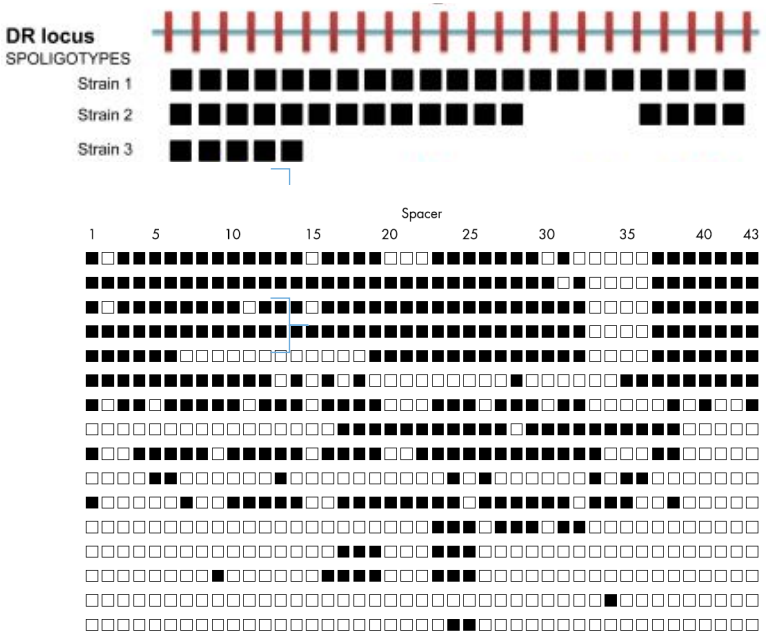
Epidemiology



Spoligotyping

Sp (acers) oligo (nucléotides) typing

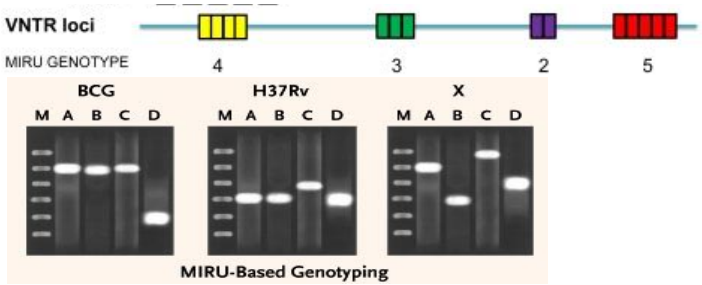
Présence ou absence de spacer (n=43)



MIRU-VNTR

Mycobacterial interspersed repetitive units-variable number tandem repeats

Comparaison du nombre de répétitions (24 locus)

