Recent revolution in diagnostic bacteriology

Prof. Gilbert GREUB, MD PhD

Director of the Institute of Microbiology of the University of Lausanne, Head of diagnostic microbiology at the University Hospital Center, Lausanne, Switzerland
Conflict of interests?

In Lausanne, we are using the WASP inoculation system, the Bruker MALDI-TOF, the BD-FX blood cultures automated system, the Vitek phenotypic assay, the GenXprt, the Luminex and DSX serology automates, the Scholzen incubators, several Hamilton robots, the ABI 7900 real-time systems, the MiSeq sequencer, … but we have no specific conflict of interest with these companies.

Relationship with industry

• Ongoing research grant with SUEZ-ONDEO (France)
• Research grant until December 2016 with BD-Kiestra (USA)
Introduction

Ten years ago

Samples

- Smears exam
- Culture
- Identification
- Antibiotic susceptibility testing

Ag detection

- PCR

Serology

J0
J1
J2
J2 - J3
Introduction

Smears exam

- Culture, identification, AST by MALDI-TOF
  - Confirmatory ASTs
    - Genomics
    - Metagenomics

Samples

- Fast PCR: Ag + POCT-PCR

Automatisation

- PCR
- Serology

More information, more rapidly
Revolution or evolution?
in diagnostic bacteriology ....

**MALDI-TOF + Revolution → 20 pubmed hits; 3x in the title**

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification.

Bizzini A, Greub G.
Clin Microbiol Infect. 2010 Nov;16(11):1614-9

Mass spectrometry: a revolution in clinical microbiology?
Lavigne JP, Espinal P, Dunyach-Remy C, Messad N, Pantel A, Sotto A.

The ongoing revolution of MALDI-TOF mass spectrometry for microbiology reaches tropical Africa.
Fall B et al.
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Score > 2
Score 1.7-2
Score < 1.7

Croxatto, Prod’hom & Greub, FEMS Microbiol Reviews 2011
Microbial identification

Prospective study

Reduced time to identification

Reduced costs of identification

<table>
<thead>
<tr>
<th></th>
<th>with MALDI-TOF*</th>
<th>without MALDI-TOF</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean cost</td>
<td>4.03€</td>
<td>9.43€</td>
</tr>
<tr>
<td>annual cost</td>
<td>71’921€</td>
<td>168’094€</td>
</tr>
</tbody>
</table>

→ 2.34 fold reduction

Including acquisition (5 year amortization) & maintenance

|                     | 132’521€        | 168’094€          |

→ 1.27 fold reduction

*identification by MALDI-TOF and when needed additional approaches

Heininger, Prod’hom & Greub, submitted
Typing: discrimination between closely-related species

Identification of genomic species from the *Acinetobacter baumannii* group

Espinal P. et al.
Detection of *S. aureus* delta-toxin

Purified toxin

Routine toxin detection by MALDI-TOF


80 of 95 isolates from bone and joint infection exhibited peaks suggesting delta-toxin production → chronic infection

Valour F Pclin Micobiol Infect 2015
Carbapenemase detection

Loss of peaks at 476 Da, 498 Da and 521 Da

Burckhardt et al., J Clin Microbiol (2011) 49: 3321-4
Carbapenemase detection: better than PCR

Modified test

using a 10 μg ertapenem disk; only 1 hour incubation

<table>
<thead>
<tr>
<th>Diagnostic approach</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypic detection (Modified Hodge test)</td>
<td>90.5% (19/21)</td>
<td>86% (24/28)</td>
</tr>
<tr>
<td>Phenotypic detection (IP/IPI)*</td>
<td>54.5% (6/11)</td>
<td>92.1% (35/38)</td>
</tr>
<tr>
<td>PCR-Check MDR Carba</td>
<td>90.5% (19/21)</td>
<td>100% (28/28)</td>
</tr>
<tr>
<td>Microarray</td>
<td>90.5% (19/21)</td>
<td>100% (28/28)</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>100% (21/21)</td>
<td>100% (28/28)</td>
</tr>
<tr>
<td>MS/MS</td>
<td>100% (21/21)</td>
<td>100% (28/28)</td>
</tr>
</tbody>
</table>

