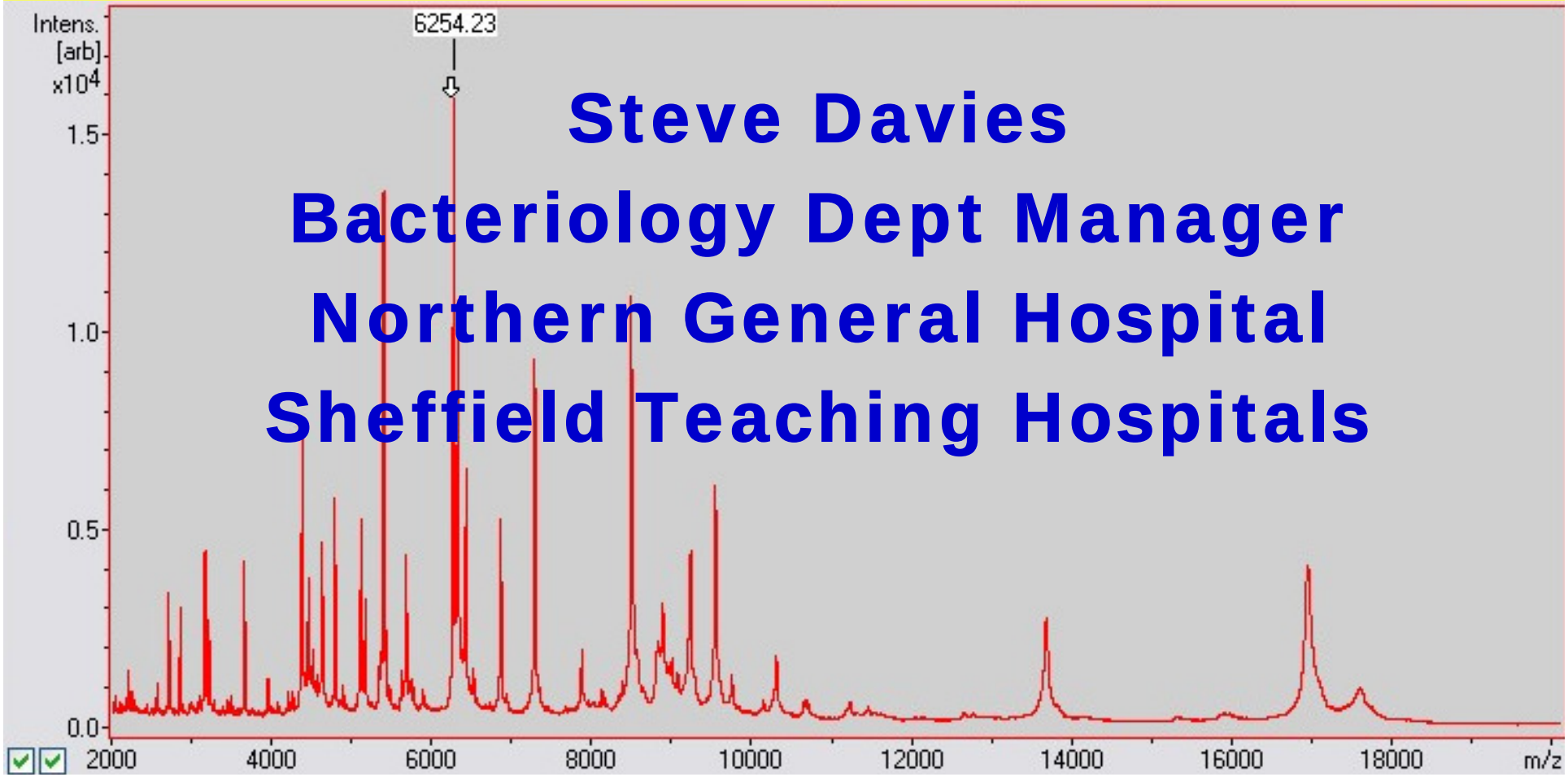
The role of MALDI-TOF in Clinical Microbiology, Including the Rapid Identification of Isolates from Positive Blood Cultures



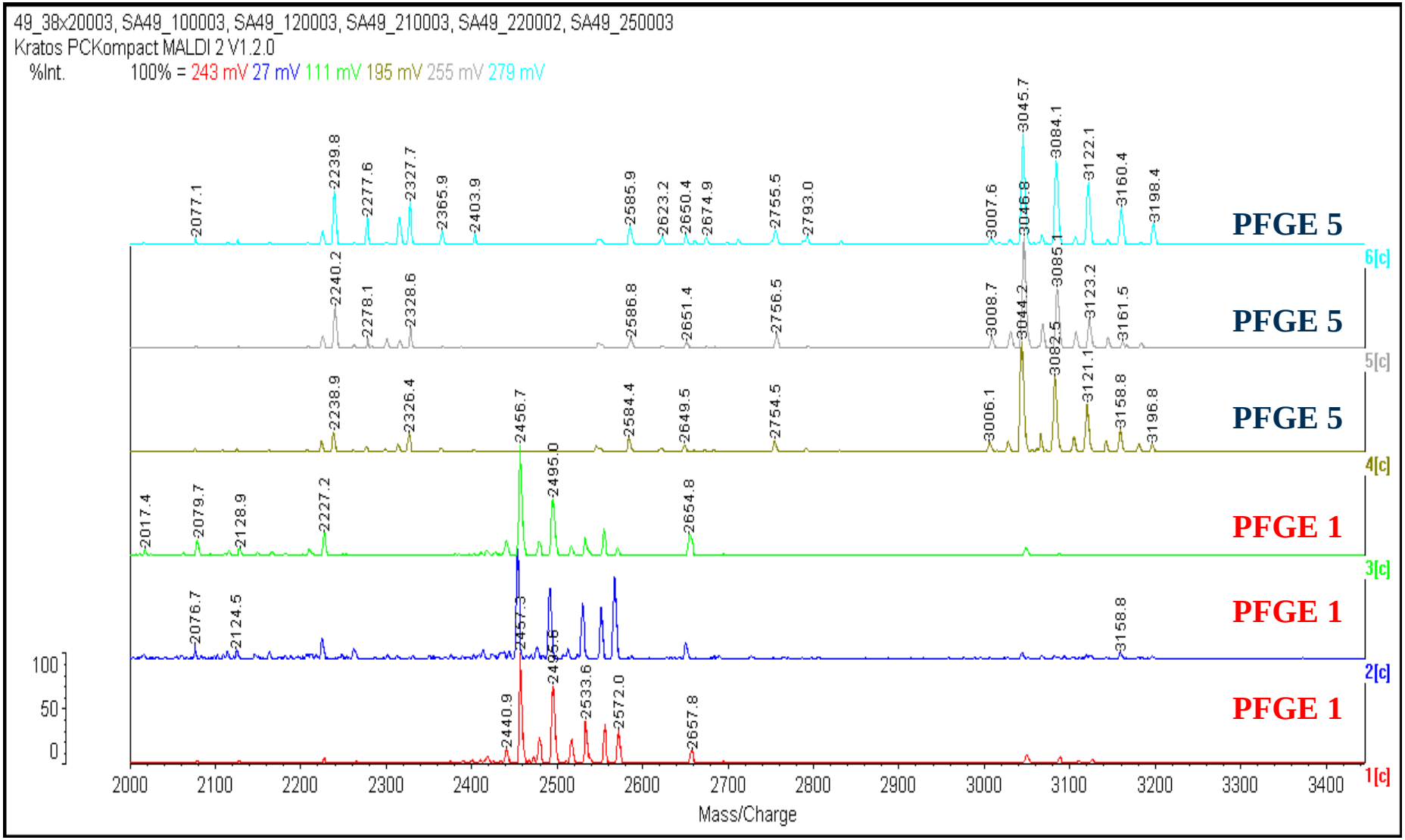
Steve Davies

Bacteriology Dept Manager

Northern General Hospital

Sheffield Teaching Hospitals

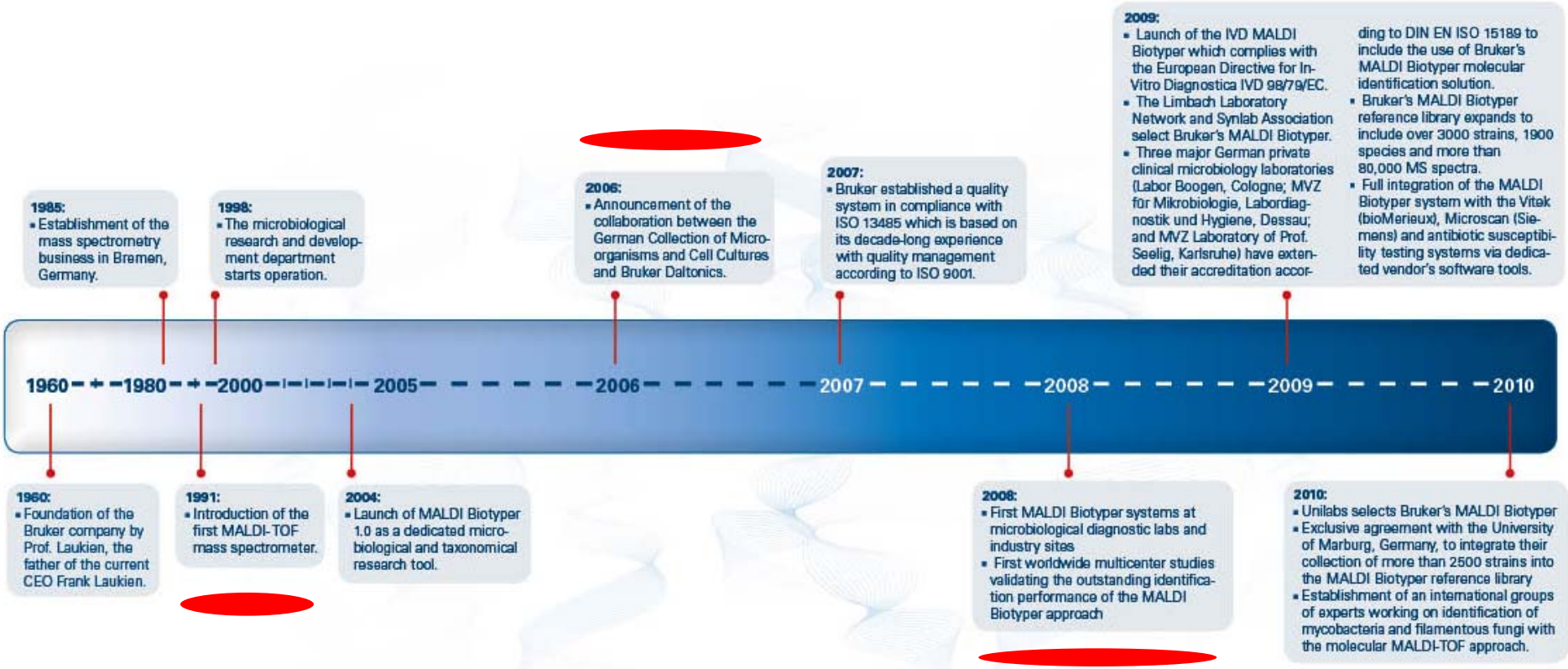
MALDI- spectra, EMRSA 15 (PFGE 5) and EMRSA 16 (PFGE 1)



The Ninth Great British Research and R&D Show and National Competition for the 2007 Westminster Awards (donated by GlaxoSmithKline at Lunchtime)

Commendation Prizes of £250 each were won by **Tom Bishop** (Edinburgh University, RS, "Restoring Blurry Photos"), **David Kelly** (Heriot-Watt University, PDRA "Identifying Depleted Appliance Trap Seals"), **Dr Katharina Mahn** (MRC & Asthma UK Centre, London, PDRA, "Asthma – the Role of Calcium Handling by Airway Smooth Muscle"), and **Pranav Somaiya** (University of Sheffield, SF/UL, "Rapid Diagnosis of MRSA).

