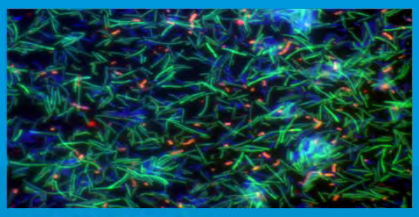
Future developments in molecular diagnostics

Paul Savelkoul



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Dept. medical microbiology
Maastricht University

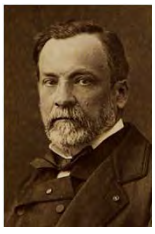
First microbiologist

1632-1723

Antoni van Leeuwenhoeck

Start discipline of medical microbiology

1822-1895



Louis Pasteur

1843-1910

Origin of many of the currently laboratory techniques

Phases of development in microbiological diagnostics

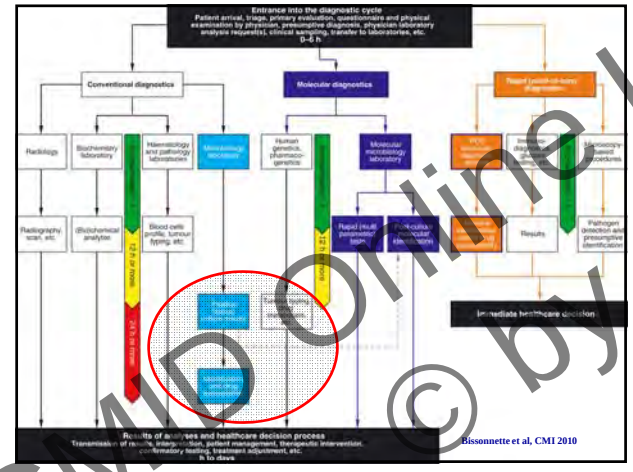
First important fact: the microscope & micro-organisms

First period of medical microbiology : classical technics for isolation and identification

Second period : improvement sampling methods, development antibiotic sensitivity testing

Third period: rise of molecular biology and molecular epidemiology

Fourth period: new systemic MDx technics, miniaturisation, automation



Current situation in the medical microbiological laboratory

Classical technics:

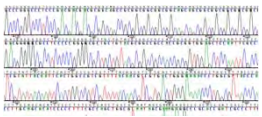
- culturing
- blood culture
- serological testing
- identification & AST
- staining

Current changing situation in the microbiological laboratory

Molecular technics:

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



Current practical changes in the microbiological laboratory

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

First initiative on patient driven applications

Roche

FLOW Primary Sample Handling System | MagNA Pure 96 System | FLOW PCR Setup System | LightCycler® 480 System

FLOW from Roche NL

The new era in Medical microbiology

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

New molecular methods

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

Respiratory POC systems

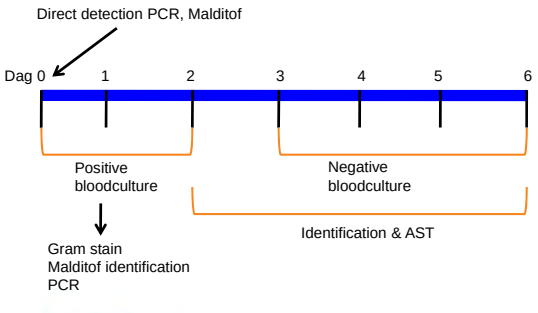
Unyvera

Parallel multiplex Real time PCR

Chamber 1	Chamber 2	Chamber 5	Chamber 6
gyrA (E. coli)	*gyrA (E. coli)*	*ermC*	*shv*
S. marcescens	*S. maltophilia*	*P. jirovecii*	*gyrA (P. aeruginosa)*
kpc	*A. baumanii*	*gyrA (P. aeruginosa)*	*M. morganii*
ctx-M	*ermB*	*E. coli*	*oxa51-like*
	L. pneumophila	*dha*	*H. influenzae*
Chamber 3	**Chamber 4**	**Chamber 7**	**Chamber 8**
Proteus sp.	*M. catarrhalis*	*mecA*	*P. aeruginosa*
S. aureus	*sul1*	*K. pneumoniae*	*K. oxytoca*
gyrA (P. aeruginosa)	*S. pneumoniae*	*mefA*	*ermA*
int1	*gyrA (P. aeruginosa)*	*parC*	*Tem1*
C. pneumoniae	*Enterobacter sp.*	*ebc*	*msrA*