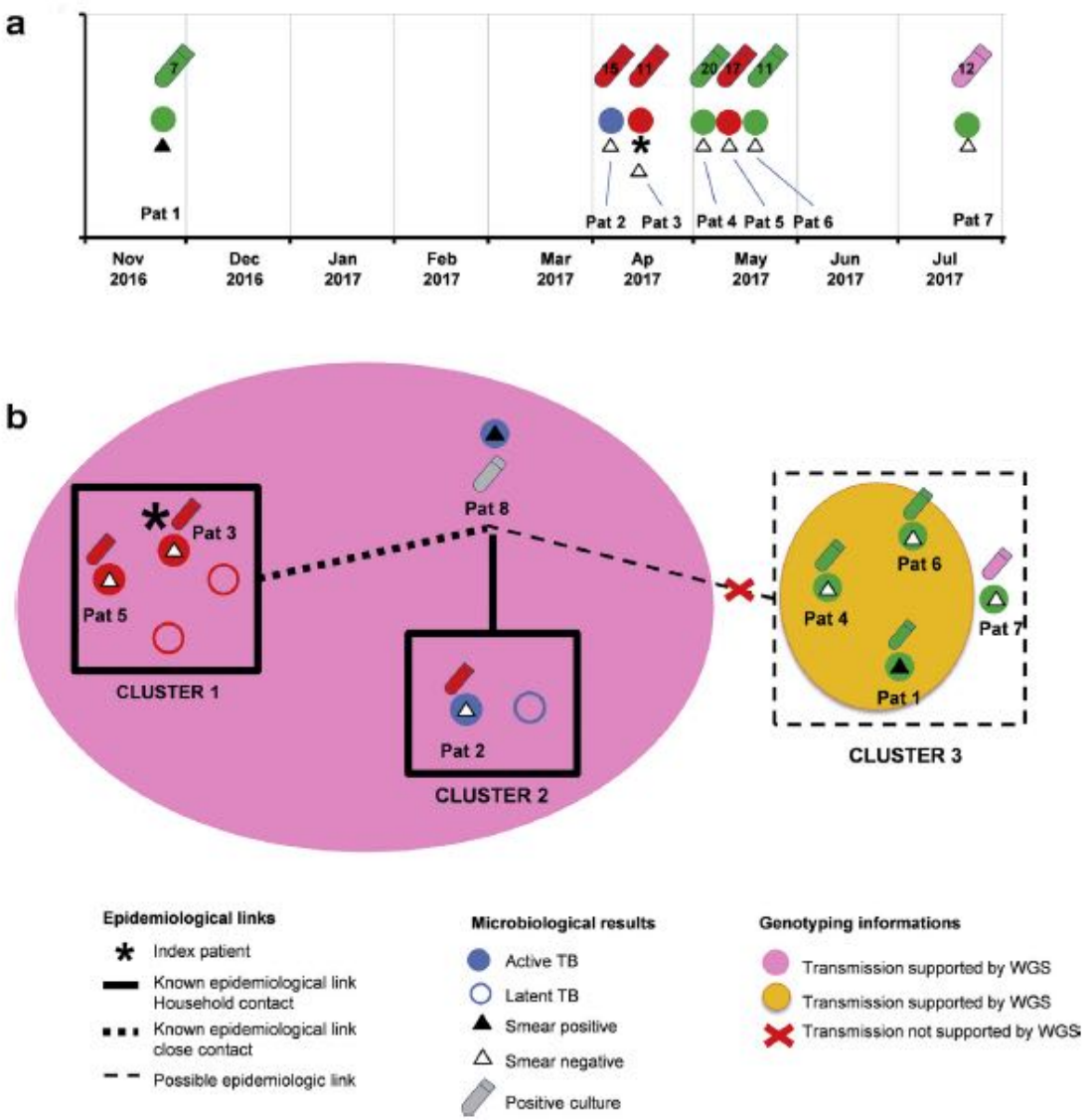


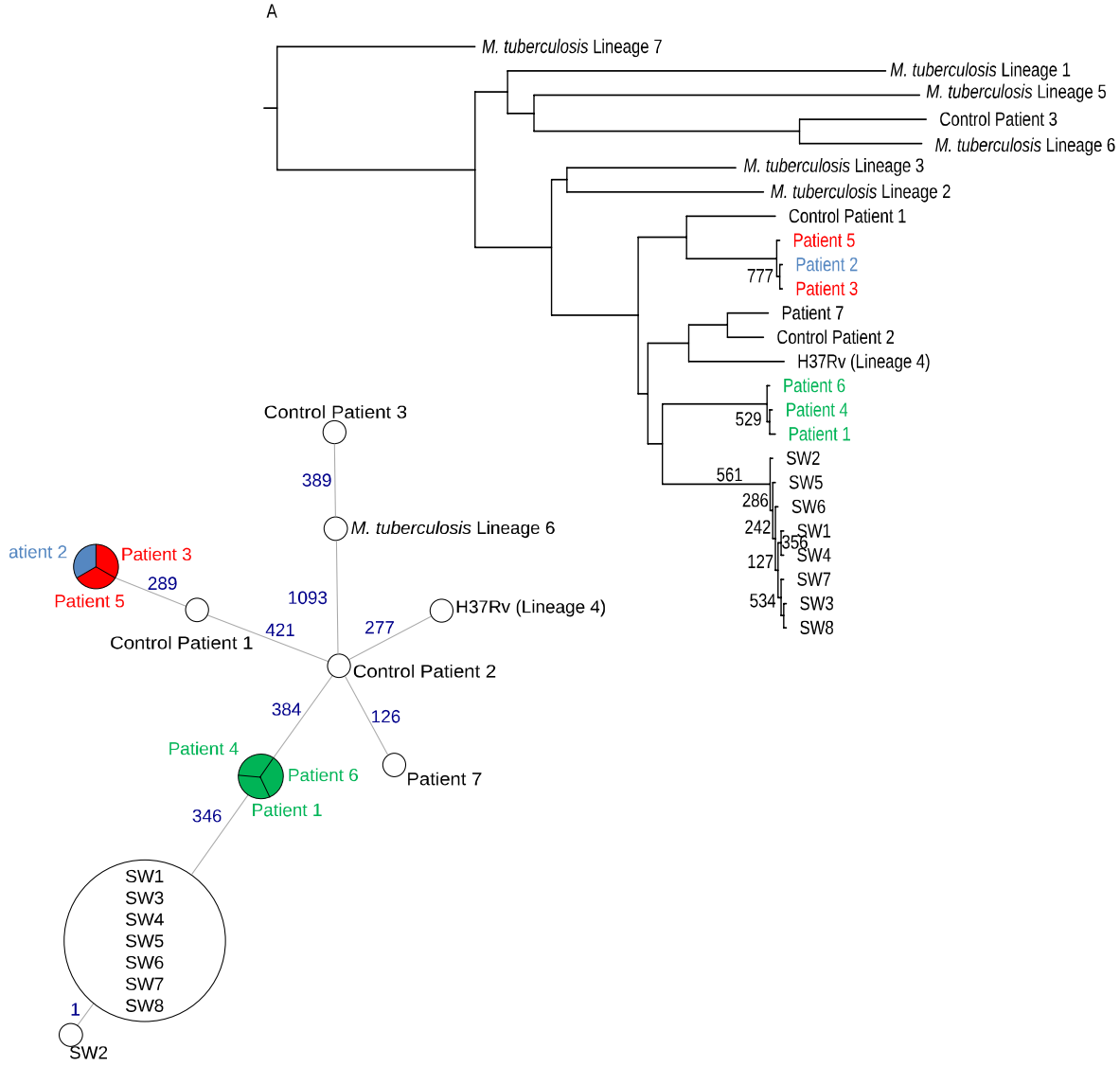
Locus																										
Repetitive number	3	3	5	4	2	4	4	5	3	2	2	3	4	4	5	3	4	6	2	3	4	3	2	2

Epidemiology

Different methods used in molecular typing of *Mycobacterium tuberculosis* (Cannas et al, 2016)

Epidemiology





Zakham, F., S. Laurent, A. L. Esteves Carreira, A. Corbaz, C. Bertelli, E. Masserey, L. Nicod, G. Greub, K. Jaton, J. Mazza-Stalder and O. Opota (2019). "Whole-genome sequencing for rapid, reliable and routine investigation of *Mycobacterium tuberculosis* transmission in local communities." New Microbes and New Infections 31: 100582.