*phenotypic detection of metallo-β-lactamases (VIM, IMP, NDM)
1. Introduction
2. MALDI-TOF
3. Etiological diagnosis of bacteremia
4. Automation
5. Rapid tests
6. Genomics
7. Metagenomics
8. Conclusions
Blood cultures

To improve diagnosis: use a pellet

Centrifugation steps
Blood cultures

**Blood culture broth**

*Escherichia coli*

*Staphylococcus capitis*

*Streptococcus dysgalactiae*

**Blood culture pellet**

# Applications of the pellet

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>n</th>
<th>Concordant ID at species level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prod’hom (2010)</td>
<td>122</td>
<td>78% (GN: 89%, GP: 72%)</td>
</tr>
<tr>
<td>Prod’hom (unpublished)</td>
<td>314</td>
<td>85% (GN: 89%, GP: 83%)</td>
</tr>
</tbody>
</table>

**Excellent accuracy**

- n=122
  - 69 (57%) > 2.0
  - 96 (79%) > 1.7

1 discordant

*S. caprae vs S. pasteuri*
<table>
<thead>
<tr>
<th>Authors</th>
<th>n</th>
<th>Concordant ID at species level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kok (2011)</td>
<td>507</td>
<td>74.8% (GN: 91.4%, GP: 67.7%)</td>
</tr>
<tr>
<td>Schubert (2011)</td>
<td>500</td>
<td>86.5% (GN 89.8%, GP 86.3%)</td>
</tr>
<tr>
<td>March-Rossello (2013)</td>
<td>100</td>
<td>~98% (GN: 97.3%, GP: 98.4%)</td>
</tr>
<tr>
<td>Clerc (2013)</td>
<td>202</td>
<td>86.7% (only GN)</td>
</tr>
<tr>
<td>Rodrig-Sanchez (2014)</td>
<td>1084</td>
<td>63.9% (81.4% after extraction)</td>
</tr>
<tr>
<td>Jakovlev (2015)</td>
<td>152</td>
<td>81.9% (up to 89.3%)</td>
</tr>
<tr>
<td>Randazzo (2016)</td>
<td>266</td>
<td>65.8% (up to 77.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>77.8%</td>
</tr>
</tbody>
</table>
Pre-MALDI-TOF era

- Gram + cocci arranged in chains
- Gram + cocci arranged in clusters
- Gram - bacilli

Post-MALDI-TOF era

- E. faecium
- S. aureus
- E. coli

Overnight incubation

Blood cultures

The true revolution is related to the CLINICAL IMPACT

© by author
Impact of MALDI-TOF: retrospective study

- Time to identification
  1h35 with MALDI-TOF (versus 25h43 without)

Clinical impact:
- 20/157 results led to a treatment change (in adults)
- 1/40 results led to a treatment change (in children)

Martiny et al. Clin Microbiol Infect 2013
Impact of MALDI-TOF: retrospective study

• Time to identification
  1h35 with MALDI-TOF (versus 25h43 without)

Clinical impact:
• 20/157 results led to a treatment change (in adults)
• 1/40 results led to a treatment change (in children)
• New blood cultures (n=2)
• Catheter removal (n=4)
• Additional investigations (n=3)
• Documented sample exchange (n=1)
• Exclude a contamination

Martiny et al. Clin Microbiol Infect 2013
Impact of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry on the Clinical Management of Patients With Gram-negative Bacteremia: A Prospective Observational Study

Olivier Clerc,1 Guy Prod’hom,2 Christelle Vogne,2 Alain Bizzini,2 Thierry Calandra,1 and Gilbert Greub1,2

1Infectious Diseases Service and 2Institute of Microbiology, Lausanne University Hospital Center and University of Lausanne, Switzerland
### Impact of the Sequential Reporting