Identification + <u>resistance</u>

Blood cultures



Polaris: enabling rapid and accurate MDx

5-10 ml fresh blood

standard lab equipment

Direct detection of sepsis

POC systems:
Idylla

Diagnosis of sepsis from 3- 6 days to 2 hrs

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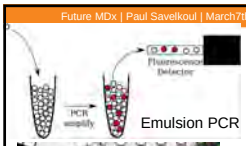
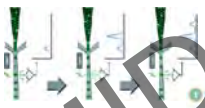


Emulsion PCR

Reliable QPCR with high sensitivity



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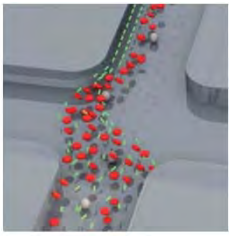
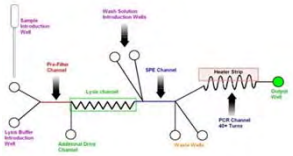
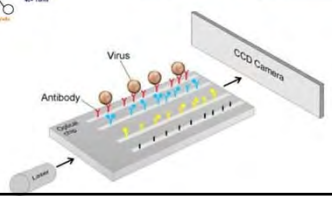
Fluidic sample types
Continuous miniaturized flow







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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)

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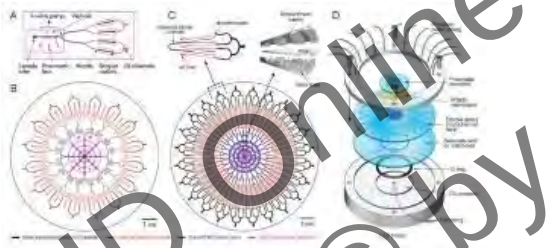
.... spirografic multi-multi applications....



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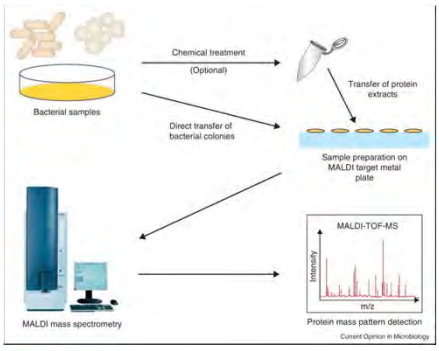
Malditof identification



Bruker
Biomerieux

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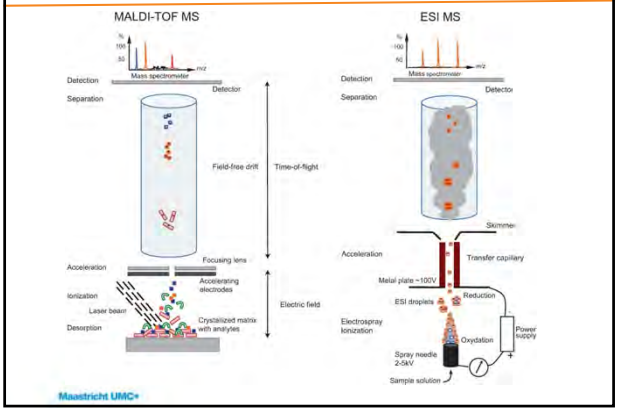
ESITOF for DNA analysis



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**

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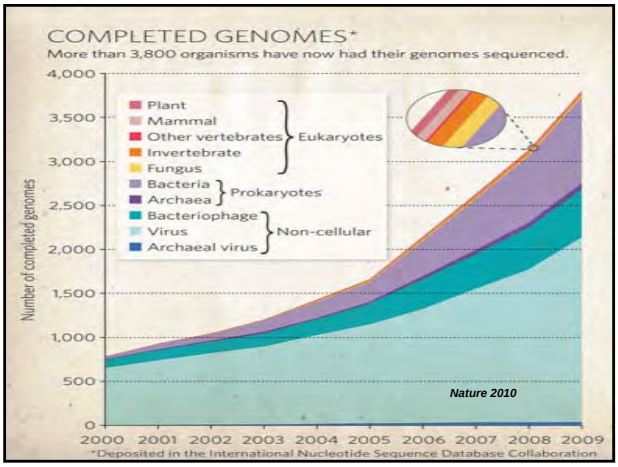