Investigation of an International Outbreak of MDR-TB among patients arriving from the Horn of Africa

30 may 2016:
German Mycobacteria reference center → independant investigation of a possible outbreak of MDR-TB in Germany

29 April 2016:
Zurich Reference Center for Mycobacteria → Investigation of a possible national outbreak of MDR-TB due to other reports in Switzerland

- From Feb. 2016 to April 2017
- 29 patients diagnose with MDR-TB
- 7 European countries
- Patients coming from Somalia, Eritrea, Djibouti Ethiopia and Soudan
- Resistance to : rifampicin, isoniazid, ethambutol, pyrazinamide and capreomycine

2016 — 2017

31 Dec 2015:
Induced sputum
Positive culture (29 days),
MDR-TB
Mutation *rpoB*
Mutation *katG*

20 June 2016:
Bronchial aspirate Asp.
Positive culture (20 days),
MDR-TB
Mutation *rpoB*
Mutation *katG*

14 Oct. 2016:
Sputum
PCR positive (~300 DNA copies/ml
Xpert MTB/RIF positif
***rpoB* mutation détected**

Investigation of an International Outbreak of MDR-TB among patients arriving from the Horn of Africa

Added-value of WGS-based molecular epidemiology

CLINICAL DATA

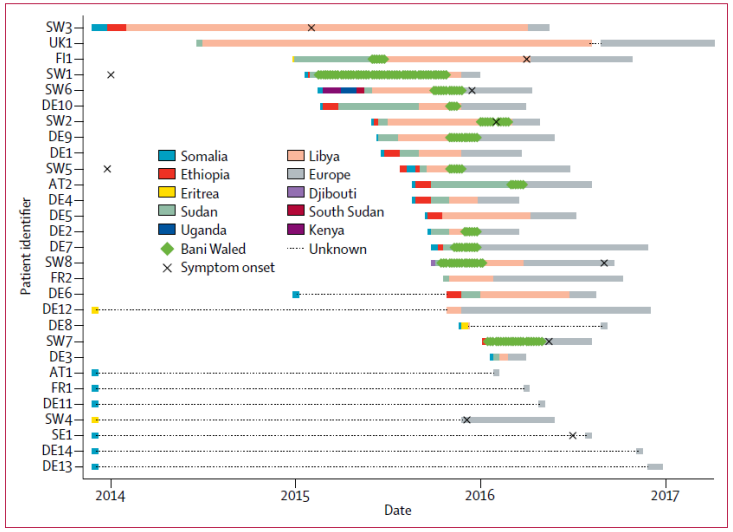


Figure 2: Timeline of patient journeys until diagnosis
The 1st of each month is shown as the country entry date. Subsequent countries visited in the same month are presented at 5-day intervals.

EPIDEMIOLOGICAL DATA

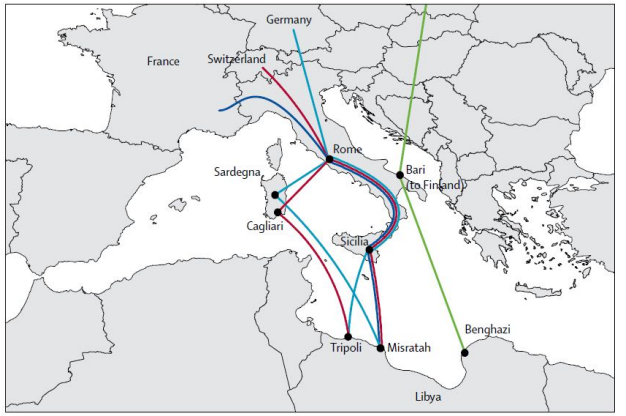
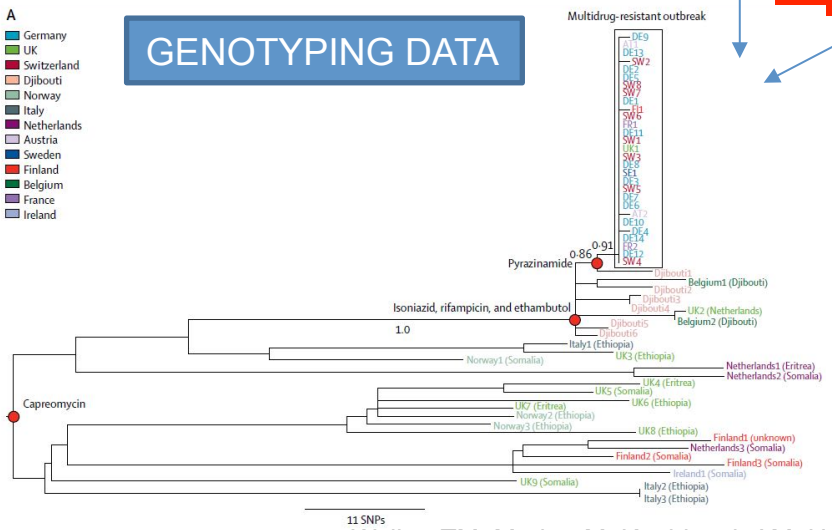


Figure 3: Reported migration routes through Italy of 29 outbreak patients with a documented migration route, August, 2014, to February, 2017