<table>
<thead>
<tr>
<th></th>
<th>N = 202</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram stain</strong></td>
<td>42 (20.8)</td>
</tr>
<tr>
<td><strong>Streamlining</strong></td>
<td>16 (7.9)</td>
</tr>
<tr>
<td><strong>Spectrum broadening</strong></td>
<td>16 (7.9)</td>
</tr>
<tr>
<td><strong>Introduction of empirical antibiotic therapy</strong></td>
<td>10 (5.0)</td>
</tr>
<tr>
<td><strong>MALDI-TOF MS</strong></td>
<td>71 (35.1)</td>
</tr>
<tr>
<td><strong>Streamlining</strong></td>
<td>22 (10.9)</td>
</tr>
<tr>
<td><strong>Spectrum broadening</strong></td>
<td>31 (15.3)</td>
</tr>
<tr>
<td><strong>Introduction of focused empirical antibiotic therapy</strong></td>
<td>18 (8.9)</td>
</tr>
</tbody>
</table>

Impact of MALDI-TOF on empirical antibiotic therapy in 35% of bacteremia
A prospective randomized trial

MALDI-TOF subgroup 1
Conventional subgroup 1
MALDI-TOF *E. coli/Klebsiella spp.*
Conventional *E. coli/Klebsiella spp.*

p < 0.001 (Log-rank test)

Cumulative optimal treatment vs. hours since blood culture draw

Osthoff et al. Clin Microbiol Infect 2017
Antibiotic susceptibility

Before 2012

Starting from a bacterial pellet

24h

MIC

Vitek

From isolated colonies

Blood cultures

- **Gram staining**
  - > 100% accurate
  - ≤1h

- **MALDI-TOF MS**
  - > 99% accurate
  - ≤1h

- **Antibiotic susceptibility testing**
  - > 99% accurate
  - 16-24h

- **POCT-PCR MRSA**
  - Sensitivity 99%
  - Specificity 100%
  - 2-3h
Blood cultures

Atomic force microscopy on blood culture pellet

Pictures from G. Dietler (Lausanne)
Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections.
Clin Microbiol Infect. 2017
Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections.
Clin Microbiol Infect. 2017
Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections.
Clin Microbiol Infect. 2017
1. Introduction
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4. Automation
   • in bacteriology
   • in molecular biology
5. Rapid tests
6. Genomics
7. Metagenomics
8. Conclusions
Laboratory automation in clinical bacteriology: what system to choose?

Another revolution in microbiology

<table>
<thead>
<tr>
<th>Level of automation</th>
<th>Inoculation</th>
<th>Partial lab automation</th>
<th>Complete lab automation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD InoquLA</td>
<td>WASP</td>
<td>WASP Lab</td>
<td></td>
</tr>
</tbody>
</table>

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ESCMID eLibrary

Automated inoculation, smart incubators and telebacteriology
Automated inoculation

Discrete colonies

**E. coli**

**E. faecalis**

**K. pneumoniae**

**S. aureus**

Difference between the systems

Bacterial species dependent

J Clin Microbiol. 2015;53:2298-307
Comparing two systems: cloudy urines

Higher number of discrete colonies with the automated inoculation systems compared to manual inoculation

### Smart incubators

<table>
<thead>
<tr>
<th></th>
<th>BD Kiestra (ReadA compact)</th>
<th>Copan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity</td>
<td>1152</td>
<td>882/1764</td>
</tr>
<tr>
<td>Plate loading</td>
<td>350 plates/h</td>
<td>360 plates/h</td>
</tr>
<tr>
<td>Plate unloading</td>
<td>250 plates/h</td>
<td>250 plates/h</td>
</tr>
<tr>
<td>Plate loading + Picture</td>
<td>250 plates/h</td>
<td>250 plates/h</td>
</tr>
</tbody>
</table>

Few differences

To be **smart**, incubators should:

- be controlled by a middleware
- be linked to a *uploading/unloading system*
- be able to *take pictures* and to sort plates according to presence/absence of colonies

**UIC**: Universal Interface Connector

**BD EpiCenter**

**WASPLab**

Laboratory automation in clinical bacteriology: what system to choose?
Croxatto A, Prod'hom G, Faverjon F, Rochais Y, Greub G.
Automation

Benefit on Full Time Equivalents

- Identification & AST: 37%
- Plates reading: 26%
- Samples arrival: 11%
- Breaks: 40%
- Microscopy: 11%
- Inoculation: 18%
- Plates sorting and incubation: 7%
- Results verification: 7%
- Parasitology: 8%
- Orientation assays: 11%
- Laboratory management: 2%
- Other: 1%

