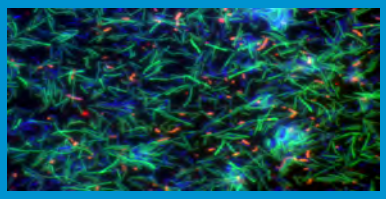
Future developments in MDx

Paul Savelkoul



Maastricht UMC+
Dept. medical microbiology
Maastricht University

First microbiologist

1632-1723

Antoni van Leeuwenhoeck

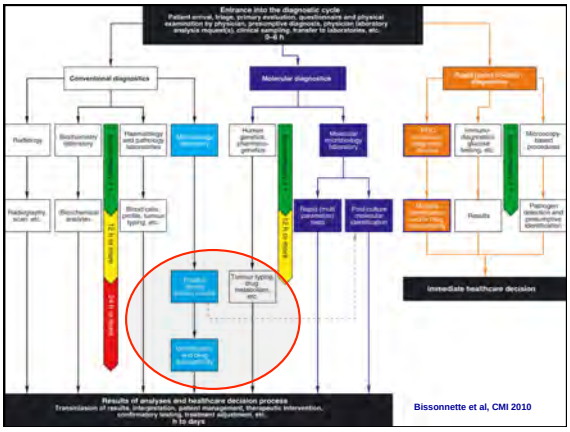
Start discipline of medical microbiology

1822-1895

Louis Pasteur

1843-1910

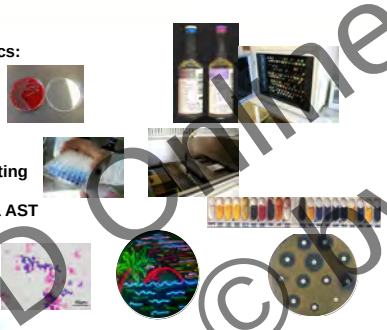
Origin of many of the currently laboratory techniques



Current situation in the medical microbiological laboratory

Classical technics:

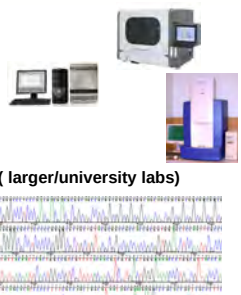
- culturing
- blood culture
- serological testing
- identification & AST
- staining



Current changing situation in the routine microbiological laboratory

Molecular technics:

- DNA isolators
- Real Time PCR
- Malditof
- Sanger sequencer (larger/university labs)



Consequences in the routine microbiological laboratory

- Viruses: virusculture replaced by RTPCR
- Parasites: more and more RTPCR
- Fungi: Increasing molecular testing
- Bacteriology: Malditof & RTPCR & BRPCR
- Resistence: More molecular detection

What is the most common situation on the sample level?

Hospital:

High throughput: Large sample size, planned, sample driven (e.g. hpv, CT, NG, HIV)

Middle thoughput: Complex, speed, combined tests, patient driven
(e.g. all clinical diagnostics

Low throughput: Urgence, rapid result, patient driven (e.g. ICU patient)

General practitioners :

Most samples outsourcing to laboratories
Insourcing rapid easy to go POC tests

Testing at home:

Patient at home: self sampling and/or self testing

The new era in Medical microbiology

Crossing points with regard to
spread of resistence, new molecular technologies, bioinformatics

A new era in medical microbiology

MDx: enabling analysis of total human microbiome

Determining health & disease

-Diagnostics of chronic infectious diseases

-Prevention of exacerbations

-Personalized diagnosis & therapy

-Monitoring of therapy

-New (preventive) treatment strategies

The new paradigm of medical microbiology

One disease : One pathogen?