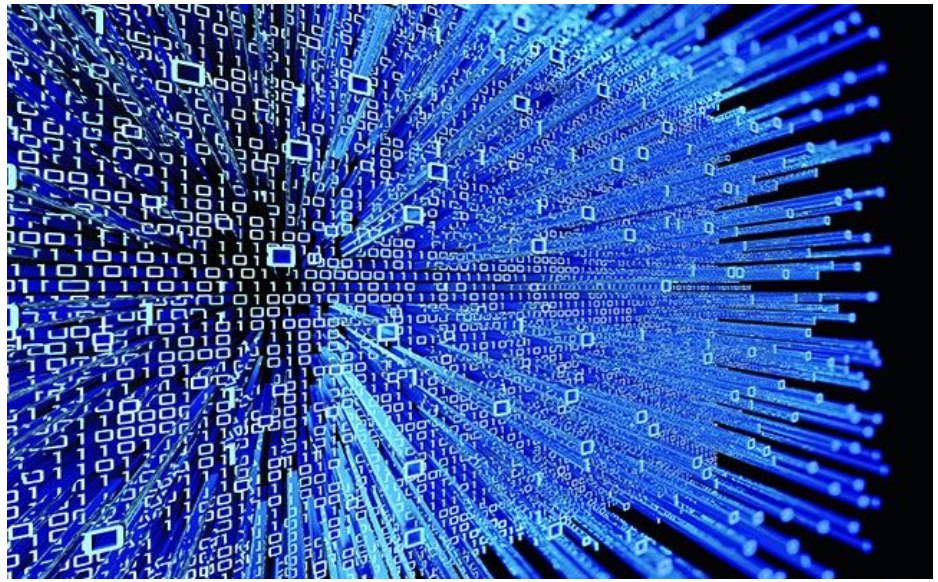
Rapid and reliable understanding of the situation

GENOTYPING DATA



Walker TM, Merker M, Knoblauch AM, Helbling P, Schoch OD, van der Werf MJ, et al. **A cluster of multidrug-resistant *Mycobacterium tuberculosis* among patients arriving in Europe from the horn of africa: A molecular epidemiological study.** *The Lancet Infectious Diseases* 2018.

Epidemiology

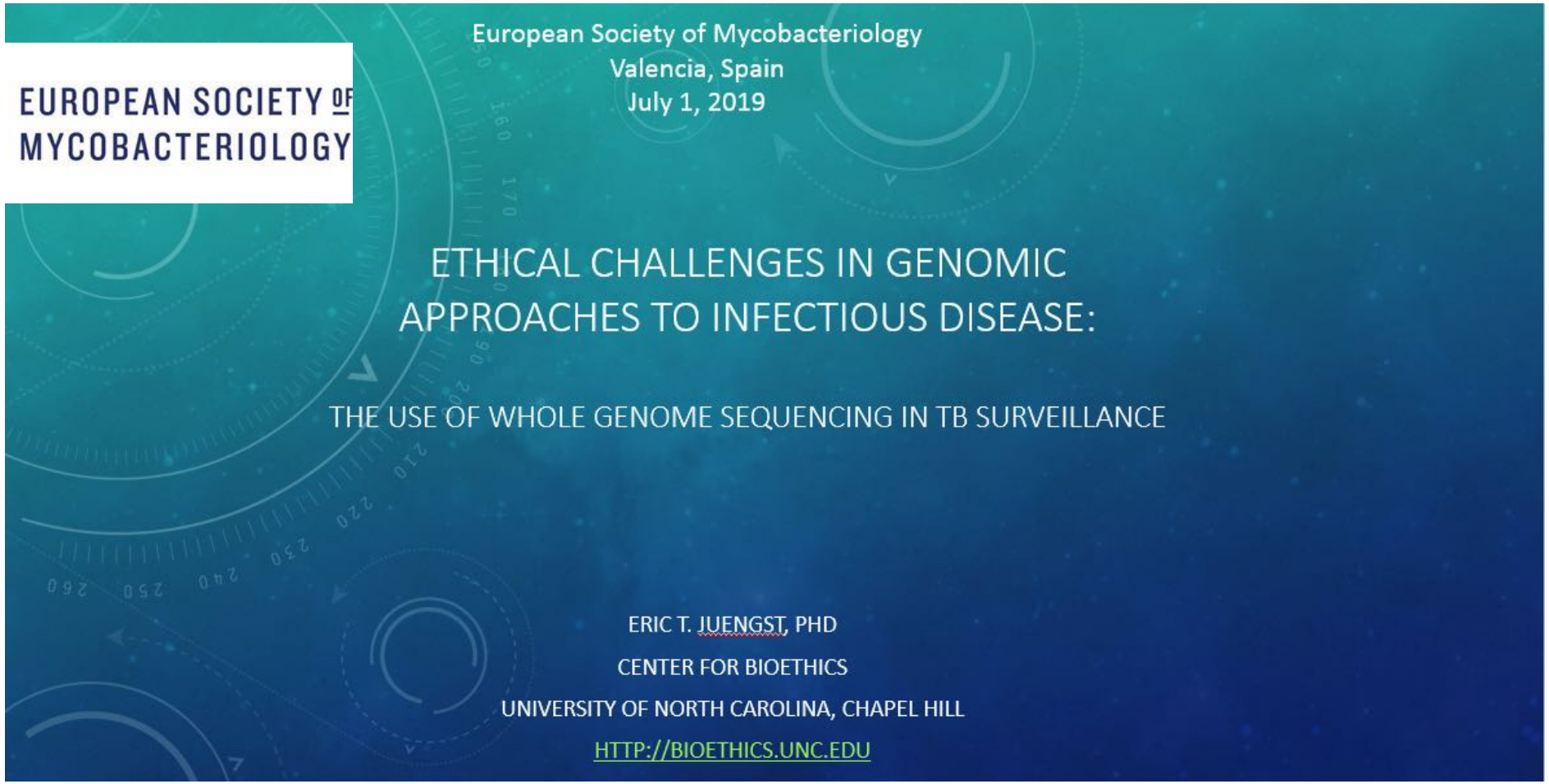


More data more rapidly,
Resolution power,
Cost effective

More accessible
More attractive

- Responsibility of microbiologist
  - Management of the data
    - Security: Where to store the data
    - Who can have access to the data
  - Management of the obtained results
  - Anticipation of the results
    - unwanted or unexpected results
  - Management of the information
    - Who can have access to the information

