ROYALFREE
world class care and expertise

Simon Rattenbury
Head of Laboratory Service
simon.rattenbury@nhs.net

The Toronto Invasive Bacterial Diseases Network Education Day
November 2012

- **The Royal Free hospital was founded in 1828 to provide free hospital care to those who could not afford treatment. The title 'Royal' was granted by Queen Victoria in 1837 in recognition of the hospital's work with cholera victims.**
- **For many years the Royal Free was the only hospital in London to offer facilities for clinical instruction to women**.

- **550 beds Reduced from 1200.**
- **700,000 patients a year from all over the world.**
- **Employ around 4,600 people and have a turnover of about £450m.**
- **Major A&E.**
- **All branches of surgery and medicine**

- Joined in 1998
- Multidisciplinary
- Varied Roles
- Molecular Microbiology
- Automation
- My remit was to introduce modern ways of working, bring together the academic & service elements of the department

Workload & Staffing

Year	**06/07**	**07/08**	**08/09**	**09/10**	**10/11**
Workload	250,000	300,000	350,000	550,000	650,000
Staffing	50	50	49	47	47

Microbiology at the Crossroads

- "It is cheaper at the moment to do the test than to argue about whether it is necessary or not. We have to change that"
- Health Service Journal supplement 3 November 2011

Current Service Provision

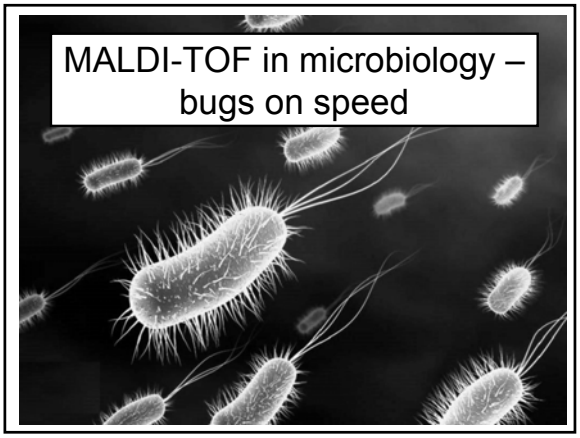
Demand Managed

- Routine bacteriology & Automation
- Automated blood culture
- Automated bacterial AST
- TB liquid culture and molecular resistance
- TB find and treat programme

Current Service Provision

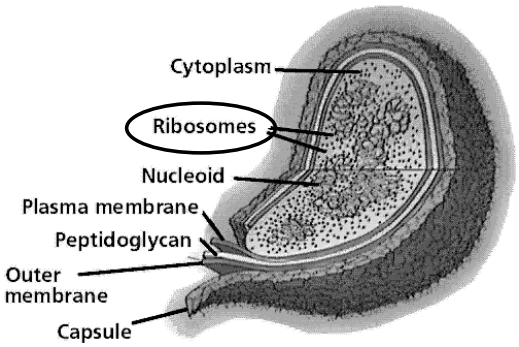
Molecular

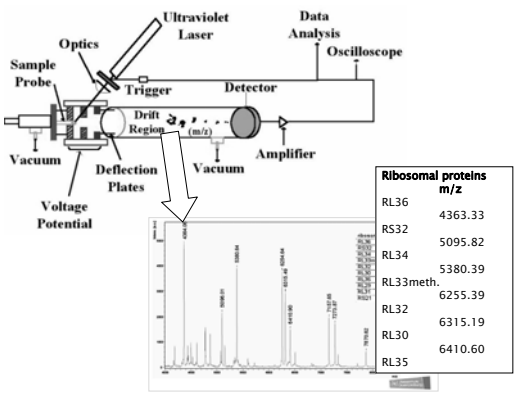
- Chlamydia/GC
- MRSA
- Enteric Panel Real time
- 16s RNA sequencing
- Fungal 18ITs Sequencing
- Typical & Atypical respiratory
- C difficile toxin