From pathogen detection toward microbiome detection:

Specific -> Syndrom -> Systemic

one bacterium -> known set -> microbiome

And beyond……

New approaches

From sample driven towards patient driven analysis

From one test towards monitoring infection

From single pathogens towards communities

From pathogen detection towards commensal detection

From testing towards testing based patient specific treatment

From acute infections towards chronic inflammatory diseases

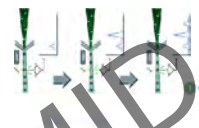
MDx future lines

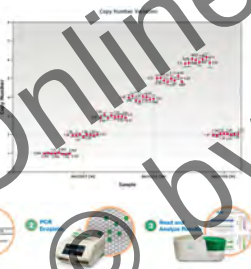
- New rapid RTPCR applications
- POC systems
- Masspectrometry systems (Maldi/Esi tof) (metabolites & DNA)
- Broad Range DNA amplification (gutflora, biofilm) (NGS, bacterial profiling)
- Next generation DNA sequencing (species & strain typing/resistome)
- Electronic nose (VOC)

Digital PCR

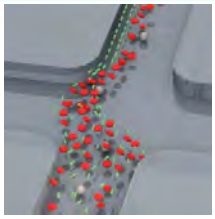
Emulsion PCR

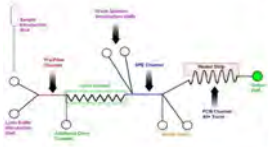
Reliable QPCR with high sensitivity

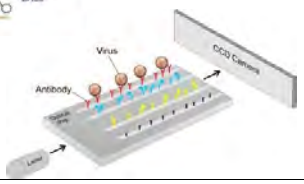




Fluidic sample types
Continuous miniaturized flow



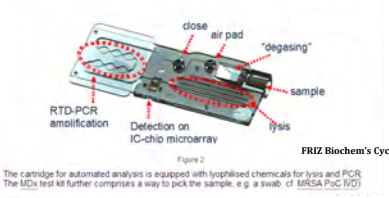




Miniaturisation

IC-CHIP POWERED NUCLEIC ACID MD

MDx technology based on electrical readout
lab on a chip cartridge



FRIZ Biochem's Cycle® technology

Figure 2
The cartridge for automated analysis is equipped with lyophilised chemicals for lysis and PCR
The MDx test kit further comprises a way to pick the sample. e.g. a swab. cf. MRSA PoC IVD)

Blood cultures

Direct detection PCR, Malditof

Dag 0 1 2 3 4 5 6

Positive bloodculture

Gram stain
Malditof identification
PCR

Negative bloodculture

Identification & AST

Direct detection of sepsis

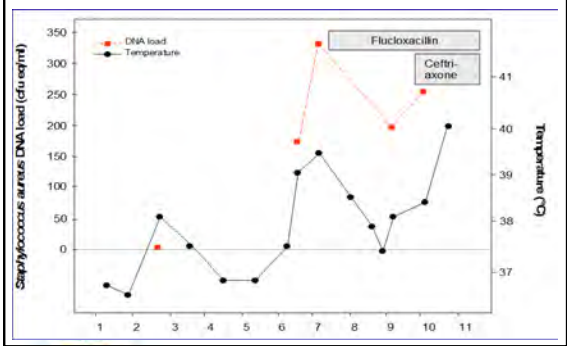
5-10 ml fresh blood

standard lab equipment

POC system:

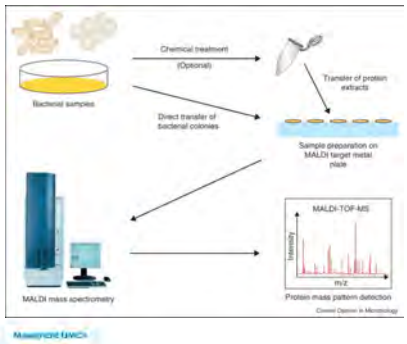
Diagnosis of sepsis from 3- 6 days to 2 hrs