Ethical Challenges in Genomic Approaches to Infectious Disease

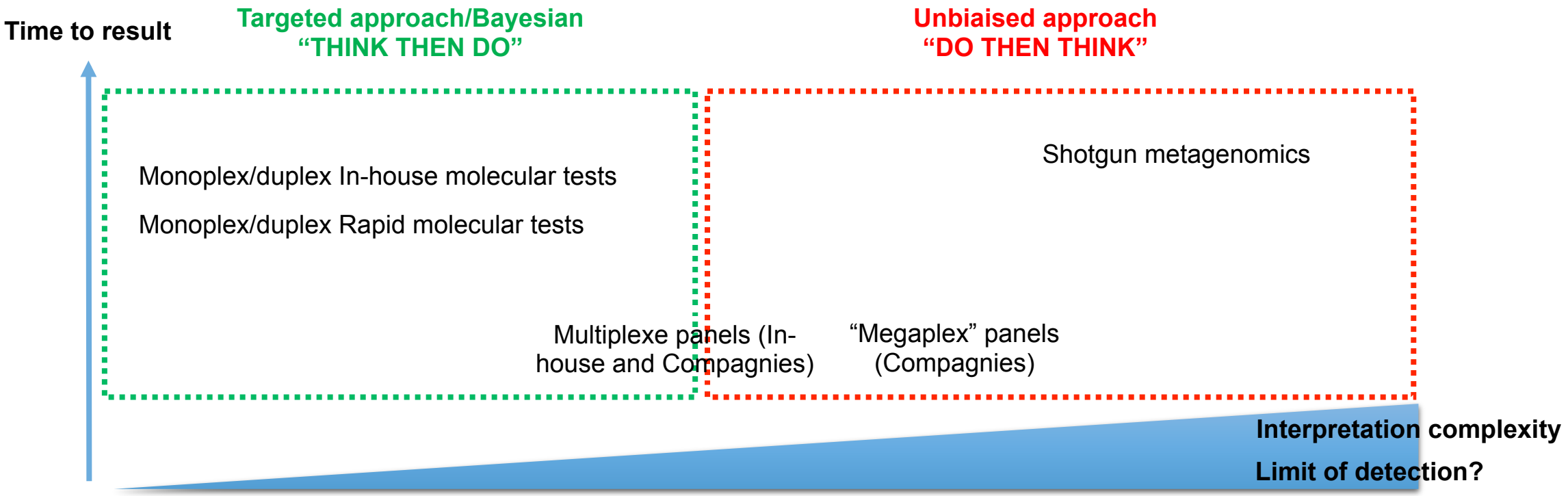


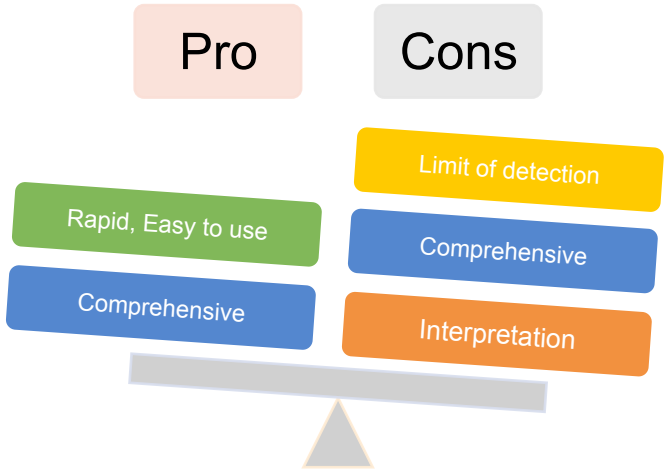
- Four illustrative cases and the ethical considerations they highlight
- Eight key considerations for developing anticipatory policies and practices
- Round table discussion and audience feedback.

Ethical Challenges in Genomic Approaches to Infectious Disease

- **8 key points for discussion**
  - ❑ Public health mandate for actionable real-time Mtb WGS
  - ❑ Mtb WGS without individual consent
  - ❑ Promoting social justice and global solidarity
  - ❑ Insuring confidentiality and security
  - ❑ Two-step data sharing between public health institutions
  - ❑ Personal disclosure and group interest: building patient partnerships
  - ❑ Privacy and consent in disclosure of transmission events
  - ❑ Professional transparency in public communication.

New approaches in clinical microbiology?