Next gen seq applications in the field of medical microbiology

1416 bacterial genomes
2340 viral genomes
1042 plasmids

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

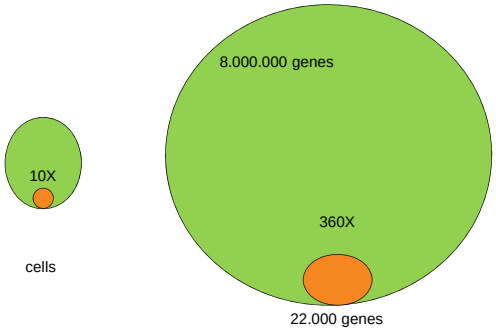
Resistome

Micro-organisms may carry resistance genes

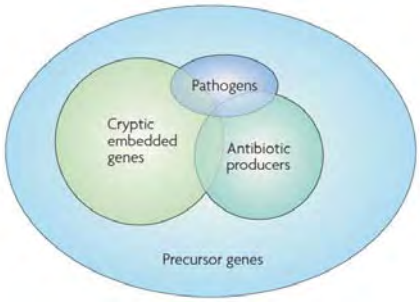
Resistome = the collection of antibiotic resistance genes in both pathogenic and non-pathogenic bacteria"

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

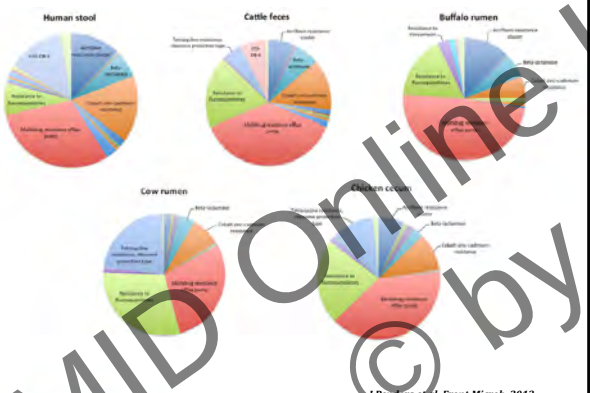
Wright, G.D. Nature Reviews Microbiology. 2007;5:175-186



The antibiotic resistome



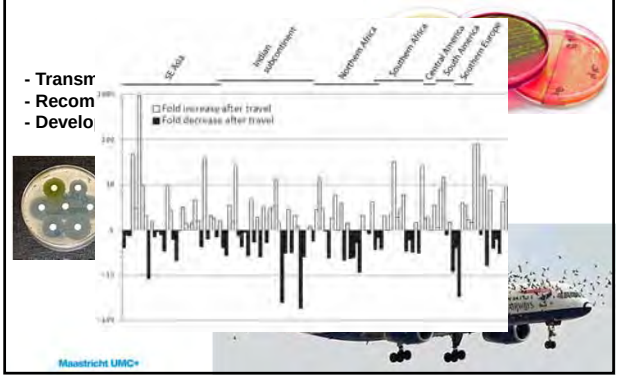
GD Wright, Nature reviews, 2007



J Penders et al, Front Microb, 2013

Transmission

- Transm
- Recom
- Develo



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?

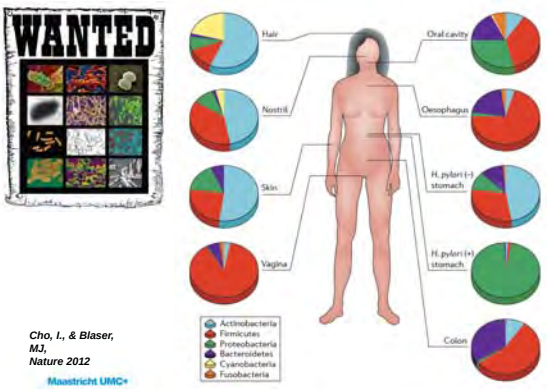
From pathogen detection toward microbiome detection:

Specific -> Syndrom -> Systemic

one bacterium -> known set -> microbiome

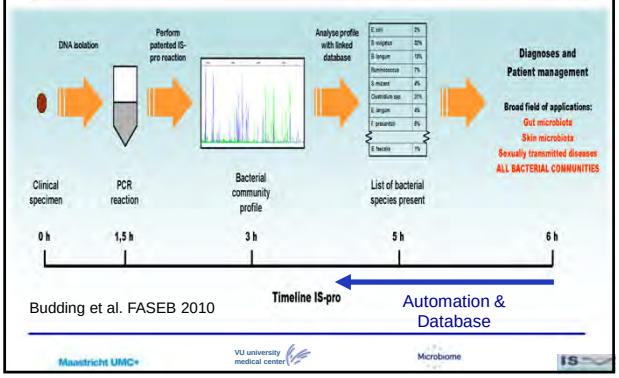
And beyond…….