MALDI Biotyper – a Success Story



20th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)
10.04.2010 - 13.04.2010

Sunday, April 11, 2010

16:00 - 18:00 Lecture Hall C

- **Official Symposium: MALDI-TOF in clinical microbiology**

  E. Nagy (Szeged, HU)
  J. Vila (Barcelona, ES)

Monday, April 12, 2010

13:30 - 14:30

- **Poster topic 86: MALDI-TOF**

Tuesday, April 13, 2010

09:00 - 11:00 Lecture Hall E2

- **Bruker Daltonik Symposium: Research and routine applications of MALDI-TOF mass spectrometry in microbiology**

  M. Bonten (Utrecht, NL)
  M. Kostrzewa (Bremen, DE)

13:30 - 15:30 Lecture Hall C

- **Oral Session: What do we expect from MALDI-TOF?**

  E. Nagy (Szeged, HU)
  Tbc

Total of

7 hours

Monday, April 12, 2010
13:30 - 14:30

Poster topic 86

MALDI-TOF

- **Detection of highly pathogenic bacteria by MALDI-TOF MS** P 1773

  M. Blaschitz*, L. Meidlinger, U. Sagel, G. Wewalka, F. Allerberger, A. Indra (Vienna, AT)

- **Development and evaluation of automated sample preparation for bacterial identification with MALDI-TOF MS** P 1774

  C. Lang*, O. Dubuis, E.H. Viollier (Basel, CH)

- **MALDI Biotyper, experience in routine clinical bacteriology in a university hospital** P 1775

  E. Bessede*, M. Angla-Gré, Y. Delagarde, S. Sep Hieng, A. Menard, F. Megraud (Bordeaux, FR)

- **Two-year experience with MALDI-TOF MS in a routine microbiology department of a laboratory in Germany** P 1776

  C. Boogen, M. Kostrzewa, U. Weller* (Cologne, Bremen, DE)

- **Rapid identification using MALDI-TOF MS for routine bacterial identification** P 1777

  S. Bocher, R. Abdul-Redha* (Copenhagen, DK)

- **Performance of MALDI-TOF MS for the identification of routine and difficult to identify bacterial strains isolated in a clinical microbiology laboratory** P 1778

  A. Bizzini*, K. Jaton-Ogay, C. Durussel, J. Bille, G. Greub, G. Prod'hom (Lausanne, CH)

- **Reassessment of conventional identification of clinical non-fermenting isolates excluding Pseudomonas aeruginosa and Stenotrophomonas maltophilia from cystic fibrosis patients using the MALDI-TOF system** P 1779

  A. Fernandez-Olmos*, M. García-Castillo, M.I. Morosini, A. Lamas, L. Máiz, R. Cantón on behalf of the CIBERESP

- **The experience of a 2-year application of MALDI Biotyper technique in a routine setting** P 1780

  A. Borovskaya, E. Ilina, S. Sidorenko, A. Kruglov, D. Mudrak, T. Savinova, T. Maier, M. Kostrzewa,

So why am I here?????

I. Wybo*, A. De Bel, I. Van Cauwenbergh, K. Vandoorslaer, P. Rosseel, D. Piérard, S. Lauwers (Brussels, BE)

- **Rapid identification of bacteria from positive blood culture bottles by MALDI-TOF MS fingerprinting** P 1791

  M. Christner*, H. Rohde, M. Wolters, I. Sobottka, K. Wegscheider, M. Aepfelbacher (Hamburg, DE)

- **High-speed blood culture diagnostic with MALDI-TOF MS** P 1792

  C. Litfin*, A. Sohns, A. Koch (Karlsruhe, DE)

- **Use of tubes equipped of separating gels for MALDI-TOF assisted bacterial identification in blood cultures** P 1793

  G. Prévost*, W. Moussaoui, B. Jaulhac, A. Hoffmann, B. Ludes, M. Kostrzevra, P. Riegel (Strasbourg, Bremen, FR)

- **Urinary tract pathogens direct identification from urine samples by MALDI-TOF MS** P 1794

  L. Ferreira, F. Sánchez Juanes, M. González Ávila, D. Cembrero Fuciños, A. Herrero Hernández, J.M. González-Buitrago Arriero, J.L. Muñoz Bellido* (Salamanca, ES)

- **MALDI-TOF ICMS: capability, potentiality and limits in the fast identification of Trichophyton rubrum from clinical cases occurrence in Portuguese health centres** P 1795

  L. Pereira, N. Dias*, C. Santos, N. Lima (Braga, Gandra, PT)

- **A MALDI-TOF assay for the rapid identification of Aspergillus and Candida sp. in clinical samples** P 1796

  L. Putignani*, L. Mancinelli, F. Del Chierico, M. Onori, L. Coltella, P. Bernaschi, E. Fiscarelli, M. Argentieri, L. Pansani, S. Ranno, B. Lucignano, L. Dimiziani, C. Russo, D. Menichella (Rome, Macerata, IT)

- **Identification of clinical fungi by MALDI-TOF MS: how to deal with growth-dependent variability in peak patterns** P 1797

  M. Erhard, U. Hipler, F. Seyfarth, Y. Gräser, M. Welker* (Potsdam, Jena, Berlin, DE)

- **Comparison of 4 different commercial identification methods and antibiotic susceptibility testing on clinical relevant coryneform bacteria** P 1798

  I. Geerts*, A. Smismans, R. Cartuyvels, H. De Beenhouwer, J. Verhaegen, E. Verhoye, J. Frans (Bonheiden, Hasselt, Aalst, Leuven, BE)

- **Rapid species identification and differentiation of Arcobacter, Helicobacter and Campylobacter by MALDI-TOF MS analysis and its clinical application** P 1799

  M. Alispahic*, K. Hummel, D. Jandreski-Cvetkovic, K. Nöbauer, E. Razzazi-Fazeli, M. Hess, C. Hess (Vienna, AT)

BD Diagnostics and Bruker Collaborate to Improve Microbial Identification and Antimicrobial Susceptibility Testing

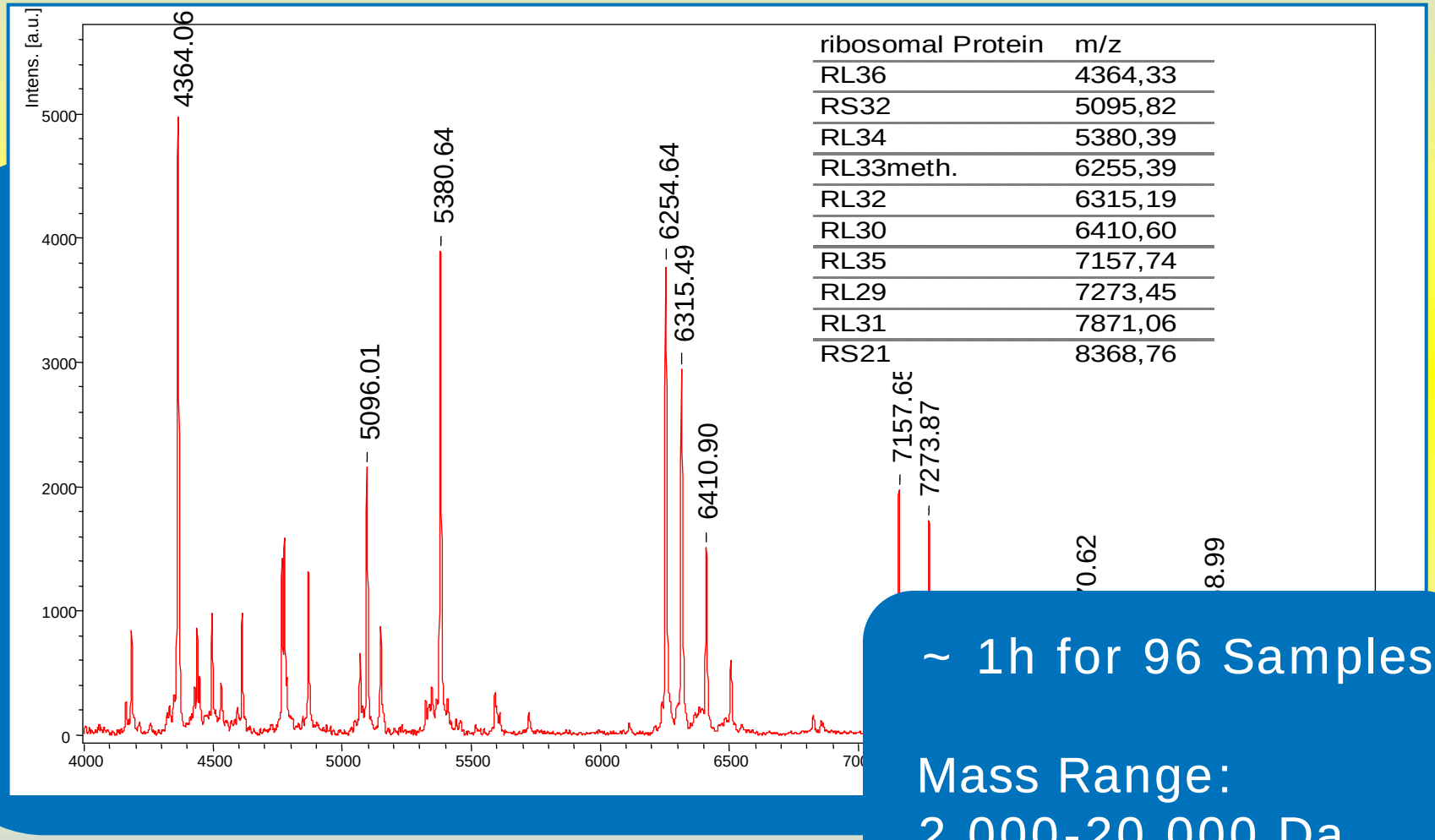
Collaboration Aims to Improve Speed, Accuracy and Efficiency in the Microbiology Laboratory

(September 29, 2010)

MALDI-TOF MS

- Matrix-assisted laser desorption ionisation – time of flight (MALDI-TOF) mass spectroscopy uses 16s ribosomal proteins
- Compares the mass peaks achieved by test strains to those of approx 3,500 known strains in the Bruker MALDI Biotyper Library
- Organism identification within 20 minutes of starting the process
- The resultant identification is meant to be robust, as it relies on high abundance proteins.

The MALDI Biotyper is Robust, as it Relies on High Abundance Proteins.



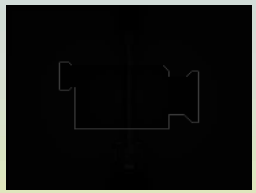
ribosomal Protein	m/z
RL36	4364,33
RS32	5095,82
RL34	5380,39
RL33meth.	6255,39
RL32	6315,19
RL30	6410,60
RL35	7157,74
RL29	7273,45
RL31	7871,06
RS21	8368,76