MALDI-TOF

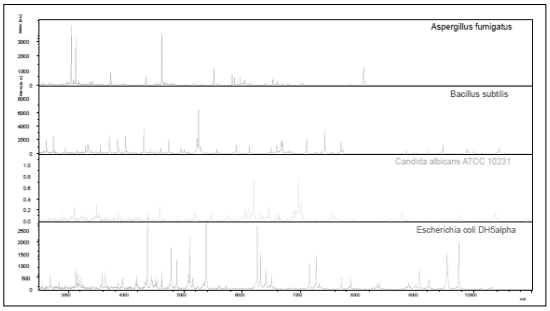
Matrix-assisted laser desorption/ionization – time of flight
Mass spectrometry





MALDI-TOF

MALDI-TOF spectra matched to database



- Although the clinical community still declares it has discovered a "new technology", the first mass spectrometry investigation of whole micro-organism analysis was published in 1970, and the first commercial protein mass fingerprinting package was introduced in 2000
- **Current Trends in Microbial Diagnostics Based on Mass Spectrometry**
  Vladimir Havlicek, Karel Lemr, and Kevin Albert Schug, *Anal. Chem.*, **Just Accepted Manuscript** • Publication Date (Web): 07 Nov 2012

Year	1993	2000	2005	2012
Papers	7	61	168	292

Why Bruker MALDI benefits

- RFH introduction of MALDI 2009
- Fast, cheap, accurate
- May expedite
  - Appropriate treatment
  - Infection control
- Comprehensive Database
  - upgradeable
  - fungi, mycobacteria, mixed cultures

Why Bruker MALDI ToF

- World wide >600 (Bruker)
- UK & Ireland ~ 50
- Most of major hospitals in London
- HPA wide contract
- As well as clinical systems then we have
- Systems in the VLA, Pharma, Quality Milk Management, Moredum

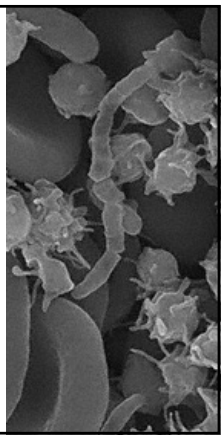
Service Provision

MALDI-ToF

- Same day / rapid Bacterial and Fungal identification <£7 = $11C
- Rapid identification of blood culture isolates
- ESBL, MRSA identification and sensitivity/resistance testing in development

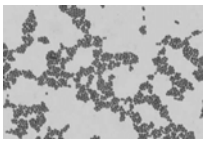
Outline

- MALDI-TOF in microbiology
- Validation results
- MALDI-TOF applications



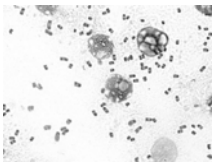
Case 1

- 82 year old man, confused, febrile
- ?chest, ?UTI, ?cellulitis
- A&E blood cultures: staphylococci in one out of two bottles
- Co-amoxiclav



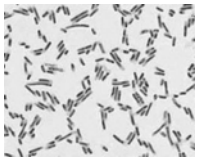
Case 2

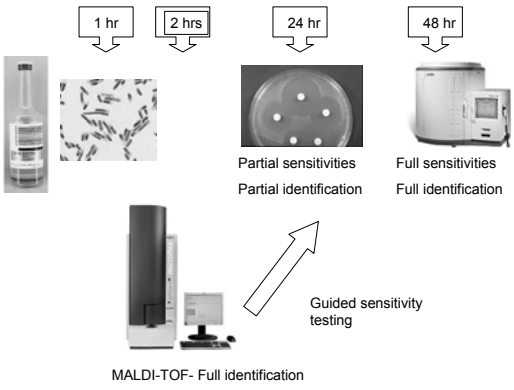
- 62 year old male, fever, abdominal pain
- ?biliary sepsis, ?gastroenteritis
- A&E blood culture: Gram negative rods
- Co-amoxiclav



Case 3

- 16 year old female, fevers, sore throat
- Previous UTIs
- A&E blood cultures: Gram negative rods
- Ceftriaxone, cephalexin