BDL & Fever

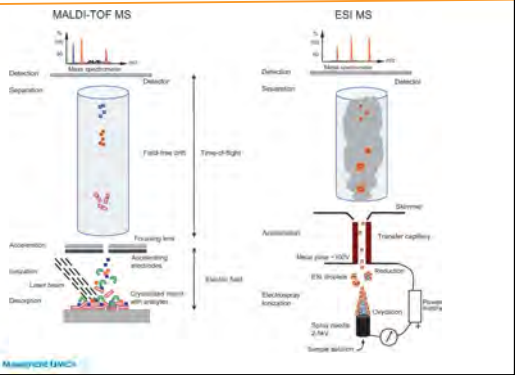


BDL & CRP

Malditof identification



ESITOF for DNA analysis



More time of flight on the horizon

***MALDI-TOF MS*, matrix-assisted laser desorption ionization time-of-flight mass spectrometry**

***MALDI-RE*, matrix-assisted laser desorption ionization resequencing**

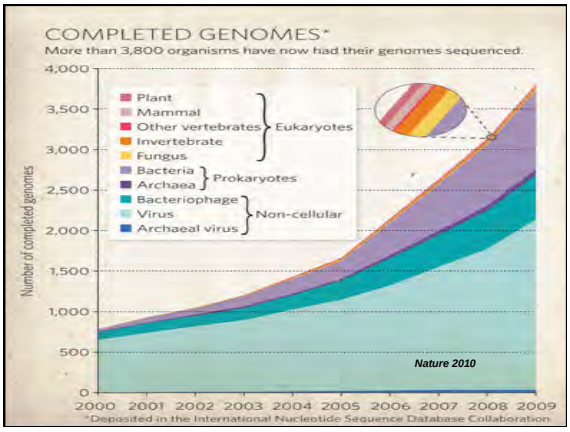
***PCR-ESI MS*, electrospray ionization mass spectrometry**

***SELDI-TOF MS*, strafcel enhanced laser desorption /ionisation tof combines a chip-based chromatographic enrichment of proteins with TOF-MS**

***MassARRAY iPLEX* single-nucleotide polymorphism (SNP) typing platform uses and the MS technology coupled with single-base extension PCR to analyze amplicons of PCR for rapid and accurate molecular identification of microorganisms**

***LC-ESI-QqQ MS*, liquid chromatography coupled to electronspray ionization triple quadrupole**

***LC-ESI-Q-TOF MS*, MALDI triple quadrupple coupled to MALDI-TOF**



Next gen seq applications in the field of medical microbiology

14,870,316 bacterial genomes
10,627 viral genomes
8,641 plasmids
767,967 fungi
84,984 archaea

Single cell sequencing
Identification and determination
Detection of resistance genes
Identification of virulence genes
Discriminating epidemical & non-epidemical strains
Strain specific characteristics (pathotyping)
Transmission routes of mobile DNA elements

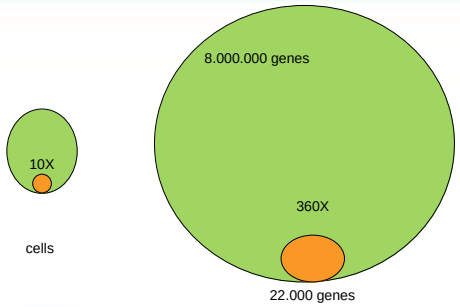
Resistome

Micro-organisms may carry resistance genes

Resistome = the collection of antibiotic resistance genes in both pathogenic and non-pathogenic bacteria"

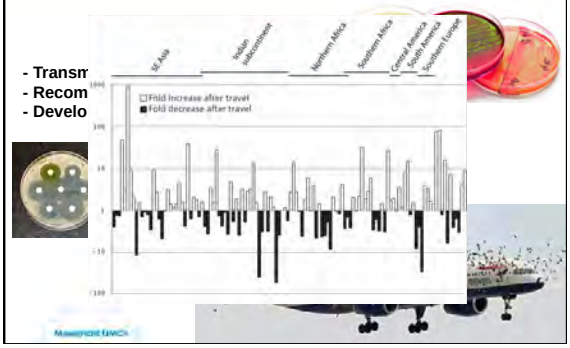
- **Why focus on the resistome?**
- Microbial communities – potential reservoirs of exchangeable resistance genes

Wright, G.D. Nature Reviews Microbiology 2007;5:175-186

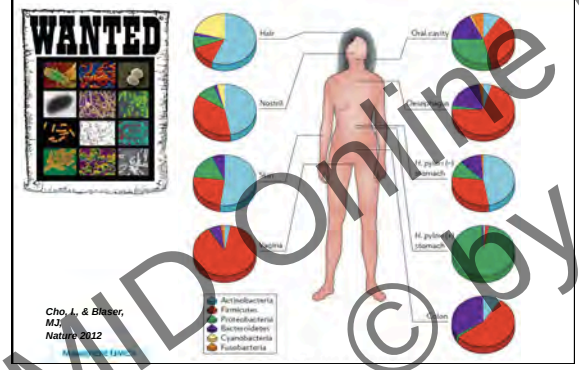


Transmission

- Transm
- Recom
- Develo



The Healthy Microbiome: Who is in there?