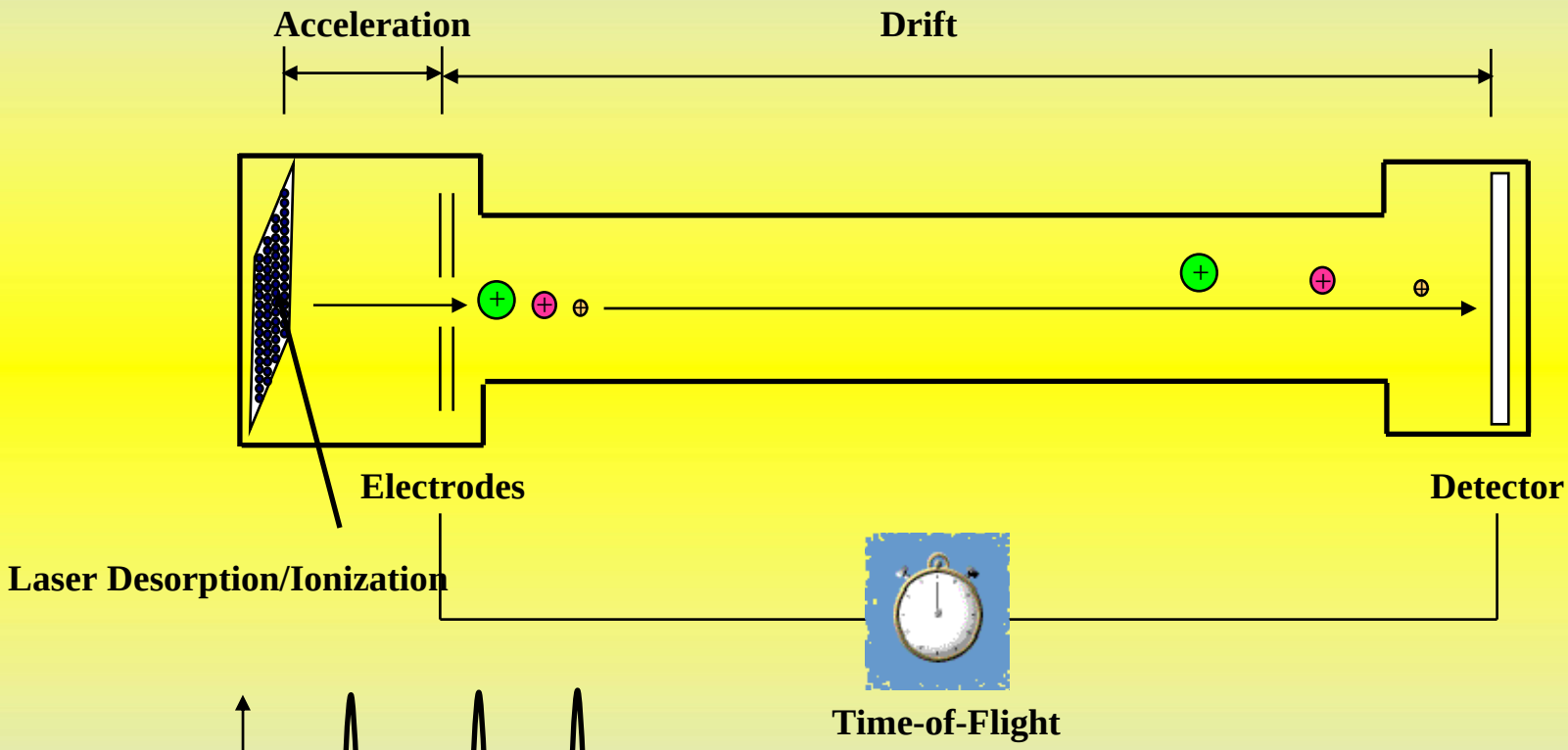
E.coli

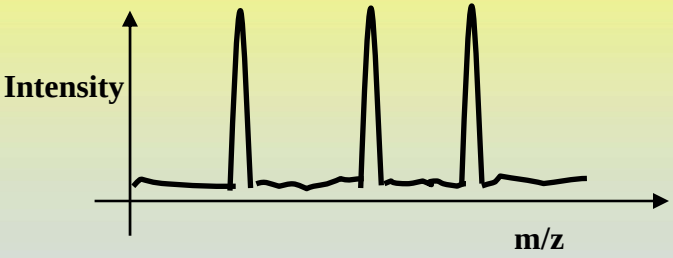
~ 1h for 96 Samples

Mass Range:
2.000-20.000 Da

MALDI-TOF Mass Spectrometry

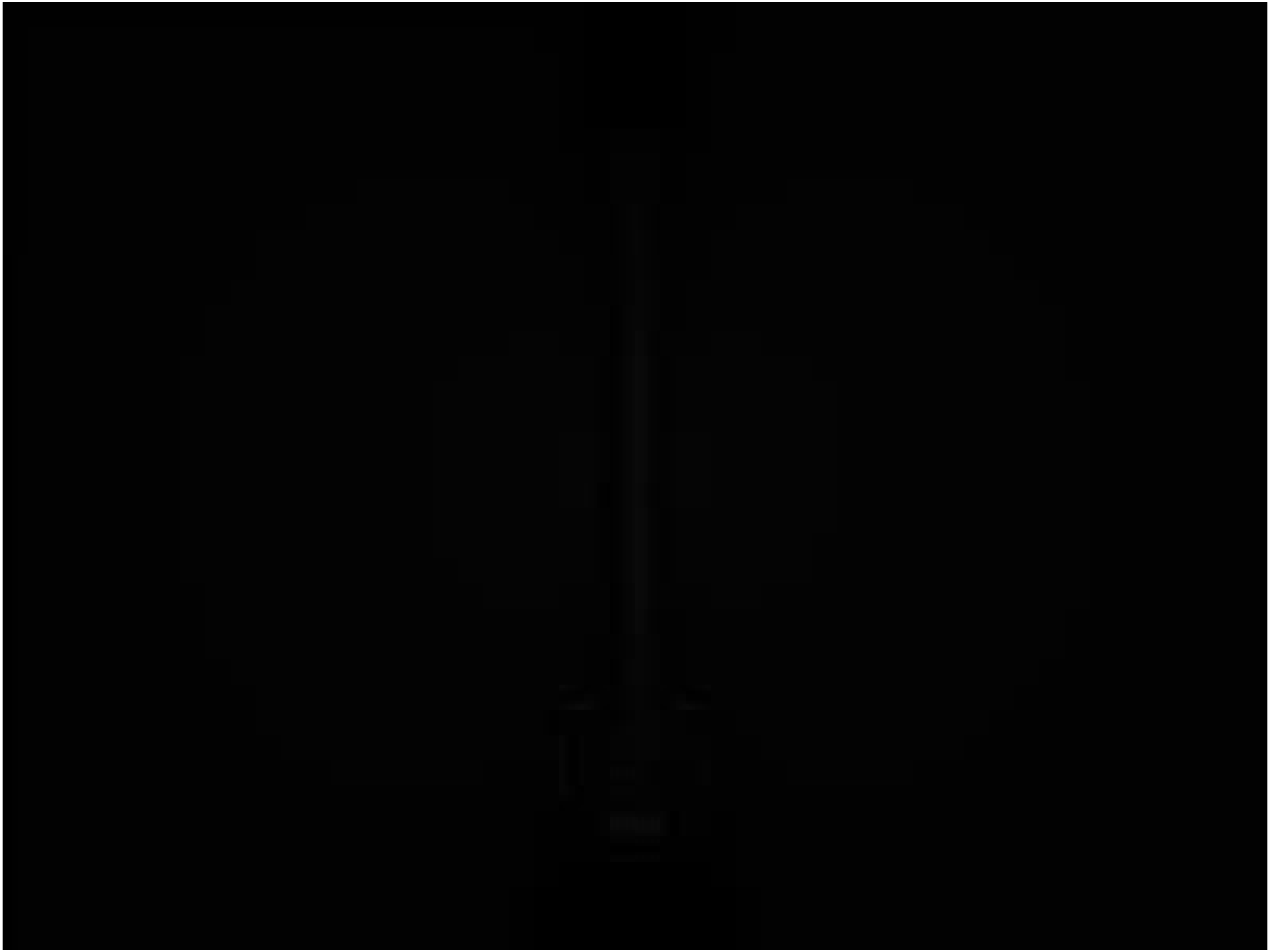


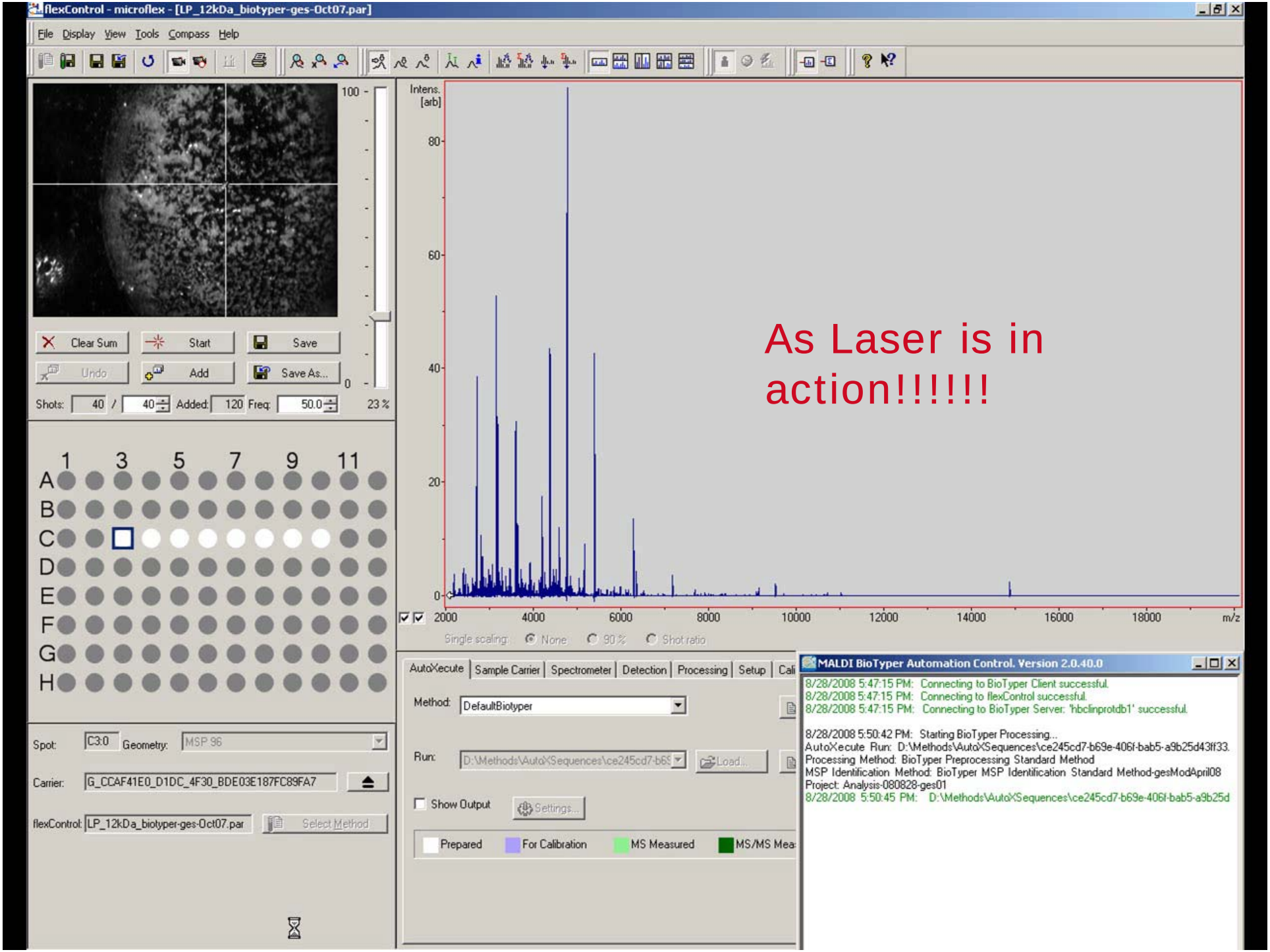




~300-500 laser shots

~15-30 sec. per sample




flexControl - microflex - [LP_12kDa_biotyper-ges-Oct07.par]
File Display View Tools Compass Help
Clear Sum
Start
Save
Undo
Add
Save As...
Shots: 40 / 40 Added: 120 Freq: 50.0
23 %
Intens. [arb]
As Laser is in action!!!!!!
m/z
Single scaling: None 90 % Shot ratio
AutoXecute
Sample Carrier
Spectrometer
Detection
Processing
Setup
Method: DefaultBiotyper
Run:
Load...
Show Output
Settings...
Prepared
For Calibration
MS Measured
MALDI BioTyper Automation Control. Version 2.0.40.0
8/28/2008 5:47:15 PM: Connecting to BioTyper Client successful.
8/28/2008 5:47:15 PM: Connecting to flexControl successful.
8/28/2008 5:47:15 PM: Connecting to BioTyper Server: 'hbclinprotdb1' successful.
8/28/2008 5:50:42 PM: Starting BioTyper Processing...
AutoXecute Run: D:\Methods\AutoXSequences\ce245cd7-b69e-406f-bab5-a9b25d43ff33.
Processing Method: BioTyper Preprocessing Standard Method
MSP Identification Method: BioTyper MSP Identification Standard Method-gesModApril08
Project: Analysis-080828-ges01
8/28/2008 5:50:45 PM: D:\Methods\AutoXSequences\ce245cd7-b69e-406f-bab5-a9b25d
Spot: C3:0 Geometry: MSP 96
Carrier: G_CCAF41E0_D1DC_4F30_BDE03E187FC89FA7
flexControl: LP_12kDa_biotyper-ges-Oct07.par
Select Method

Check Results on Automated Biotyper

Results - Colour Coded Identification & Consistency Categories

Meaning of Score Values

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

Meaning of Consistency Categories (A - C)

Category	Description
A	**Species Consistency:** The best match was classified as 'green' (see above). Further 'green' matches are of the same species as the first one. Further 'yellow' matches are at least of the same genus as the first one.
B	**Genus Consistency:** The best match was classified as 'green' or 'yellow' (see above). Further 'green' or 'yellow' matches have at least the same genus as the first one. The conditions of species consistency are not fulfilled.
C	**No Consistency:** Neither species nor genus consistency (Please check for synonyms of names or mircobial mixture).