Laboratory automation in clinical bacteriology: what system to choose?
Croxatto A, Prod'hom G, Faverjon F, Rochais Y, Greub G.
Automation in bacteriology

- Improved quality:
  • reproducibility
  • Number of discrete colonies
  • reduced time to results
  • less contamination
- Decreased workload
  • reduced costs ?
  • increased interest for work ?
- Risk for small laboratories
- Change + project management
- Lean management
A future revolution

Automated identification thank to new algorithms and chromogenic agar

May replace MALDI-TOF …

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Molecular diagnosis in Lausanne

• Hamilton robot coupled to 96-wells Magnapure
  = high throughput DNA extraction

Molecular diagnosis in Lausanne

- Hamilton robot coupled to 96-wells Magnapure
  = high throughput DNA extraction

- Two Hamilton robot + 2 ABI 7900 real-time PCR
  = high throughput Taqman PCR in 384-wells
Molecular diagnosis in Lausanne

93 PCRs
Detection of bacteria, fungi, parasites and viruses on the same microplate

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.
Due to financial constraints and resources shortage, we need to be more efficient:

- A single line for all PCR tests
- Flexibility
- High throughput - scalability
- Multiplexing
- Quality (fiability)
- Reduced costs

Only possible with home-made tests setting
Advantages of home-made tests

Thank to the **flexibility** of home-made PCRs:

- Many applications
- Pathogen detection
- Typing
- Identification
- Resistance genes
- New microbes
- Toxin detection

Impossible to have all PCRs that you would like to have with commercial tests, even when having 5 different industrial partners ...
Advantages of home-made tests

High throughput - scalability

Spring-summer 2012: Cluster of 14 human cases of acute Q fever in Lavaux


Blood donors. From 14 July to 20 August 2012, we had to test in our high throughput platform, 2393 blood donations in a prospective reactive way, to prevent Q fever transmission by blood transfusion in the setting of a local Coxiella outbreak.

Jaton, Peter, Raoult, Tissot & Greub. New Microbes New Infection 2013
Advantages of home-made tests

Multiplexing

With home-made test, you also can have a classical duplex

*C. trachomatis* – *N. gonorrhoeae* PCR

And many other duplex PCRs – according to your need

**Multiplex real-time PCR for the diagnosis of malaria**

L. Dormond¹, K. Jaton-Ogay¹, S. de Vallière², B. Genton², J. Bille¹,³ and G. Greub¹,³

*Clinical Microbiology and Infection* 2010

**Development of a duplex real-time PCR for the detection of *Rickettsia* spp. and typhus group rickettsia in clinical samples**

Stefano Giulieri¹, Katia Jaton², Alain Cometta³, Laurence T. Trellu⁴ & Gilbert Greub¹,²


**Improving the molecular diagnosis of *Chlamydia psittaci* and *Chlamydia abortus* infection with a species-specific duplex real-time PCR**

Onya Opota¹, Katia Jaton¹, James Branley², Daisy Vanrompay³, Veronique Erard², Nicole Borel⁵, David Longbottom⁶ and Gilbert Greub¹,⁷

*Journal of Medical Microbiology* (2015), 64, 1174–1185
Quality

May be achieved by a **precisely defined R&D process**:
1. Literature review
2. Choice of a target gene
3. Target primers / probe design
   - with/without locked nucleic acid
   - with/without miner grove binder probe
   - high copy number target, specific gene
   - conserved gene, not polymorphic
4. Analytical sensitivity (plasmid pos. controls), reproducibility, and analytical specificity
5. Fiability when applied to clinical samples
6. Post-implementation follow-up

**R&D meeting once a week - large R&D team**
Disadvantages of commercial tests

**Quality**

With commercial tests, NO knowledge of the target

- No possibility to check the target
- No guarantee of quality

Ex: the *Chlamydia trachomatis* Swedish mutant undetected by most commercial tests

outbreak in Sweden in 2006

(our home-made PCR was during several month the only PCR able to detect the Swedish mutant in Switzerland)

Herrmann B, Eurosurgence 2008; Reischl U Eurosurveillance 2009
Advantages of home-made tests

Reduced costs

![Graph showing reduced costs with different platforms over varying number of samples per run.](image)

- Cobas Amplicor
- Real-time PCR
- Corrected platform approach

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GeneXpert
Fully automated and miniaturized
< 2 hours