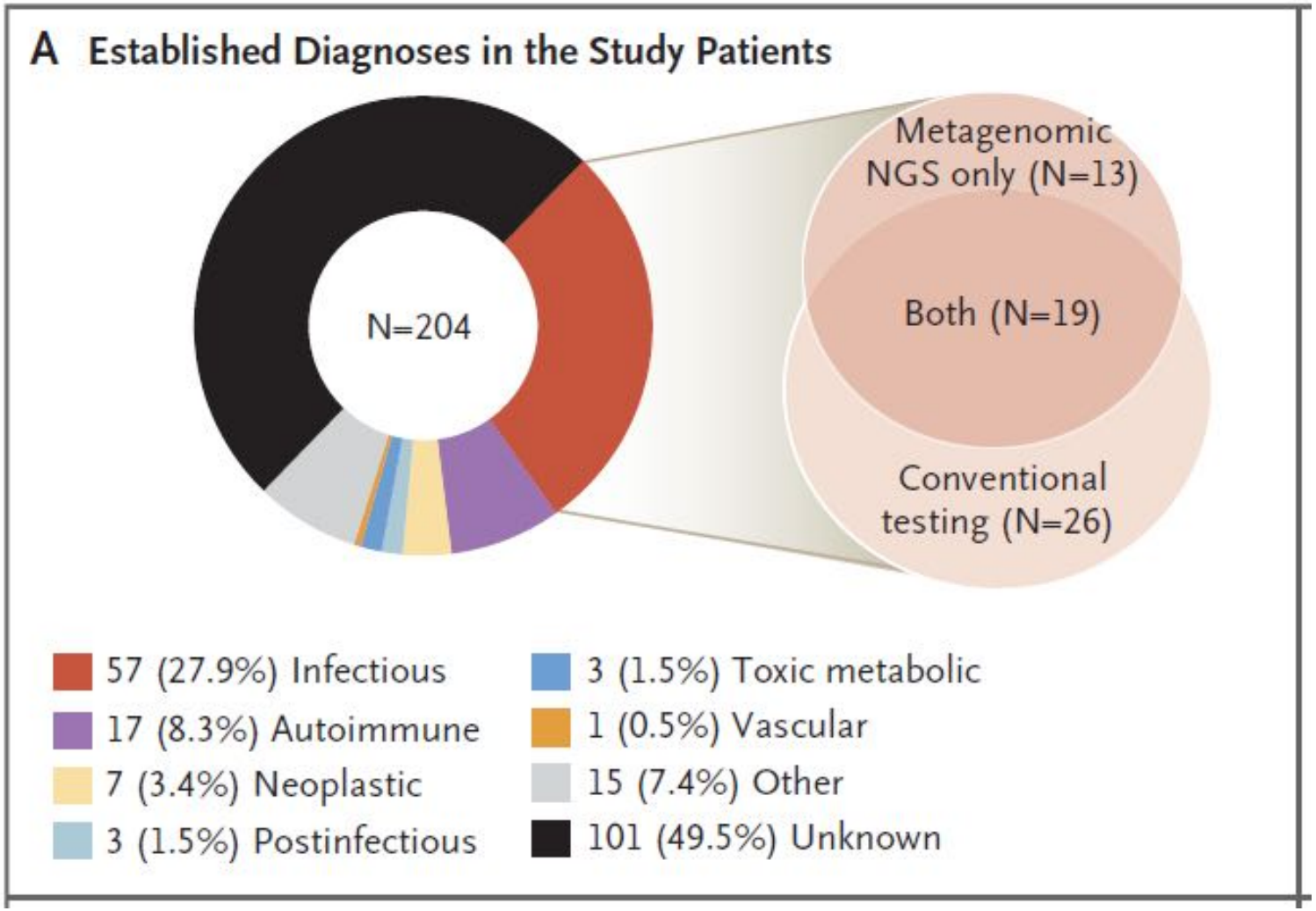




New approach in clinical microbiology?

Shotgun meta-GEN for pathogen detection and identification

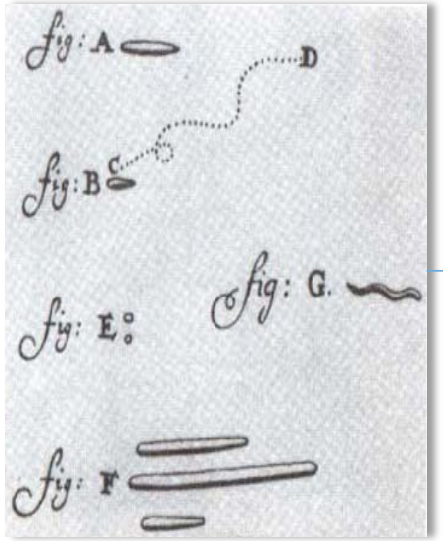
- Backgrounds:
  - Encephalitis of unknown etiology: **32%–75%**.
- Methods
  - 204 specimens, conventional culture and molecular methods versus shotgun metagenomics
- Results
  - Conventional methods + NGS resulted in **49.5%** of unknown etiology
- Conclusions:
  - Added value +/-
  - Need other studies/other syndromes
  - Other indication of metagenomics



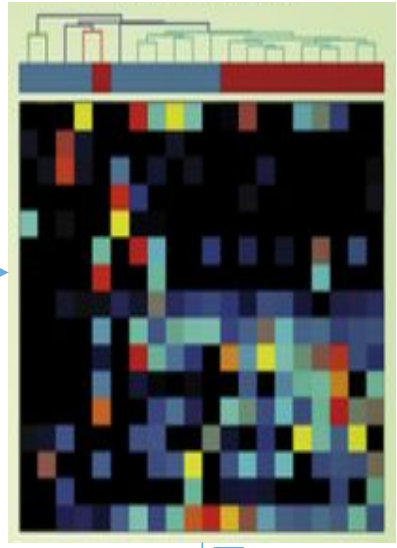
Wilson, et al., 2019. "Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis." N Engl J Med **380**(24): 2327-2340.

New approaches in clinical microbiology?

Microbial species



van Leeuwenhoek
1683



Microbial species
Microbes functions/pathways
Host cells response

Quince et al. 2017.

Same approaches?
Same objectives?
Same applications?