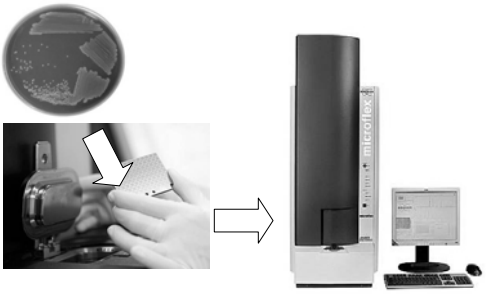


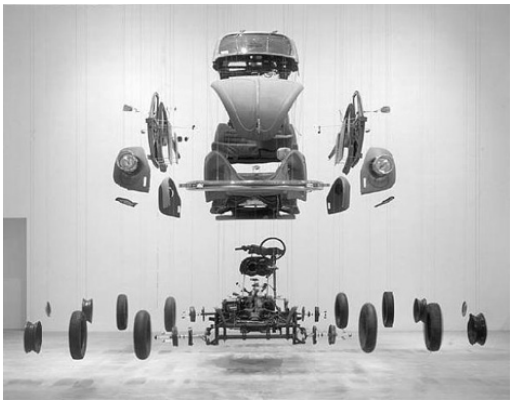


MALDI-TOF identification

- Case 1: *Staphylococcus aureus*
  - flucloxacillin
- Case 2: *Serratia marcescens* AmpC
  - ertapenem
- Case 3: *Fusobacterium necrophorum*
  - metronidazole, plus amoxycillin for Grp A Strep

MALDI-TOF identification of bacteria from colonies





Gram negative drug resistance


Amoxicillin

Gram negative drug resistance

	B-lactamase 	ESBL/AmpC 
Amoxicillin		
Ceftriaxone		
Piperacillin/ tazobactam		

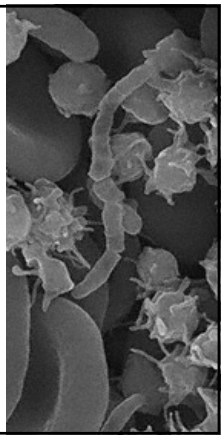
Gram negative drug resistance

	B-lactamase 	ESBL/AmpC 	*P. aeruginosa* 	*S. maltophilia* *A. baumanii* NDM 
Amoxicillin				
Ceftriaxone				
Piperacillin/ tazobactam				
Meropenem				

Identification matters

Outline

- MALDI-TOF in microbiology
- Validation results
- MALDI-TOF applications



Validation

- Published studies show:
  - 84-94% isolates identified to species level
  - 95-99% isolates identified to genus level
  - 95% concordance with Phoenix

Known issues

- Viridans type streptococci are misidentified as *S.pneumoniae*
- *Shigella spp.* cannot be distinguish from *E.coli.*
- *Stenotrophomonas maltophilia* have very similar spectra to some *Pseudomonas spp.*
- *Klebsiella oxytoca/Raoultella ornitholytica* are closely related and give similar spectra.

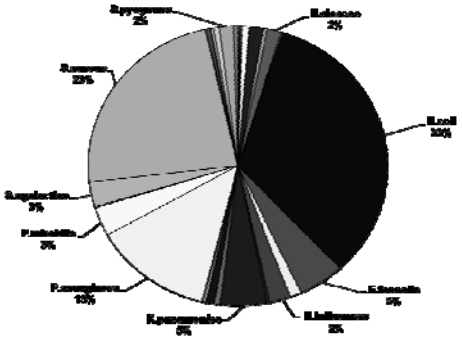
Validation

- ~40 isolates from the American Type Culture Collection were initially tested.
- 766 isolates obtained from clinical specimens were tested using MALDI-TOF MS and the identification compared to current methods e.g.. BD Phoenix.
- Isolates giving insufficient identification were retested following an extraction step.
  - **70 % Formic acid/Acetonitrile [1:1]**
- Discrepant results were retested and if not resolved underwent16S rRNA sequencing.