Reagents compartments (extraction, purification, amplification)

Optical detection

Real-time PCR (thin to allow temperature changes)

Valve (fluid movements)

Picture by G Greub

Clerc O & Greub G. Clin Microbiol Infect 2010; 16, 1054–1061
GeneXpert MTB/RIF

Bates et al, 2013
Zambia
Prospective study of inpatients (n=930) with median age 24 months (maximum 15 years) and suspected TB: in culture-positive cases (n=58), the Xpert MTB/RIF assay was more sensitive than smear microscopy when testing sputum samples (90.0% vs 30.0%) or gastric lavage aspirates (68.8% vs 25.0%) and specificity was 99.3%

Tortoli et al, Italy
2012
Study of the diagnosis of extrapulmonary TB in adults and children with a wide range of different sample types (tissue biopsies, pleural fluid, gastric aspirates, pus, CSF, and urine) that used a composite reference standard of culture, radiology, histology, and treatment response: the sensitivity in samples from children (86.9%) tended to be higher than that in samples from adults (77.6%), possibly as a result of the types of clinical samples in each group

High sensitivity of 90% on sputum samples and of 77 to 86% on extrapulmonary samples
Lawn SD et al.

Importance of such POCTs given the worldwide spread of resistant *M. tuberculosis* strain
**GeneXpert MTB/RIF**

- **Xpert MTB/RIF** to assess patients' transmission risk to quickly guide airborne isolation decisions, compared to microscopy
  - n=242 (2010 - 2014 in Lausanne)
  - Sensitivity 91.5% (65/71) vs 64.8%
  - Specificity 99.6% (170/171) vs 94.2%
  - Negative predictive value 100%
  - 11 smear negative & Xpert positive patients with a significant transmission risk

---

**Xpert-based strategy**

for a faster and more accurate management of tuberculosis patients' transmission risk

Meningitis: Enterovirus POCT-PCR

High costs of rapid molecular tests but:

Early results led to:
- reduction of hospitalisation time 5.4 vs 2.2 days
- reduction of antibiotic usage
- reduction of investigations
- costs reduction

## Meningitis: Enterovirus POCT-PCR

<table>
<thead>
<tr>
<th></th>
<th>Period A (No PCR)</th>
<th>Period B (real-time PCR)</th>
<th>Period C (POCT-PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration of antibiotic tt</strong></td>
<td>3 (1-12) day</td>
<td>1 (1-3) day</td>
<td>&lt; 1 day</td>
</tr>
<tr>
<td><strong>Duration of hospital stay</strong></td>
<td>4 (1-12) day</td>
<td>2.5 (1-26) day</td>
<td>0.45 day</td>
</tr>
<tr>
<td><strong>Costs (SFr)</strong></td>
<td>3691.-</td>
<td>2738.-</td>
<td>580.-</td>
</tr>
</tbody>
</table>

*Note: Median (range) duration of antibiotic therapy and hospital stay.*

*Note: Median costs.*

*Note: Duration of antibiotic therapy in days, duration of hospital stay in days, and cost in Swiss Francs (SFr).*

*Slide from P. Meylan et al. (Lausanne)*
Rapid tests

BD max

Pictures by G Greub
Rapid tests

**BD max**

**Commercially developed PCRs**

*Salmonella, Shigella, Campylobacter*

*Entamoeba, Giardia, Cryptosporidium*

**Open system → home-made tests**

*N. meningitidis, S. pneumoniae,*

*H. influenzae, L monocytogenes,* …

Malaria (pan-*Plasmodium*)

*Pneumocystis jirovecii* …
Future development of POCTs

- To be used in core labs or at bedside? (Keep it simple for bedside use)
- Syndrome-oriented multiplex diagnostic kits
- Different level of information on a given pathogen: presence of pathogen & AB susceptibility / virulence
- New POCT format:
  - POCT-ICT
  - POCT-PCR
  - POCT-ELISA
  - POCT-MICROSCOPY
  - NANO-POCT

Ex: MRSA, MTBC, EHEC
Rapid tests: nano-plasmonic sensors

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Genomics + Revolution + Bacteria → 98 pubmed hits; 11x in the title
The quantum leap