Samples Results – Top 2 Matches Species Only

B1 (+++)(A)	mv4072	Escherichia coli	2.347	Escherichia coli	2.194
B10 (++)(C)	mv4170_2	Citrobacter amalonaticus	2.286	Citrobacter amalonaticus	2.266
B11 (+++)(C)	4171	Enterobacter cloacae	2.473	Enterobacter cloacae	2.31
B12 (+++)(A)	4177	Acinetobacter baumannii	2.534	Acinetobacter baumannii	2.433
B2 (++)(B)	mv4126_2	Escherichia coli	2.218	Escherichia coli	2.202
B4 (+++)(A)	mv4131_2	Enterococcus faecalis	2.414	Enterococcus faecalis	2.368
B5 (+++)(A)	mv4131_3	Enterococcus faecium	2.604	Enterococcus faecium	2.566
B6 (+++)(A)	mv4139_2	Staphylococcus aureus	2.385	Staphylococcus aureus	2.301
B7 (+++)(B)	mv4155_1	Escherichia coli	2.301	Escherichia coli	2.106
B8 (+++)(B)	mv4155_2	Escherichia coli	2.387	Escherichia coli	2.363

Sample B1"+++/A" – Top 10 Matches

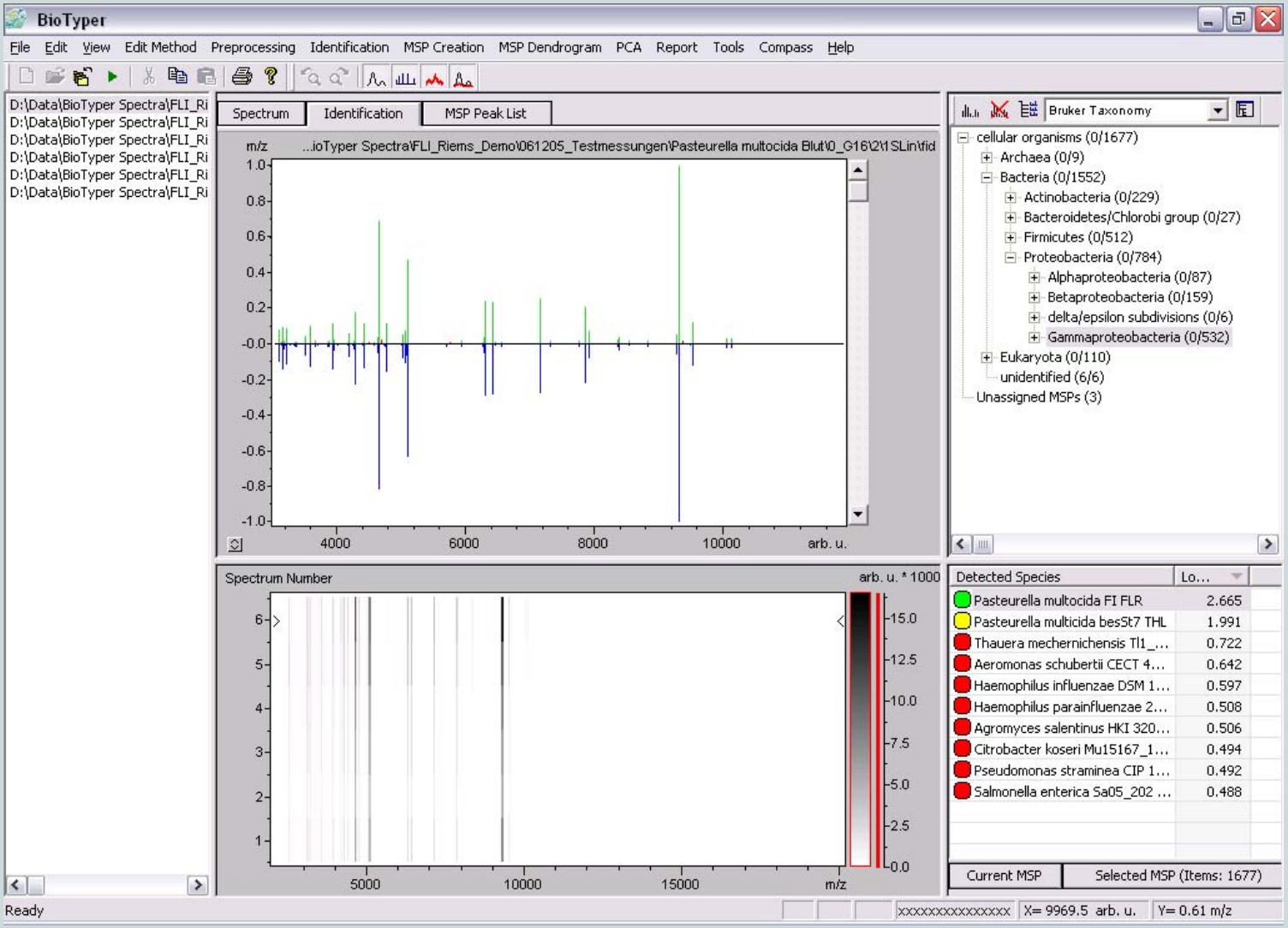
Click here!!

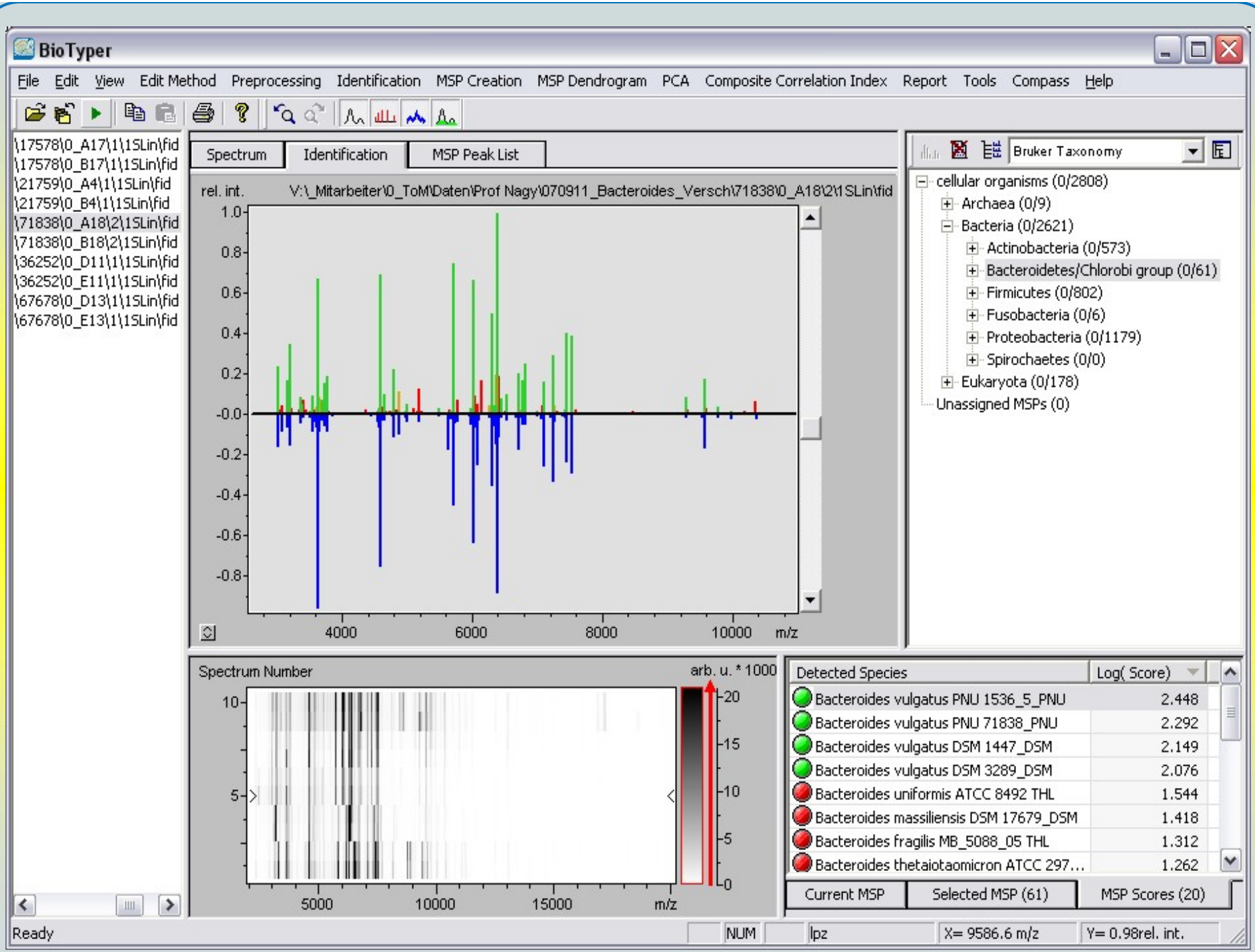
B1 (+++) (A)	mv4072	Escherichia coli	2.347	Escherichia coli	2.194

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Escherichia coli Nissl VML	2.347	562
2 (++)	Escherichia coli MB11464-1_CHB	2.194	562
3 (++)	Escherichia coli B421T_DSM 30083_UFL	2.144	562
4 (++)	Escherichia coli DSM 30083 HAM	2.096	562
5 (++)	Escherichia coli DH5alpha BRL	2.082	562
6 (++)	Escherichia coli ATCC 35218_CHB	2.006	562
7 (+)	Escherichia coli ATCC 25922_CHB	1.984	562
8 (+)	Escherichia coli ATCC 25922 THL	1.97	562
9 (+)	Escherichia coli ESBL EA RSS 1528T_CHB	1.777	562
10 (+)	Escherichia fergusonii DSM 13698 HAM	1.752	564

MALDI Biotyper - Product

Software GUI




BioTyper
File Edit View Edit Method Preprocessing Identification MSP Creation MSP Dendrogram PCA Composite Correlation Index Report Tools Compass Help
\17578\0_A17\1\1SLin\fid
\17578\0_B17\1\1SLin\fid
\21759\0_A4\1\1SLin\fid
\21759\0_B4\1\1SLin\fid
\71838\0_A18\2\1SLin\fid
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\67678\0_D13\1\1SLin\fid
\67678\0_E13\1\1SLin\fid
Spectrum
Identification
MSP Peak List
rel. int.
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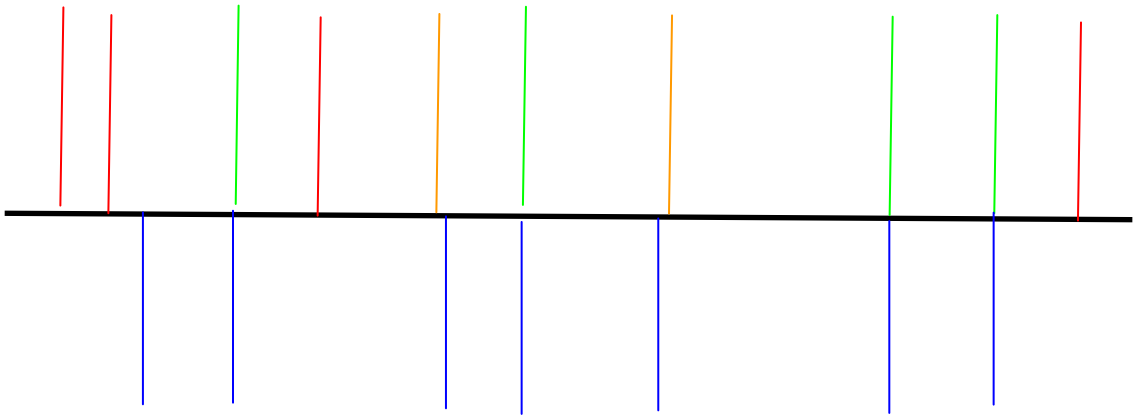
1.0
0.8
0.6
0.4
0.2
-0.0
-0.2
-0.4
-0.6
-0.8
4000
6000
8000
10000
m/z
Bruker Taxonomy
cellular organisms (0/2808)
Archaea (0/9)
Bacteria (0/2621)
Actinobacteria (0/573)
Bacteroidetes/Chlorobi group (0/61)
Firmicutes (0/802)
Fusobacteria (0/6)
Proteobacteria (0/1179)
Spirochaetes (0/0)
Eukaryota (0/178)
Unassigned MSPs (0)
Spectrum Number
arb. u. * 1000
10
5
20
15
10
5
0
5000
10000
15000
m/z
Detected Species
Log(Score)
Bacteroides vulgatus PNU 1536_5_PNU 2.448
Bacteroides vulgatus PNU 71838_PNU 2.292
Bacteroides vulgatus DSM 1447_DSM 2.149
Bacteroides vulgatus DSM 3289_DSM 2.076
Bacteroides uniformis ATCC 8492 THL 1.544
Bacteroides massiliensis DSM 17679_DSM 1.418
Bacteroides fragilis MB_5088_05 THL 1.312
Bacteroides thetaiotaomicron ATCC 297... 1.262
Current MSP
Selected MSP (61)
MSP Scores (20)
Ready
NUM
lpz
X= 9586.6 m/z
Y= 0.98rel. int.