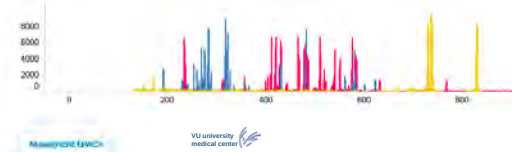


Cho, I. & Blaser, MJ. Nature 2012

Broad range DNA amplification

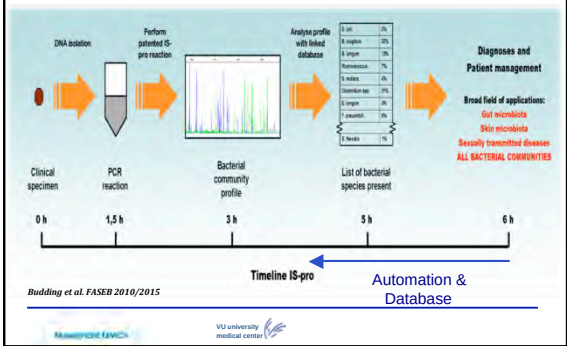
PCR Fir/Bac PCR Proteo

Bacterial profiling: IS-pro*tm*



VU university medical center

Overview ISpro® technology



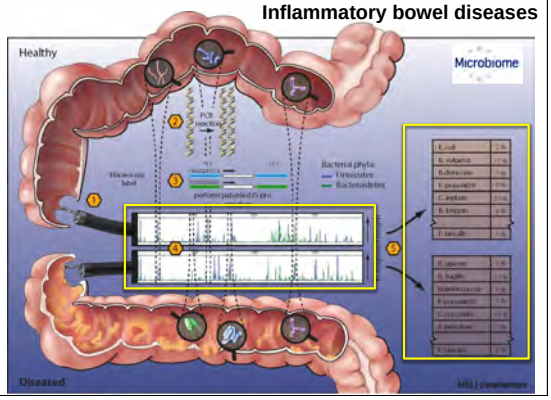
Budding et al. FASEB 2010/2015

VU university medical center

In the clinic

VU university medical center

Inflammatory bowel diseases



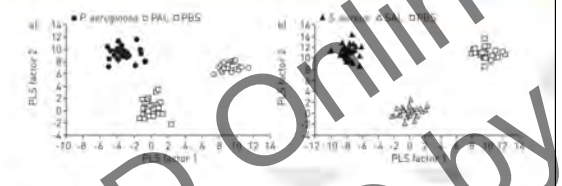
Medical applications of VOCs

VOCs in medicine

Neoplastic	Exposure assessment	Screening	Therapy response
Infectious	Pathogen identification	Host response	Monitoring
Inflammatory	Exposure assessment	Stratification and therapy titration	Monitoring for exacerbations

Pneumonia - Host response assessment

VOCs in medicine



Bean et al, ERJ, 2015

NEC - Monitoring for disease onset

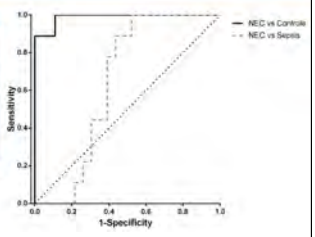
VOCs in medicine

Table III. Performance characteristics of focal VOC analysis for the discrimination of NEC, sepsis, and controls

Time window	AUC ± 95% CI	P value	Sensitivity	Specificity	+LR	-LR
NEC vs control						
$T_{0,-1}$	0.96 ± 0.04	>.001	88.9	89.9	8.1	0.1
$T_{-1,-3}$	0.77 ± 0.21	.024	83.3	75.0	3.3	0.2
$T_{-3,-5}$	0.65 ± 0.25	.257	60.0	60.0	1.5	0.7
NEC vs sepsis						
$T_{0,-1}$	0.84 ± 0.18	.210	88.9	66.5	2.1	0.20
$T_{-1,-3}$	0.80 ± 0.17	.004	83.3	75.0	3.3	0.2
$T_{-3,-5}$	0.52 ± 0.23	.886	50.0	40.0	0.8	1.3

+LR, positive likelihood ratio; −LR negative likelihood ratio

Sensitivities, specificities, and positive and negative likelihood ratios are reported for the optimum cut-points.



de Meij et al, J Ped, 2015

Crohn's Disease - Disease activity?