Validation

Total isolates	766	
	-32	Conventional ID not done
	-7	Sample errors
	-32	Extraction needed
Total for analysis	**696**	**100.0**
1. Species match directly	685	98.4
2. Genus match, acceptable	4	0.6
3. 1+2	689	99.0
4. No genus match, acceptable	4	0.6
5. Unacceptable genus match	3	0.4

Organisms tested



Genus matches – clinically acceptable ID (4)

Phoenix ID	MALDI-TOF ID
S.haemolyticus	*S.lugdenensis*
C.brakkii	*C.freundii*
S.capitis	*S.epidermidis*
E.cloacae	*E.aerogenes*

No genus match – clinically acceptable ID (4)

Phoenix ID	MALDI-TOF ID
C.farmeri	*E.cloacae*
P.mirabilis	*M.morganii*
P.rettgeri	*M.morganii*
C.freundii	*E.cloacae*

For repeat Phoenix and MALDI from saved isolates

Genus matches – unacceptable ID (3)

Phoenix ID	MALDI-TOF ID
S.aureus	*S.epidermidis*
S.aureus	*S.capitis*
C.sporogenes	*C.perfringens*

For repeat Phoenix and MALDI from saved isolates

Validation conclusion

- 99% species or acceptable genus match
- Valid for routine colony ID

Outline

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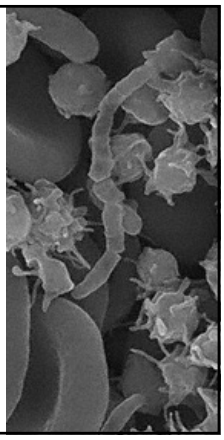
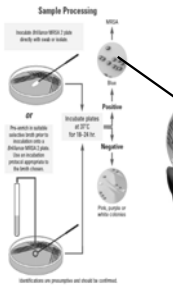


MALDI-TOF direct from positive blood culture bottles

Direct BC results

- 60-80% acceptable ID directly
- Same problem with α-haem Streps, etc.
- Difficulty with mixed cultures
  - being addressed with next update
- Validation currently being completed

Figure 1 Illustration from Oxoid showing how the MRSA selective agar should be used.



Saving 24hrs

Figure 2 : MRSA Brilliance agar and the blue colonies of MRSA

Direct ID from Urine

Saving 24hrs

- Bone & Joint

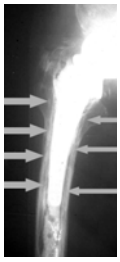
PJI - Diagnosis

- Pre-op
  - Heat, sinus, fevers
  - CRP/ESR
  - Imaging
  - Aspiration
  - Biopsy

- Post op
  - Histology
  - Cultures
  - PCR
  - CRP/ESR
  - Wound healing

PJI - surgical samples

- 5 or more samples
  - 2 indistinguishable: probable
  - 3 or more: definite infection
- Histology correlates well
- Prolonged cultures
- +/-PCR
- +/-Sonication of implant



Atkins, J Clin Micro 1998; 36(10): 2932

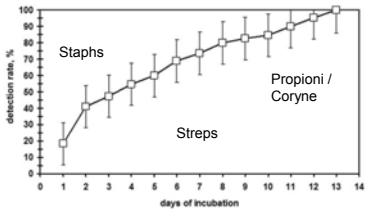
Prolonged culture required

- Latency phase of previously non-replicating bacteria
- Slowly replicating bacteria
- Problems
  - Delays for patient diagnosis
  - Contamination on sub-culturing
  - RCM may look falsely cloudy – negative on subculture

Prolonged Bacterial Culture to Identify Late Periprosthetic Joint Infection: A Promising Strategy

Peter Schäfer,[1] Bernd Fink,[2] Dieter Sandow,[1] Andreas Margull,[1] Irina Berger,[3] and Lars Frommelt[4]

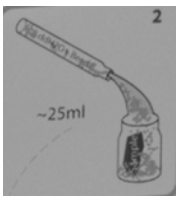
[1]Ambulatory Healthcare Center, Labor Ludwigsburg, Ludwigsburg, [2]Clinic of Joint Replacement, General and Rheumatic Orthopaedics, Orthopaedic Clinic Markgröningen, Markgröningen, [3]Institute of Pathology, Klinikum Kassel, Kassel, and [4]ENDO-Klinik, Hamburg, Germany

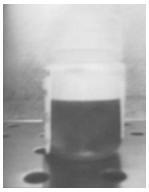


Significant positive cultures up to day 14.