Calculation uses Multiplies % of peaks present and Intensity of those peaks



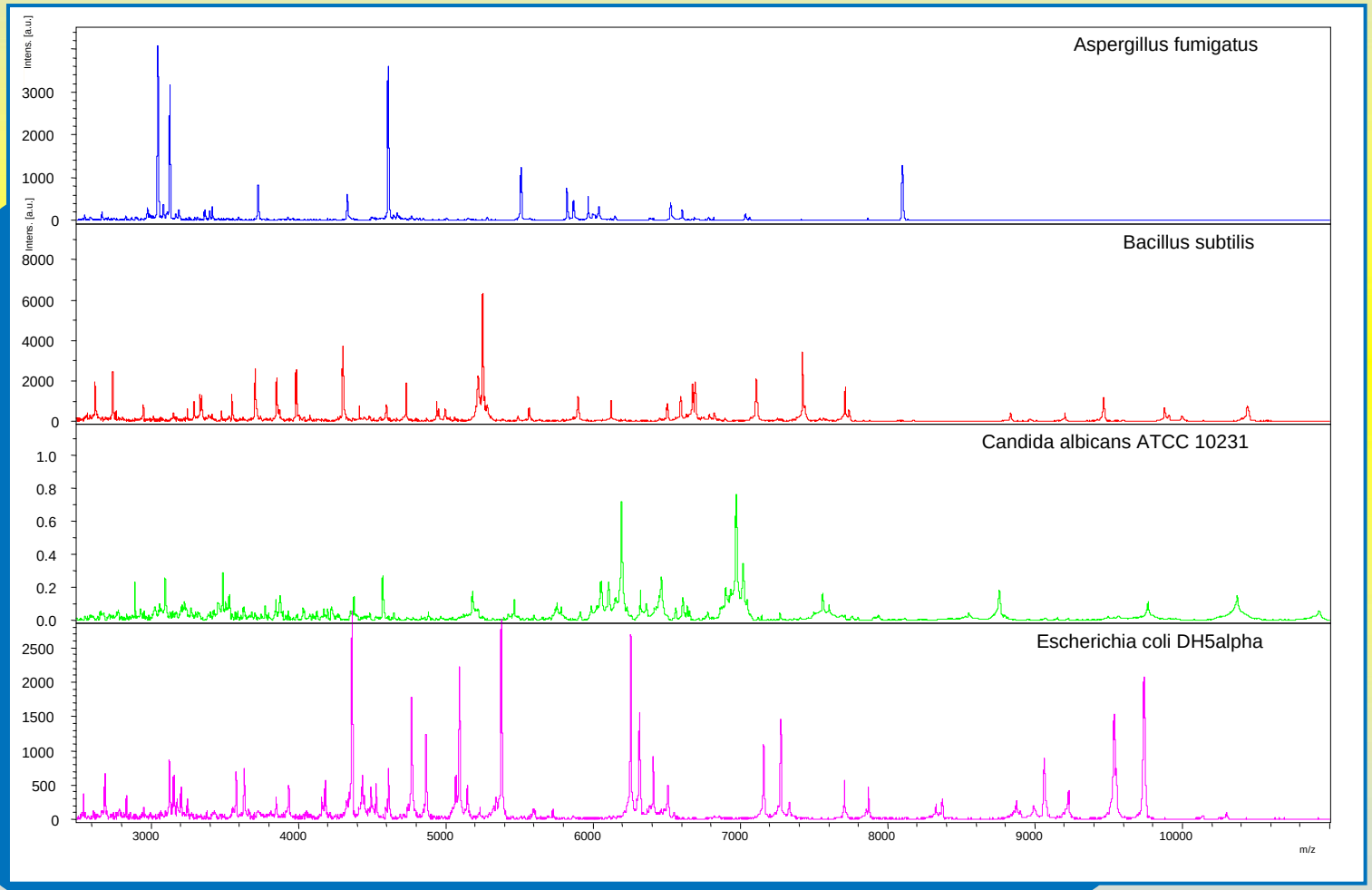
Maximum value 1000

= Log_{10} (1000) = 3.00

Log_{10} (200) = 2.30 (Acceptable species ID)

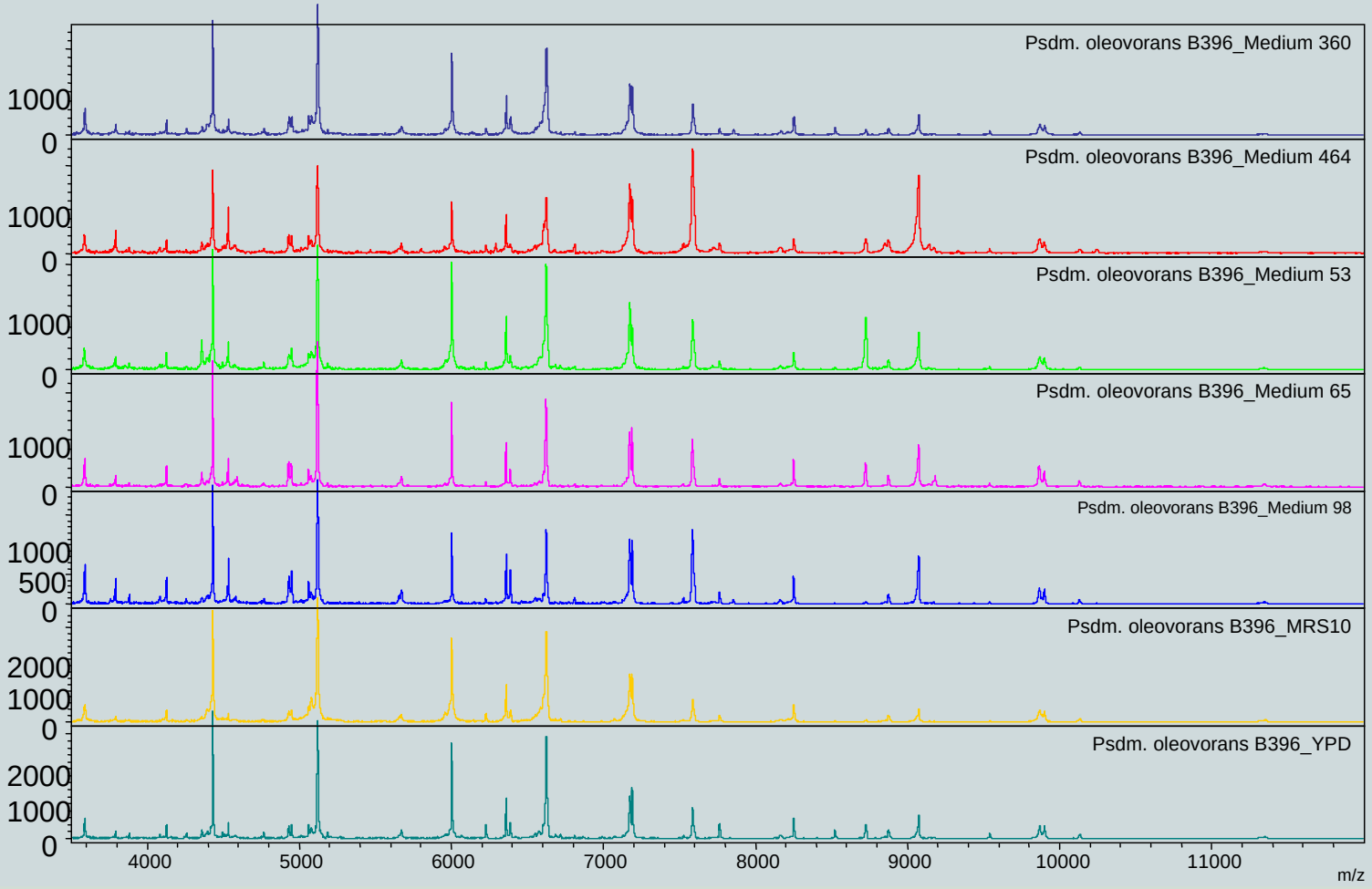
Broad Applicability of MALDI-TOF MS Profiling

Filamentous fungi, yeast, gram+ and gram- bacteria



Low influence of culture conditions

Pseudomonas oleovorans grown on different media



Three Crucial Stages

Preparation of the Organic Solvent (OS)

The composition of OS is 50% acetonitrile (AN) / 2.5% tri-fluoroacetic-acid (TFA)

The **OS** is used in making up the Matrix and the bacterial test standard (BTS)

COSHH and Risk Assessments

α-Cyano-4-Hydroxycinnamic Acid portioned (HCCA matrix)

Add 250 µl "OS" to one tube of "HCCA matrix portioned" and vortex until all matrix crystals are completely dissolved, this may take several minutes but is important to completely dissolve.

Prepared matrix MUST be stored in the **dark** at room temperature and can be viable for up to 2 weeks (“best before”).

Role of Matrix

To break open cell wall, crystallises proteins within seconds.

Needs to be in solution with correct amount of AN (No Pre-Crystals!)

Bacterial Test Standard (BTS)

Using the Bruker BTS portioned sample add 50µl of Organic Solvent (OS) to the pellet and dissolve by Pipetting up and down for at least 20 times.

Allow the BTS to stand at room temperature for 5 minutes and then repeat Pipetting up and down for at least 20 times

BTS is has a combination of 6 *E coli* peaks and a RNAse A and Myoglobin to give a couple of higher mass peaks

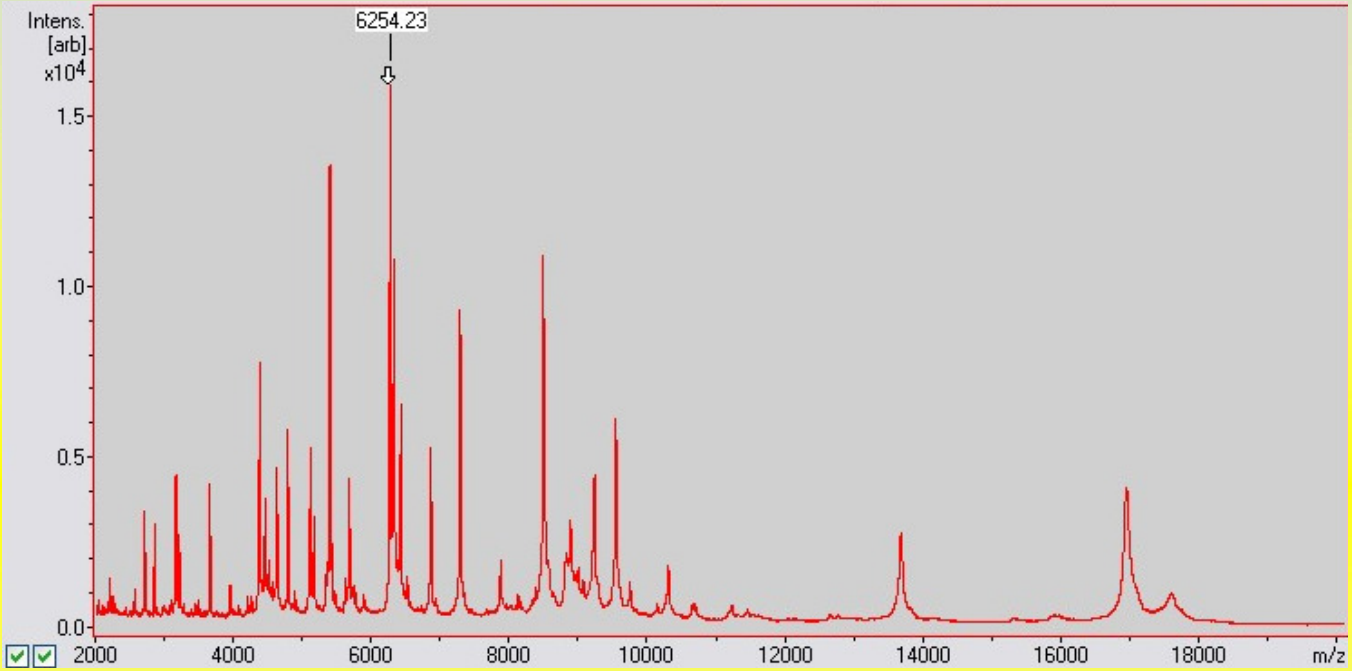
Role of BTS

Ensure spectrum is consistent

To recalibrate weekly

Use any standard organism as daily control!