VOCs in medicine

Table 2. Performance characteristics for the discrimination of ulcerative colitis, Crohn's disease and healthy controls by faecal VOC analysis.

	AUC ± 95% CI	p-Value	Sensitivity	Specificity	+ LR	- LR
Distinguishing ulcerative colitis from healthy controls						
Active disease	1.00 ± 0.00	< 0.001	100	100	N.A.	N.A.
Remission	0.94 ± 0.05	< 0.001	94.4	94.4	16.86	0.06
Distinguishing Crohn's disease from healthy controls						
Active disease	0.89 ± 0.05	< 0.001	86.3	66.7	2.59	0.21
Remission	0.94 ± 0.06	< 0.001	93.8	94.4	16.75	0.07
Distinguishing ulcerative colitis from Crohn's disease						
Active disease	0.96 ± 0.03	< 0.001	96.6	92.0	12.08	0.04
Remission	0.81 ± 0.08	0.000	87.5	72.2	14.86	0.06

Sensitivities, specificities, positive and negative likelihood ratios are reported for the respective optimum cut points. Remission was defined as a Physician Global Assessment value of 0. Abbreviations: AUC, ± 95% CI, area under the curve with 95% confidence interval; + LR, positive likelihood ratio; - LR, negative likelihood ratio.

de Meij, J Crohn Colitis, 2014

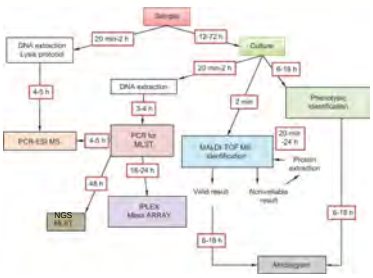
Current challenges in VOC research

VOCs in medicine

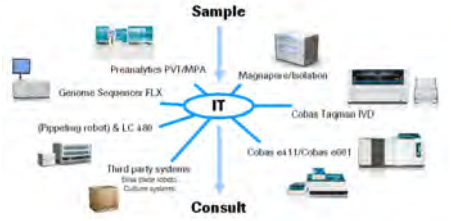
• Selecting the right population	Intention to diagnose population
• Lack of standardisation	Lack of reproduction and data-sharing
• Potential confounders	Usability versus scientific rigor
• Aspecificity of some markers	Carefully select clinical application
• Reliability of sensor platforms	Intra- / Inter-device variability, drift
• Selectivity of analytical approach	Balancing selectivity (local, systemic, exo) with portability
• Lack of biological validation	More insights into biomarker origins needed

Agnes Boots, Trends in Molecular Medicine, 2015

Direct detection from clinical samples
Detection of resistance
Detection of virulence factors
Typing of strains

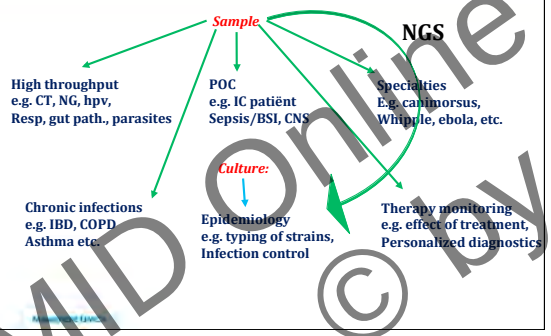


MDx: Automation



- Need for speed
- Need for connective databases
- Need for accessibility
- Need for specialised molecular microbiologists
- Need for molecular epidemiologists
- Need for bioinformaticians

The future routes of MDx



The coming years….

More rapid MDX systems (shift from culture -> MDx)

Fully automation (culture + MDx)

Focus from pathogen detection towards microbiome detection

Focus from infection towards prevention (typing & transmission)

New treatment strategies to prevent disease and spread of resistance

From disease towards personalised health (& systemic medicine)