More sequences

Cheaper

More rapidly

Raw daily output

454 Genome Sequencer: 30 Mb
Sanger: 1 Mb

Turnaround time: bacterial genome

Sanger: 60 days
454 Genome Sequencer: 2 days

Illumina HiSeq2000: 14 days (2 days)
454 Genome Sequencer: 2 days

Illumina MiSeq: 1 day (<1 day)
ION PGM: 1 day (<1 day)
(Oxford Nanopore: 2 hours)

Cost per Mb assembled sequence

Sanger: $10,000
454 Genome Sequencer: $1,000

© by author
Increased number of genomes

- **Sanger sequencing**
  - First bacteria: *H. influenzae, M. genitalium*
  - Human genome draft sequence
  - The Global Ocean Sampling for metagenomics

- **High throughput sequencing**
  - Pyrosequencing (454)
  - First personal genome of James Watson
  - Rapid sequencing during an outbreak of EHEC O104:H4

- Number of completed sequencing projects:
  - Complete genomes
  - Draft genomes

- Equal to dirty genomes
New high-throughput sequencing technologies

Development of new diagnostic approaches

Genotyping

Taxogenomic

Therapy

Prognosis

Resistance genes

Virulence genes

Development of new diagnostic approaches

New ELISA

New PCR

Greub G
Clin Microbiol Infect 2013
Enterohemorrhagic *E. coli*

*E. coli* O104:H4

Useful to develop a strain specific PCR

Mellmann et al PLOS One 2011
Enterohemorrhagic *E. coli*

*E. coli* O104:H4

Identified virulence factors:

- **Shiga toxine** = verotoxine
- Two new plasmids:
  - Coding for AAF/I fimbriae (83 kb) = adhesin
  - Coding for TEM-1 & CTX-M-15 beta-lactamases (90 kb) = resistance to antibiotics

Useful to identify virulence factors

Mellmann et al PLOS One 2011
Genomics

Genomic of medical importance
Institutional project since 2012

For selected cases:
- Pathogenicity
- Clinical picture
- Outbreak
- Specific AB resistance
- Need of diagnostic tools

Goal: results in 48 to 72 hours

Clients:
- microbiologists
- medical doctors

Institutional project since 2012

Fragment analyzer
MiSeq (Illumina)

© by author
Bacteremia in a newborn receiving probiotics

*Bifidobacterium longum*

Strains isolated from:
- Blood cultures (Lausanne)
- Capsules from Lausanne
- Capsules from Berlin

Bertelli et al, Clinical Infectious Diseases, 2015
**Neisseria meningitidis**

*ctrA* gene for *Neisseria meningitidis*

Corless *et al* J Clin Microbiol 2001

False negative PCR due to a gene polymorphism in *ctrA* gene of *Neisseria meningitidis*

Jaton, Ninet, Bille & Greub. J Clin Microbiol 2010

Similar polymorphism also observed in Italy

Budroni S *et al*. PNAS 2011

Out of 2602 genes in the *N. meningitidis* genome, only 12 genes common to all 183 genomes available and not present in any other bacterial species → New targets for specific *N. meningitidis* PCRs

A large success locally

possible thank to chlamydial genomics background (genomes of various chlamydia-related bacteria)

about 5 years of institutionnal project
several hundreds genomes for > 30 clinicians-driven questions

Provided the minimal bioinformatic team (4 to 6) to also develop:
- direct genome sequencing from clinical samples
- 16S PCR + NGS analysis of microbiota
- direct metagenomics

Need of trained bioinformaticians
Sequence a genome: a practical course

Sequencing and characterizing the genome of *Estrella lausannensis* as an undergraduate project: training students and biological insights

Claire Bertelli¹,², Sébastien Aeby¹, Bérénice Chassot³, James Clulow³, Olivier Hilfiker³, Samuel Rappo³, Sébastien Ritzmann³, Paolo Schumacher³, Céline Terrettaz³, Paola Benaglio⁴, Laurent Falquet⁵,², Laurent Farinelli⁶, Walid H. Gharib⁷,², Alexander Goesmann⁹, Keith Harshman⁹, Burkhard Linke⁸, Ryo Miyazaki¹⁰, Carlo Rivolta⁴, Marc Robinson-Rechavi⁷,², Jan Roelof van der Meer¹⁰ and Gilbert Greub¹ *