MALDI Biotyper Calibration Procedure



Name	Ref. Mass/Da	Cur. Mass/Da	Err/ppm	Err/Da
✓ RL36	4365.30000	4363.53095	-234	-1.01985
✓ RS22	5096.80000	5096.01431	69	0.35027
✓ RL34	5381.40000	5380.61863	93	0.50081
✓ RL33meth	6255.40000	6254.23121	87	0.54565
✓ RL29	7274.50000	7272.71226	54	0.39570
✓ RS19	10300.10000	10296.84751	8	0.07971
✓ RNAse A	13683.20000	13676.63933	-175	-2.38898
✓ Myoglobin	16952.30000	16949.28910	91	1.53693

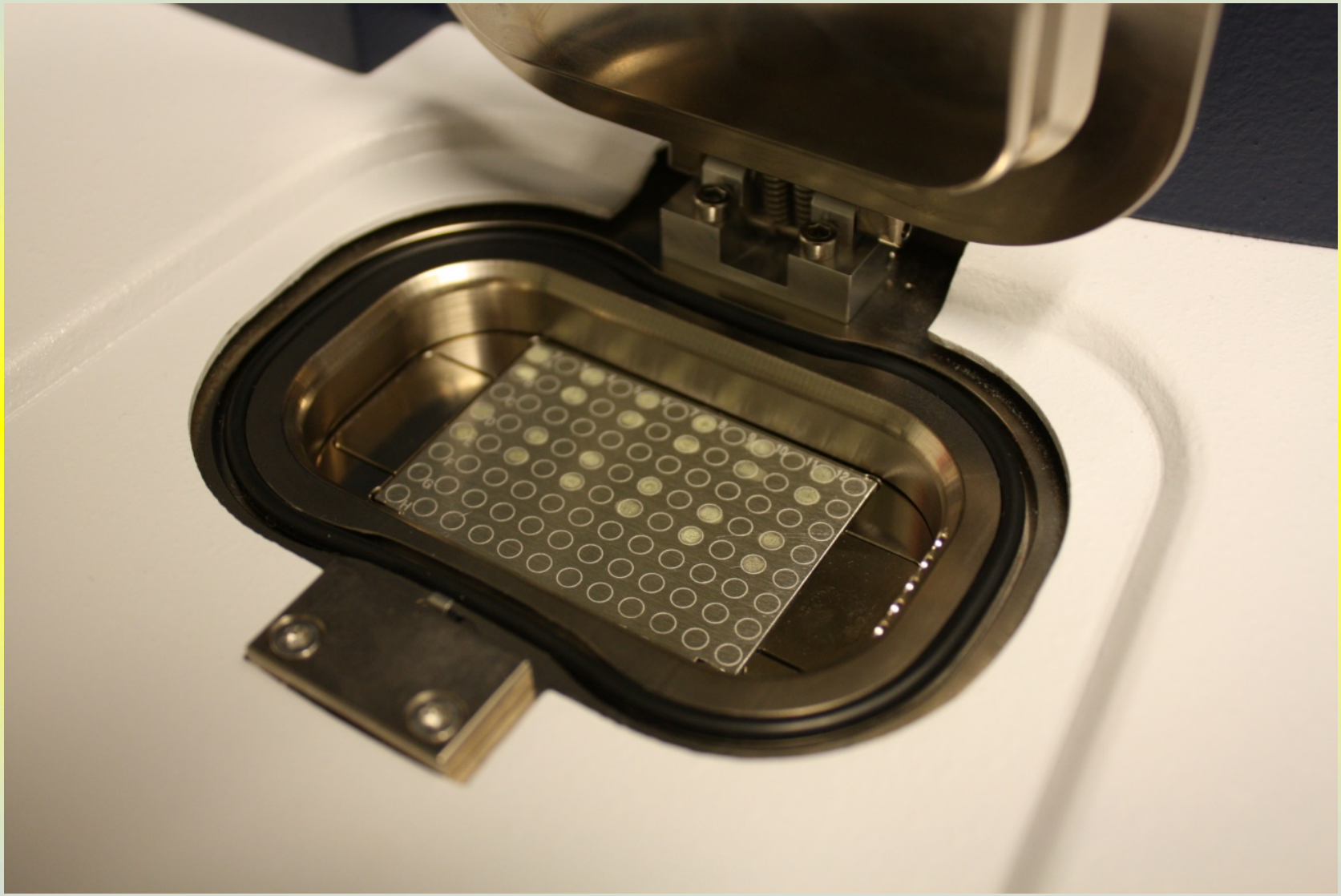
Sample preparation

Direct Smear

- Pick colony
- Smear onto target
- Allow to dry
- Add matrix
- Allow to dry
- Analyse

Extraction

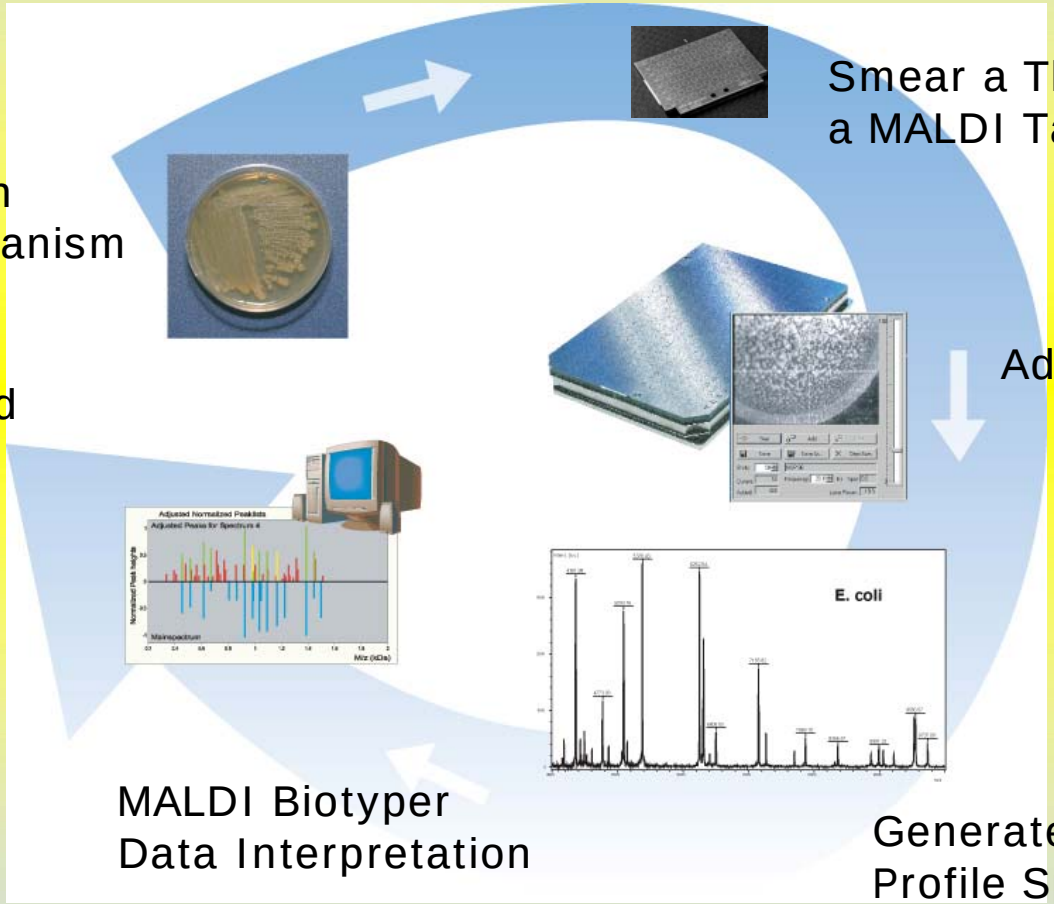
- Pick colony
- Resuspend
- Add ethanol
- **Inactivation / storage / shipment**
- Add formic acid and ACN
- Centrifuge
- Pipette supernatent onto target
- Allow to dry
- Add matrix
- Allow to dry
- Analyse



MALDI Biotyper

Workflow

Select a Colony



Smear a Thin-Layer onto a MALDI Target Plate

Add MALDI Matrix

Generate MALDI-TOF Profile Spectrum

MALDI Biotyper Data Interpretation

Identified Species

Unknown Microorganism

Consumable Cost/test – 5–10p

MALDI Biotyper – Customer Evaluation Studies

Clinical Routine 2007

Bacterial Group	N	Accuray (%)	
		Species	Genus
Enterobacteriaceae	262	96	100
Nonfermenters	63	79	100
Pseudomonas aeruginosa	33	100	100
Staphylococci	116	98	100
Enterococci	31	100	100
Streptococci	21	100	100
others	42	95	100
total	**535**	**95**	**100**

LABOR LIMBACH
HEIDELBERG

Ludwig Maximilians Universität München — LMU

	identical results	MALDI superior	biochemical test superior	total No.
enterobacteriaceae	196 (98 %)	2 (1 %)	2 (1 %)	200
non-fermenting gramnegative rods	192 (96 %)	6 (3 %)	2 (1 %)	200
staphylococci	80 (100 %)	0	0	80
streptococci	73 (91%)	5 (6 %)	2 (3 %)	80
grampositive rods	24 (60%)	2 (5 %)	14 (35 %)	40

ECCMID 2008, U. Eigner et. al. & S. Schubert

NGH Initial Testing

Candida species	Number
C. Albicans	3
C. parapsilosis	3
C. glabrata	8
C. krusei	2
C. tropicalis	1
C. lusitaniae	1
C. guillimondii	1
Sacchromyces cerevisiae	1

(Also confirmed Susceptibility on Vitek 2)

Now introduced Cross City

Many other routine isolates (both G+ve and G-ve) – All matched conventional or reference laboratory id results.

HACEK organisms – Identified all correctly

Vibrio albensis

Interesting Cases

Salmonellas on Christmas eve

Unusual Gram Pos Rods –

Turicella otitidis (From ear)

Turicella otitidis is a non-fermenting Gram-positive bacillus isolated almost exclusively from ear exudates. Its significance in acute or chronic otitis media is controversial

Corynebacterium macginleyi (eye swab)

Fifteen strains of ***Corynebacterium macginleyi*** were exclusively isolated from conjunctival swabs of patients with either conjunctivitis or corneal ulcers

GITU patients with possible Coliform/Steno/Acino (Co-trimoxazole or Meropenem)

Coliform on TKR awaiting ID. MALDI – *Enterobacter cloacae* and patient immediately switched to Meropenem instead of Pip/Tazo. Patient went home after couple of days on Ciprofloxacin. Savings in Bed Costs for Hospital??

Restrictions of MALDI Biotyper Identification Status 06/09

- **Shigella** is not included in the MALDI Biotyper database as it has to be considered as a part of the ***E. coli*** species phylogenetically, and accordingly gives no different pattern.
- ***Streptococcus pneumoniae*** identification, a second test has to be used for confirmation. As S. pneumoniae is very closely related to the S. mitis group, there might occur misidentifications, mostly with low ID scores. 16S rDNA sequencing is not sufficient for differentiation, too! From our current observations, no false ID from a *S. pneumoniae* as a S. mitis-group member does occur.
- ***Bordetella pertussis*** and ***Bordetella bronchioseptica*** are closely related and show very similar pattern.
- ***Stenotrophomonas maltophila***: three “Pseudomonas” species have to be considered as very closely related to Stenotrophomonas maltophila and accordingly may appear as “mis”identification result: *Ps. hibiscola*, *Ps. geniculata*, and *Ps. beteli*. Most biochemical tests will identify all these microorganisms as “S. maltophila”.
- A couple more!