Schafer CID 2007; 47: 1403

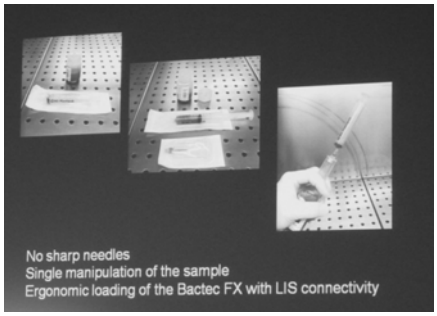
Homogenisation with Ballotini beads

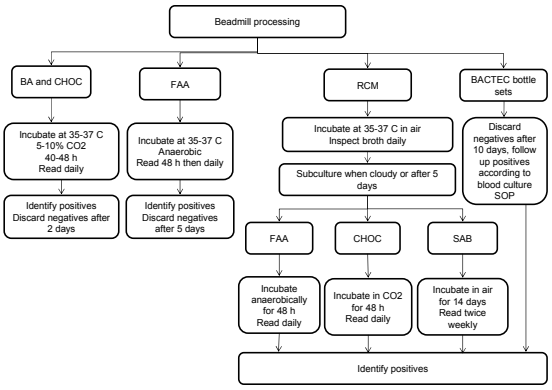




Gram stain only if grossly purulent
Add sterile mixture of beads and saline
Use vortex or orbital shaker to homogenise into a suspension for culture

Bactec innoculation





Culture follow up

- Gram positive isolates
  - MALDI identification
  - Phoenix sensitivities
  - Rifampicin disc
  - ID and sensitivities for each sample
  - Mixed CNS may be significant – might be able to cross-reference these for same patient

Culture follow up

- Gram negative isolates
  - MALDI identification
  - Phoenix sensitivities
  - Additional sensitivities
  - ID and sensitivities for each sample
  - Mixed infections may be significant – might be able to cross-reference these for same patient

Oxford Bactec study

- 10 months: 141 cases, 20 infections (14%)
  - 9 on primary culture plates
  - 11 more on RCM and Bactec
  - Sensitivity/specificity same for RCM and Bactec
  - Need to use both Bactec bottles
  - Bactec faster than RCM
  - Oxford planning to stop RCM

Hughes, CMI, September 2011

The usual suspects

- Coagulase negative staphylococci
- *S. aureus*
- Corynebacterium species

Skin flora

- Streptococci
- Gram negatives
- Candida species
- Anaerobes
- Mixed infections

MALDI-TOF
Yeast Identification

191 yeasts analysed

- 96 % (184/191) of yeasts were identified by MALDI-TOF.
  - Conventional formic acid extraction – 15 minutes.
  - Using a log score >1.9 for species ID.
  - Repeating extraction where 'no peaks found' or low log score.
- 4% (7/191) not identified by MALDI-TOF despite good spectra
  - ITS rRNA sequencing demonstrated that the biochemical test had misidentified all 4% (7/191).

MALDI-TOF & Biotyper 3.1™ database

- Identifies >95% of clinical yeast isolates correctly.
- Does not misidentify yeast isolates.
- Is more rapid and accurate than biochemical testing.

MALDI-TOF
Mould Identification

Preliminary study 42 moulds

- 74% (31/42) moulds identified correctly by MALDI-TOF.
- 14% (6/42) moulds misidentified compared with ITS rRNA sequencing.
- 12% (5/42) gave no ID despite good spectra.