¹ Center for Research on Intracellular Bacteria, Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland
² SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland
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1. Introduction
2. MALDI-TOF
3. Etiological diagnosis of bacteremia
4. Automation
5. Rapid tests
6. Genomics
7. Metagenomics
8. Conclusions
A 14-year-old boy
4 months with fever and headache that progressed to hydrocephalus and status epilepticus
Brain biopsy examination was unrevealing
Next generation sequencing of the cerebrospinal fluid identified 475 of 3,063,784 sequence reads (0.016%) corresponding to Leptospira infection
PCR and serology done at CDC confirmed Leptospira infection

Metagenomics to detect novel emerging pathogens?

- Keywords: “new pathogen”, metagenomics: n=122
  
  Rather unexplored field?
  Few success story?

- Many case reports

- Really few “new pathogen” discovery
  (often detection of known species)
Necrotizing enterocolitis (NEC)

Severe disease in premature neonates due to impaired blood flow in enteric vessels, likely due to an infection ...

www.momjunction.com

www.pediatricsurgerymd.org

www.chla.org
Clostridium butyricum

Bacterial stool microbiota of 30 preterm neonates with and without necrotizing enterocolitis (NEC)

**Clostridium butyricum**

Specific qPCR on 363 stool samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with NEC (n = 93)</td>
<td>74 (79.6)</td>
<td>19 (20.4)</td>
</tr>
<tr>
<td>Controls (n = 70)</td>
<td>9 (12.8)</td>
<td>61 (87.2)</td>
</tr>
<tr>
<td>Infants with gastroenteritis (n = 100)</td>
<td>7 (7)</td>
<td>93 (93)</td>
</tr>
<tr>
<td>Infants without symptoms (n = 100)</td>
<td>4 (4)</td>
<td>96 (96)</td>
</tr>
</tbody>
</table>

**Strong association (p<0.001)**

Clinical metagenomics

Mainly PCR-based NGS

Clients:
- Endocrinology → obesity, diabetes, ...
- Dermatology → atopic dermatitis
- Obstetrics → miscarriage
- Oncology → effect of immunotherapy
- Forensics → identification of twins
- Intensive care → skin of burn patients
- Infectiology → unknown etiology, virulome, resistome
Gut microflora diversity of Wild type & mutant mice on normal and High Fat Diet (HFD)

<table>
<thead>
<tr>
<th>WT</th>
<th>Knock-out mutant -/-</th>
<th>Chow diet</th>
<th>HFD diet</th>
</tr>
</thead>
</table>

β-Klotho deficiency protects against obesity through a crosstalk between liver, microbiota, and brown adipose tissue.
Somm E et al.
JCI Insight. 2017 Apr 20;2(8) [ahead of print]
14 healthy men

**Metagenomics**

**Dietary restrictions + activity monitoring**

**Group A**
- Acidified milk
  - Run-in: 4 wks
  - Wash-out: 3 wks

**Group B**
- Probiotic Yoghurt
  - Wash-out: 3 wks

**Post-study**
- 4 wks

**Before phase**
- 4 wks

**Stools**
- V1
- V2
- V3
- V4
- V5

**Burthon et al, in press**
Influence of processing on microbiota profile derived from 16S rRNA - NGS studies

ILLUMINA Metagenomic App, species level

USEARCH, operational taxonomic unit (OTU) level

Effect of the analytical pipeline

Metagenomics

Metagenomics

Effect of the DNA extraction

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Several recent revolutions in diagnostic microbiology

Technological revolution …

More data
More rapidly
Increased quality
Lower costs

But needs flexibility + adaptation …
New skills
Regulatory issues, quality issues …
Several recent revolutions in diagnostic microbiology

• Need to extend our network outside infectious diseases ➔ serving also endocrinologists, pneumologists, …
• Need to prepare our team to all changes ➔ change management ➔ project management
• Need to define best protocols & develop quality controls ➔ quality management
• Need to develop new skills ➔ ex.: practicals “to sequence a genome” ➔ team management, mentor-mentee relationship*

* Opota O and Greub G, Clin Microbiol Infect, submitted
Thank you

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