Microbiology

Clinical microbiology

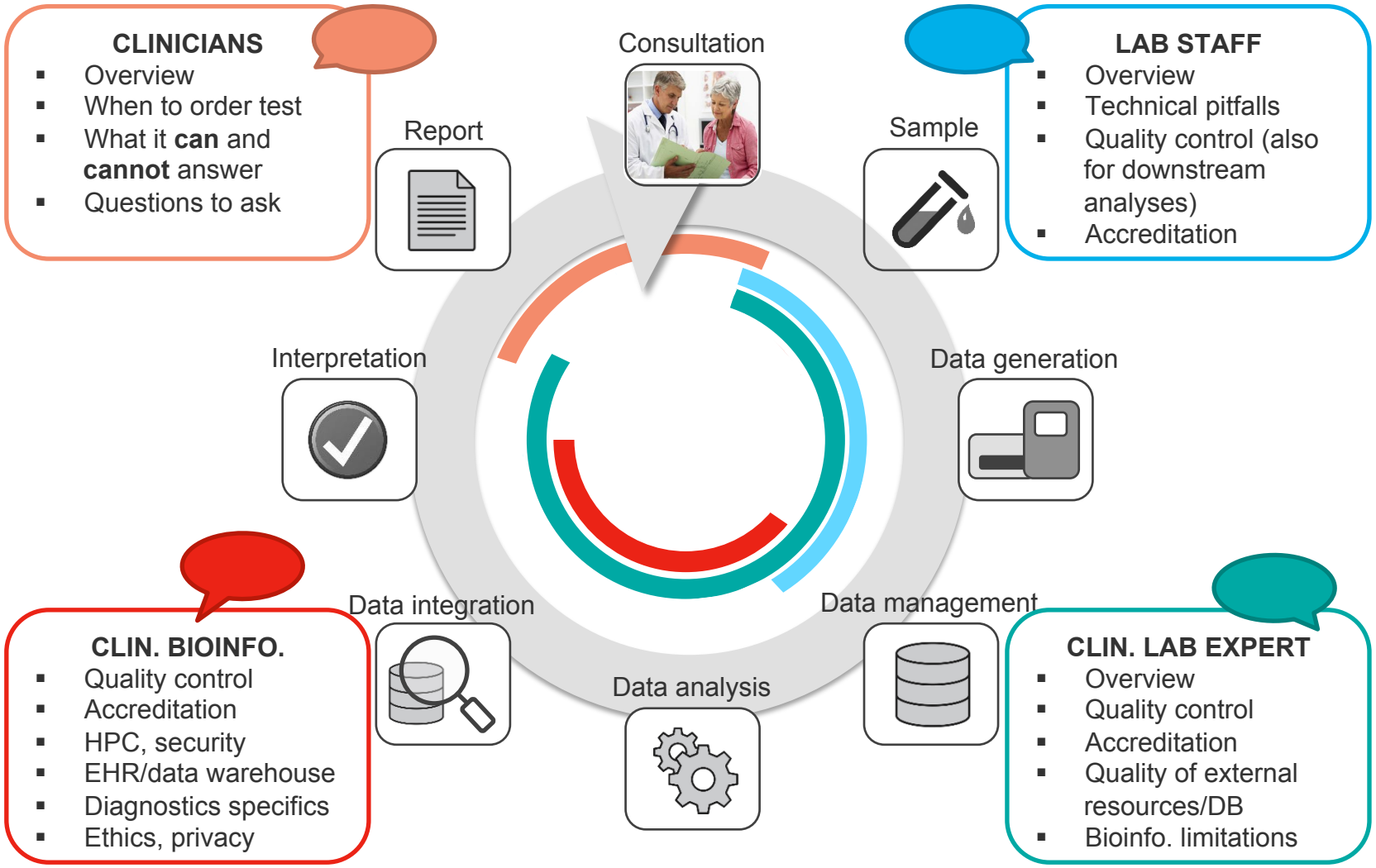
New applications,
New approches

Identification	Resistance	Carriage	+	Prognosis	Anticipation	Personalized clinical microbiology
Immune status	Infection course	Epidemiology		Severity	Forensic	…?

Conclusions

- Clinical Microbiologist
  - is not only a service provider
  - participates in the prescription of the biologic analysis and can, by dialoguing with the clinician, modulate this one. REMIC (Referential of Medical Microbiology of the French Society of Microbiology).
- Importance of training to maintain knowledge and skills
  - expertise for dialogue
  - to anticipate future changes

Conclusions



Examples of profile-specific training needs (not exhaustive)

Dre Aitana Lebrand

Conclusions

- ❖Management of increasing informations
- ❖Important role of the CM
  - Data management
  - Data interpretation integration
  - Reporting results
- ❖New approaches? New tools?
  - Digitalization and infectious diseases to improving patient outcome in the age of big data
- ❖Societal and ethical Challenges
  - Personalized medicine
  - Genomic approaches to infectious disease