- Preliminary data is encouraging
- Potential role in centres with a lack of mycology experience
- Further database expansion and interrogation is required

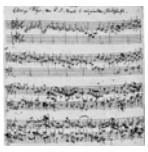
Mycobacteria

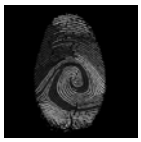
- Currently working on Ensuring that the mycobacteria are killed during preparation
- Direct ID from MIGT

Future applications

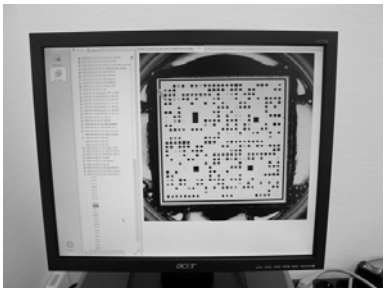
- Resistance testing
- Strain typing
- Virulence factors
- Micro Array
  - Blood culture
  - Urines
  - Fluids

Multiplex arrays for Genotyping Complex Information





Automated Analysis of the Array



Antimicrobial Resistance Markers +ve & -ve

- A system of DNA micro-arrays for the simultaneous detection of multiple genes in bacteria including sub-typing, antimicrobial resistance genes, toxin- and virulence genes.
- This genetic fingerprinting can support infection control in hospitals, communities and reference labs. Rapid strain identification helps to trace outbreaks or epidemiological changes without sequencing.

β-lactamase Detection

- **An automated evaluation algorithm for the MALDI-TOF MS based functional β-lactamase assay**
  - resistance mechanism of *Enterobacteriacae* is the expression of ß-lactamases.
  - These enzymes are able to inactivate ß-lactam antibiotics by hydrolyzing the ß-lactam ring
  - The hydrolysis is characterized by a distinctive change of the molecular mass of the respective antibiotic which can be easily monitored by MALDI-TOF MS

ESβ-lactamase Detection

- Carbapenems, such as imipenem and meropenem, are often used to treat infections caused by extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria. A new class of bacterial enzymes capable of inactivating carbapenems, known as *Klebsiella pneumoniae* carbapenemases (KPCs)

Food for thought!!

- Do we need all the sensitivity testing
- Is ID sufficient
  - Trending monthly
  - Target high risk such as ITU etc
- Gram film or direct to MALDI ToF

Thank you Canada

References

- Applied and Environmental Microbiolgy, Sept. 2007, p.5692-5697
  Pathotyping Escherichia coli by using Miniaturised DNA Microarrays Muna F Anjum, Muriel Mafura, Peter Slickers, Karin Ballmer, Peter Kuhnert, Martin J Woodward and Ralf Ehricht.
- International Journal of Antimicrobial Agents 31 (2008) 440-451
  Development of a miniaturized micro-array for the rapid identification of antimicrobial resistance genes in Gram-negative bacteria Miranda Batchelor, Katie L Hopkins, Ernesto Liebana, Peter Slickers, Ralf Ehricht, Muriel Mafura, Frank Aarestrup, Dik Mevius, Felicity A Clifton-Hadley, Martin J Woodward, Rob H Davies, E John Threfall, Muna F Anjum
- Journal of Clinical Microbiology, May 2005, p.2291-2302
  Microarray-based Detection of 90 Antibiotic Resistance Genes of Gram-positive Bacteria.Vincent Perreten, Lorainne Vorlet-Fawer, Peter Slickers, Ralf Ehricht, Peter Kuhnert and Joachim Frey.
  Matrix-assisted laser-desorption/ionization BIOTYPER: experience in the routine of a University hospital E. Besse´de1,2,3, M. Angla-gre1, Y. Delagarde1, S. Sep Hieng1, A. Me´nard1,2,3 and F. Me´graud1,2,3
- CHU de Bordeaux, Ho˜pital Pellegrin, Laboratoire de Bacte´riologie, Bordeaux, 2) Universite´ Victor Segalen Bordeaux 2, Laboratoire de Bacte´riologie and 3) INSERM U853, Bordeaux, France