The Healthy Microbiome: Who is in there?



Cho, I., & Blaser, MJ, Nature 2012

Overview ISpro® technology

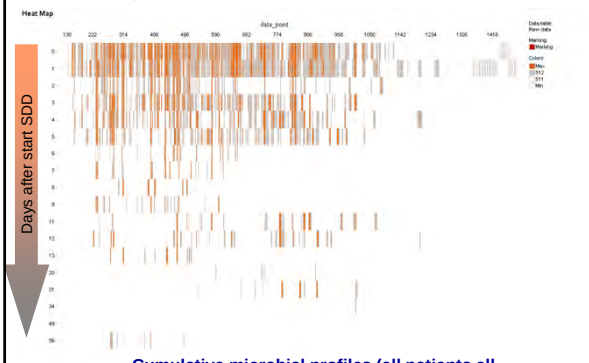


Budding et al. FASEB 2010

OBESE <-> LEAN

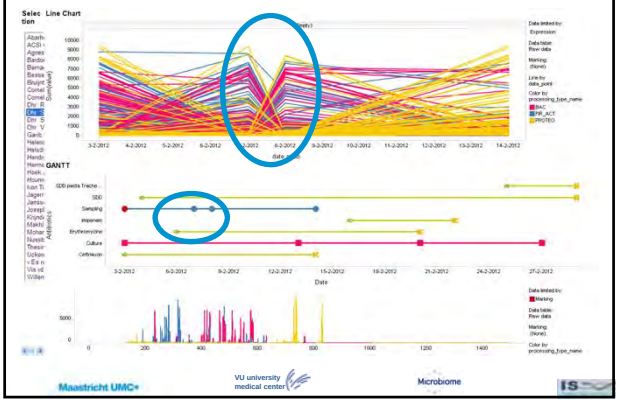
Role gut microflora: auto immune diseases, behaviour, CFS etc.

Diversity over time

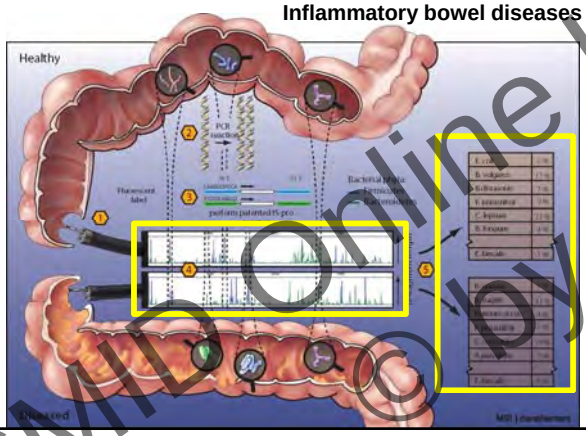


Cumulative microbial profiles (all patients all timepoints)

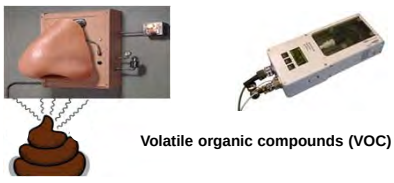
In the clinic



Inflammatory bowel diseases



VOC systems



Volatile organic compounds (VOC)

Smelling of COPD, Crohn's disease, colonic cancer etc.

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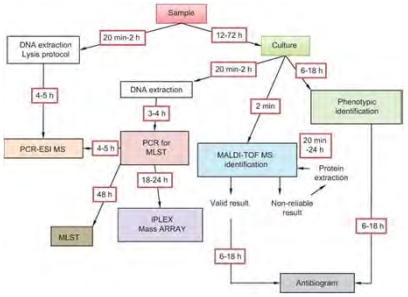
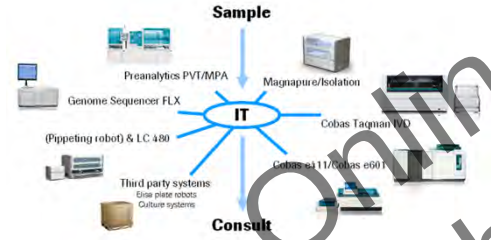
Direct detection from clinical samples
Detection of resistance
Detection of virulence factors
Typing of strains



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 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)