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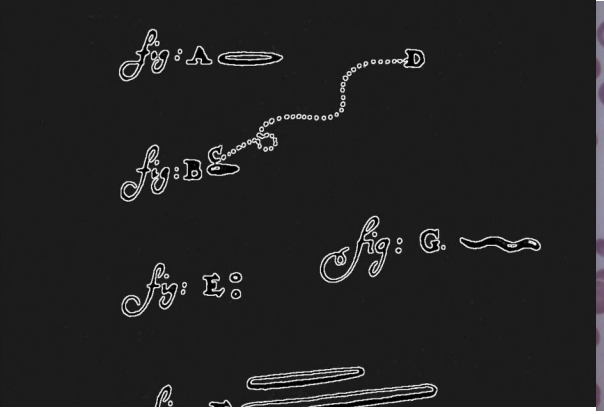
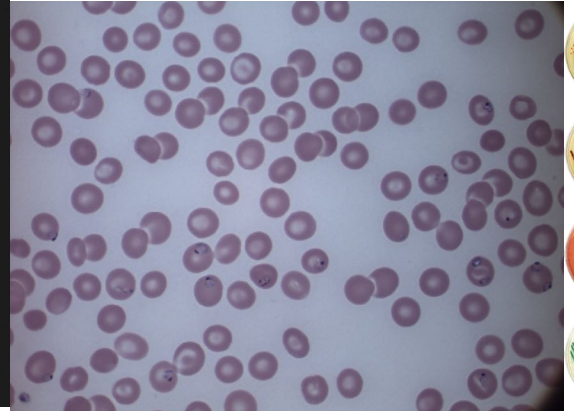
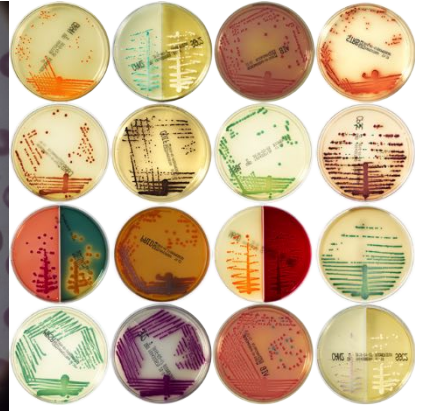
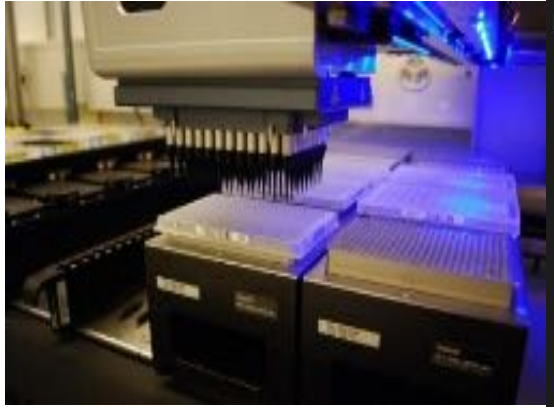
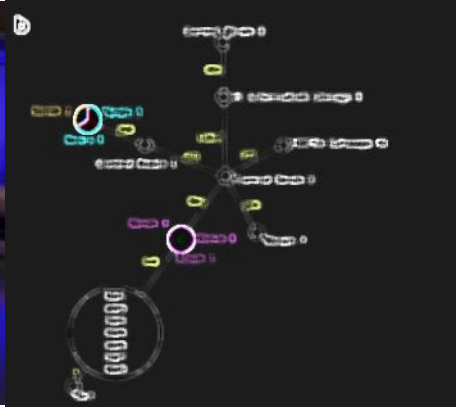
Dr Jessica Mazza-Staldder

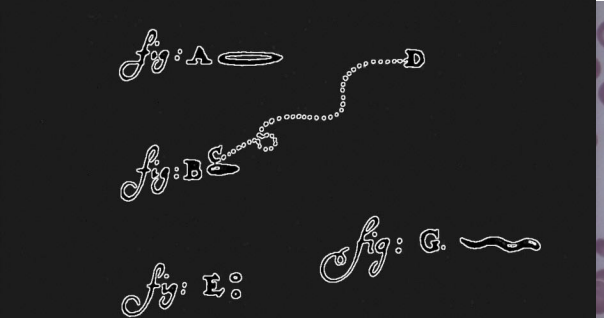
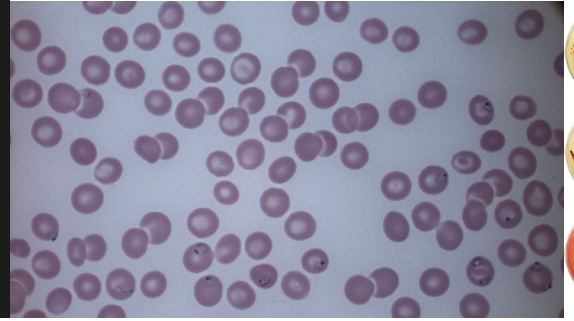
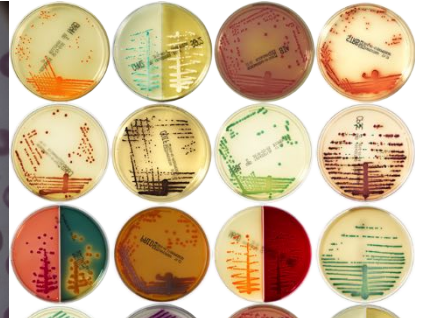
Laboratory of Physics of Living Matter, BSP, EPFL
Prof Giovanni Dietler, Dr Sandor Kasas,
Dr Petar Stupar

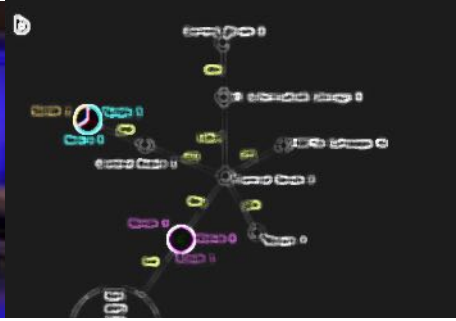
Swiss Government Excellence Scholarships for Foreign Scholars and Artists



“Education is the passport to the future, for tomorrow belongs to those who prepare for it today” Malcolm X (Malcolm Little)



From the clinic: The changing profession of the clinical microbiologist

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