Rapid identification of microorganisms using MALDI Biotyper (Bruker-Daltonics) MALDI-TOF mass spectrometry system

Eugene Rees[1], Stuart Johnston[1], Khalid El-Bouri[1], and Dietrich Mack[1,2]

[1]Bacteriology, Singleton Hospital, Microbiology Swansea A.B.M Trust, Swansea, [2]Medical Microbiology and Infectious Diseases, Institute of Life Sciences, School of Medicine, Swansea University, Swansea

1,775 sequential, clinically isolated organisms identified by conventional methods, were investigated using a MALDI-TOF system MALDI Biotyper (Bruker Daltonics, Germany). Gram-negative bacteria were identified by either API20E, API20NE or BD Phoenix.

Conclusion – MALDI Biotyper is a reliable system for identification of clinical microorganisms, is extremely fast compared to conventional methods, and requires significantly less staff time and negligible consumable costs than conventional method

Water based Blood Culture Method!

Figure 1	Direct blood culture		
	Acceptable ID	No reliable ID[1]	No Spectrum found
O2 Bottle	7	5	6
AnO2 Bottle	13	5	8
Paediatric	2	0	1
Organisms	Number		
E coli	8		
Serratia marscesens	1		
Salmonella sp	1		
Staph aureus	4		
Staph epidermidis[2]	5		
Strep agalactiae	2		

Comments

There is a definite potential for rapid identification of organisms from positive blood cultures. In our experience, just over half the bottles tested give a positive result

Blood Culture Results - Swansea

Organism in blood culture	1ml	5ml	All
S. aureus	3	7	10
S. epidermidis	6	6	12
S. haemolyticus	0	2	2
S. capitis	0	1	1
S. hominis	1	1	2
S. simulans	0	1	1
E. coli	12	12	24
K. pneumoniae	1	1	2
K. oxytoca	1	3	4
P. mirabilis	0	3	3
Serratia marcescens	1	2	3
Serratia sp	0	1	1
Acinetobacter sp	1	0	1
S. maltophilia	0	1	1
Salmonella cholerae suis	1	0	1
E. faecalis	1	5	6
E. faecium	0	1	1
Group A streptococcus	0	1	1
Group B streptococcus	1	2	3
S. anginosus	0	2	2
No reliable information	12	4	16
No peaks found	10	4	14
	51	60	111

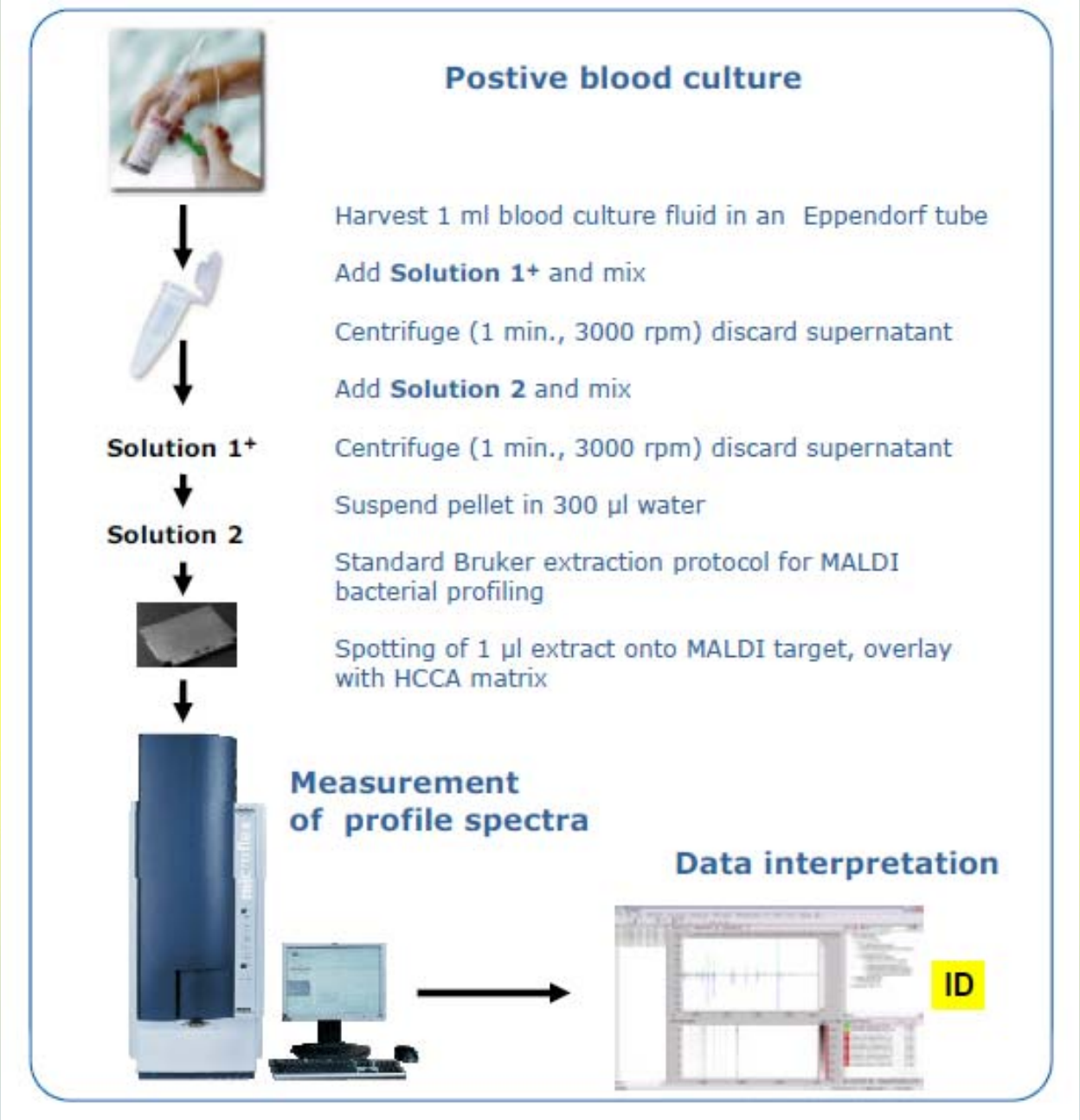
Method 1ml 73%

Method 5ml 90%

If they can get a decent Gram film then they can get a good Biotyper ID

45 mins!

New Blood Culture Assay



Initially tested G-ve Positive Blood Cultures

Gram negative bacteraemia cases continue to rise in the England, Wales and Northern Ireland (HPA 2010), as does resistance to many first line systemic antibiotics, made worse by the apparent increases in ESBL/Amp C producing Gram negative strains.

Over reliance using more broad spectrum antibiotics, especially the Carbapenems. This has both cost implications and the possibility of helping to create resistance to this class of antibiotics.

The practice of de-escalating to a narrower range antibiotic is to be encouraged, but at present this is only possible a minimum of 24 hours after the diagnosis of a Gram negative.

Over a three month period, positive blood culture bottles from 53 different patients, each containing Gram negative bacilli were investigated.

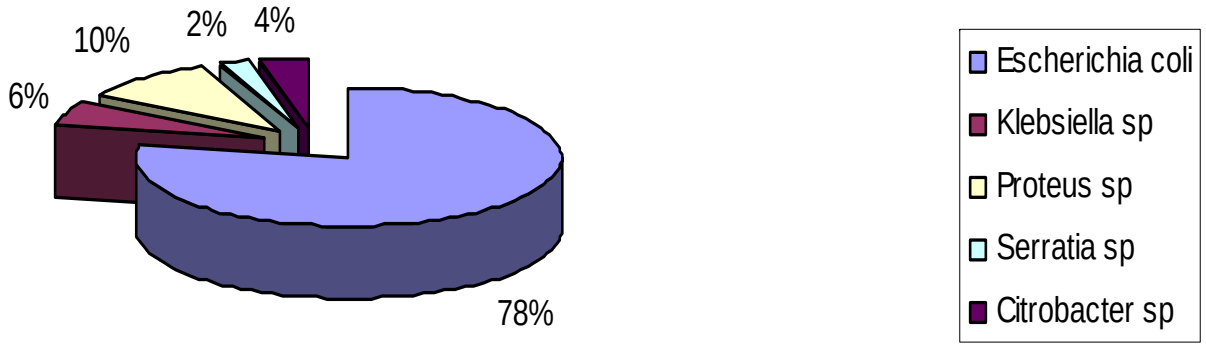
Identification was attempted using three different methodologies.

1) Positive broth using MALDI
2) The Blood culture isolate using MALDI
3) Traditional laboratory biochemical techniques.

The laboratory currently uses a BD Bactec Fx instrument and Bactec Plus bottles (both aerobic and anaerobic).

All broth cultures were taken off the Bactec Fx machine as soon as practicable and extraction done within 4hrs of taking the bottle of the machine.

Sheffield Northern General Hospital Isolates



Total Gram negative isolates for England,Wales & Northern Ireland (2007)

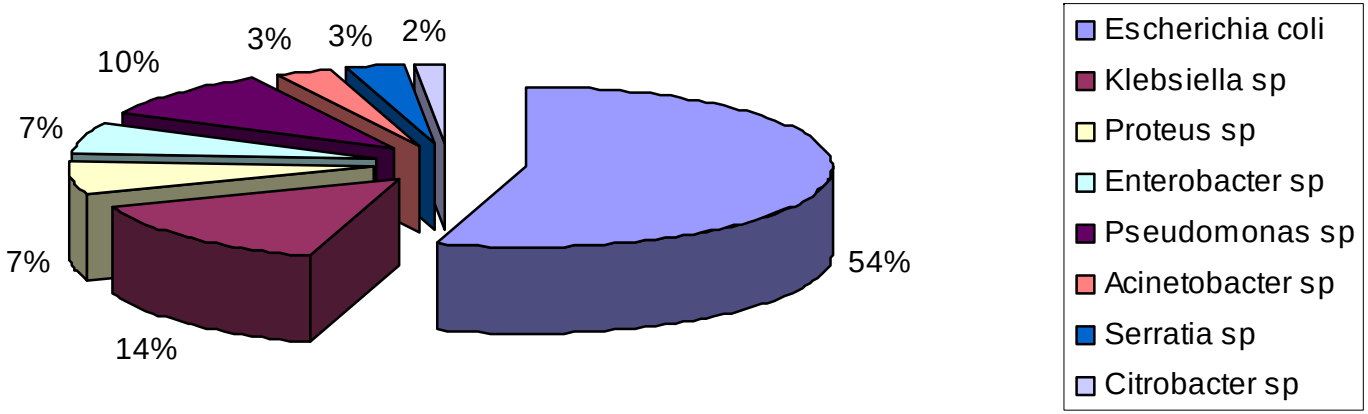


Table 1 – Identification given direct from broth, colony and using traditional methods of 53 Gram negative positive blood cultures

Direct Blood Culture ID by MALDI	MSP Value Range	Number	Direct colony MALDI ID	MSP Value Range	Laboratory ID
Escherichia coli	2.109-2.483	37	Escherichia coli	2.048-2.451	Escherichia coli
Escherichia coli	1.907-2.215	2	N/A	N/A	*
No reliable ID	<0 - 1.311	2	Escherichia coli	2.221-2.251	Escherichia coli
Citrobacter freundii	2.299	1	Not performed	Not performed	Citrobacter freundii
Citrobacter koseri	2.377	1	Citrobacter koseri	2.331	Citrobacter koseri
Klebsiella oxytoca	2.264	1	Klebsiella oxytoca	2.426	Klebsiella oxytoca
Klebsiella pneumoniae	1.968-2.353	3	Klebsiella pneumoniae	2.196-2.468	Klebsiella pneumoniae
Proteus mirabilis	2.126-2.335	5	Proteus mirabilis	2.252-2.393	Proteus mirabilis
Serratia ureilytica	2.318	1	Serratia marcescens	2.219	Serratia marcescens

Blood Culture Results

Direct analysis of blood cultures resulted in 48 (90.7%) correct identifications to species level and 49 (92.5%) to genus level.
Four samples gave discordant results.
Two gave no reliable identification despite growing *Escherichia coli*
One identified as *E. coli* but a mixture of *Proteus* species also grew
One gave a good *E. coli* peak despite giving no growth on culture.
(The latter is assumed to be a false positive due to a laboratory error, cross contamination or insufficient cleaning of the metal target between repeated use).

B/C Conclusion

- MALDI-TOF MS profiling enables a very quick and reliable identification of Gram negative bacteria from positive blood culture samples.
- Identification results are available at least 24hrs earlier than conventional techniques.
- Rapid accurate identification allows for a more informed decision when deciding antimicrobial therapy whilst awaiting susceptibility results and may significantly improve patient care.
- Further work may show that accurate identification coupled with rapid susceptibility testing of Gram negative bacilli using the E Test may provide a full identification and susceptibility profile within 6hrs.

66% from Direct

Staphylococci (15 isolates)

Lab Identification	Direct Maldi	Colony Maldi
MSSA x 3	STAU (2.24 – 2.36)	STAU (2.3 x 3)
MRSA	STAU (2.2)	STAU (2.4)
CNS x 11	S. epidermidis x 4 (1.89 – 2.29)	S. epidermidis x 4 (2.3 – 2.4)
	S. capitis (2.03)	S. capitis (2.09)
	S. haemolyticus (1.89)	S. haemolyticus (2.01)
	No Peaks x 2	S. epidermidis x 2 (2.3)
	No Reliable ID x 3	S. haemolyticus (1.97)
		S. saccharolyticus (1.99)
		S. hominis (1.93)

73% from Direct.

1 Misleading

HSA x 4	S. pyogenes x 3 (2.11-2.31)	S. pyogenes x 4 (2.2 – 2.5)
	No Reliable ID x 1	
HSB	S. agalactiae (2.46)	S. agalactiae (2.3)
HSG	No Reliable ID	S. dysgalactiae (1.8)
S. pneumoniae x 3	S. pneumoniae x 2 (1.69 – 1.9)	S pneumoniae x 3 (2.24 – 2.46)
	Clot in solution	
Strep gallolyticus (bovis) (VITEK 2)	S. gallolyticus (2.19)	S. gallolyticus (2.2)
Strep milleri group	No Reliable ID	S. constellatus (2.12)
Viridans Streptococci	S. pneumoniae (1.67)	S. pneumoniae (1.87)
(Optichin Resistant)		

Candida species (6 isolates)

Lab Identification	Direct Maldi	Colony Maldi
Candida albicans x 2	C. albicans x 2 (1.4 - 1.8)	C. albicans x2 (2.08 – 2.12)
Candida glabrata x 2	C. glabrata x 2 (1.5 – 2.0)	C. glabrata (2.01 – 2.13)
Candida parapsilosis x 2	C. parapsilosis (1.6) No Reliable ID	C. parapsilosis (2.28) C. metapsilosis (2.01)

91% from Direct

Enterobacteriaceae (69 isolates)

	(1.31) x 1	
	No Peaks x 4	
Klebsiella pneumoniae x 4	K. pneumoniae x 4 (2.0 -2.4)	K. pneumoniae x 4 (2.2 – 2.5)
Klebsiella oxytoca x 2	K. oxytoca x 2 (2.3 – 2.4)	K. oxytoca (2.4 – 2.5)
Proteus mirabilis x 5	P. mirabilis x 5 (2.13 – 2.35)	P. mirabilis x 5 (2.25 – 2.38)
Citrobacter freundii	C. freundii (2.29)	C. freundii (2.30)
Citrobacter koseri	C. koseri (2.37)	C. koseri (2.33)
Enterobacter cloacae x 2	E. cloacae (2.18)	E. cloacae x 2
	E. ludwigii (2.3)	(2.2 – 2.4)
Enterobacter aerogenes	No Peaks	E. aerogenes (2.34)
Serratia marcescens	S. ureilyticum (2.3) (S. marcescens 2.2)	S. marcescens (2.23)

Unusual (6 Isolates)

Lab Identification	Direct Maldi	Colony Maldi
Acinetobacter baumanii	No Peaks	A. genomospecies (2.17)
Bacteroides sp	No Peaks	B. fragilis (2.3)
Fusobacterium species	F. necrophorum (1.44) No RId	F. necrophorum (2.16)
Pasteurella multocida	P. multocida (1.4) No RId	P. multocida (2.2)
Propionibacterium species	P. acnes (1.24) No RId	P. acnes (2.14)
Veillonella sp	No Peaks	Veillonella parvula (2.3)

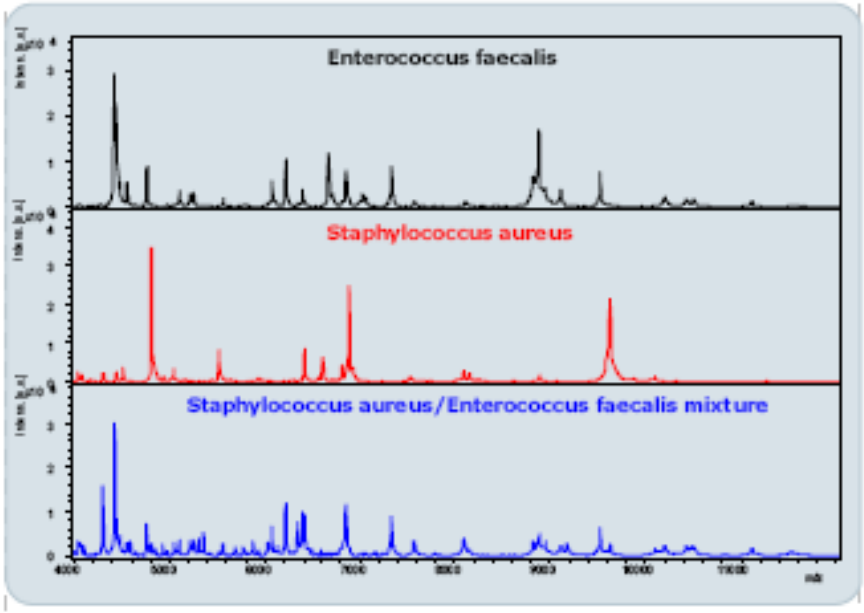
Misleading Results (5 occasions)

Lab Identification	Direct Maldi	Reason
Mixed Anaerobes	E. coli (1.9)	New April 2010 or Dodgy Matrix
Mixed Proteus x 2 and E. coli	E. coli (2.2)	More E. coli
Mixed S. aureus and S. haemolyticus	S. haemolyticus (2.19) O2 Bottle	Mixture in AnO2 bottle only
Mixed CNS x 2	S. epidermidis (2.0)	Missed lesser CNS
	S. hominis (2.2)	Missed lesser CNS

Automated detection of mixed cultures of microorganisms using MALDI-TOF mass spectrometry

Thomas Wenzel, Stefan Klepel, Thomas Maier, Simone Stumpf, Beatrix Wegemann, Markus Kostrzewa

Sample No.	Standard Analysis MALDI Biotyper 2.0 (log(score))	Identification using the novel control algorithm (log(score))
1	Enterococcus faecalis (2.225), Staphylococcus aureus (2.069)	Enterococcus faecalis + Staphylococcus aureus (2.647)
2	Escherichia coli (2.271), Enterococcus faecalis (1.915)	Escherichia coli + Enterococcus faecalis (2.618)
3	Escherichia coli (2.363), Pseudomonas aeruginosa (1.750)	Escherichia coli + Pseudomonas aeruginosa (2.652)
4	Staphylococcus aureus (2.216), Escherichia coli (1.792)	Staphylococcus aureus + Escherichia coli (2.641)
5	Staphylococcus aureus (2.186), Staphylococcus epidermidis (1.625)	Mix culture not recognised automatically, Staphylococcus aureus (2.186)



Conclusions

- Detection of bacterial mixtures reliably down to a ratio of 1:5 with high sensitivity
- Rapid spectra check of real-life samples for probable existence of mixed cultures
- Applicability of automated mixed culture identification for positively flagged blood cultures
- Possible incorporation of the algorithm into MALDI Biotyper 2.0

Doesn't Currently pick up *S. aureus/S. epidermidis* mixture well so blocked alert!

Interesting B/C Case Studies

- Streptococcus – Patient with possible necrotising fasciitis (small area on leg) or O157.

  Strep in one bottle only

  MALDI result – Group A strep – Immediately started on high dose Pen and Igs.

  Patient treated with Igs 18hrs earlier

  Responded very well
- *Salmonella paratyphi A*
- *Enterococcus faecium* endocarditis

Current Routine Use!

Two Blood Culture runs – 8:30am and 1:30pm

Two Direct runs –

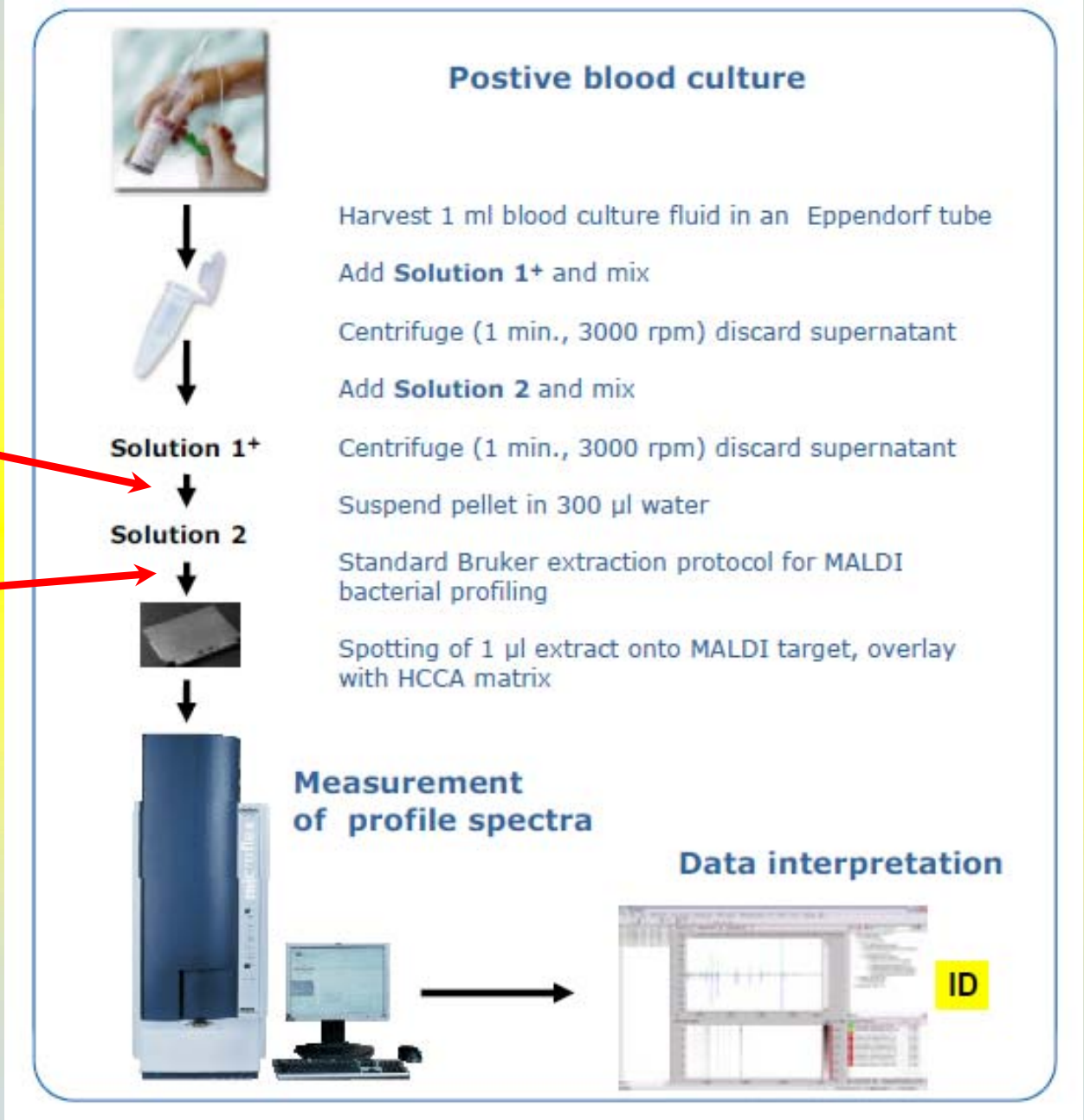
- 10:00am - Respiratory samples
- 2:00pm - Interesting and difficult to id organisms including significant yeasts from systemic sites.

(Background of Cheap Multipoint Identification for ID results)

Any other Issues!

Slime

Beads



Fast and Easy Microorganism Identification

The MALDI Biotyper Solution

Data Acquisition

- Benchtop instrument
- Automated system
- Unattended Operation

MALDI Biotyper Reference Library

- Ready-to-use library
- Real-Time analysis
- Create your own Libraries

Sample Preparation

- Optimized quality
- Robust
- 5 min Direct Protocol
- 20 min EtOh Protocol

MALDI Biotyper Data Analysis

- Automated data processing
- Signal identification
- Pattern matching
- Compare strains from same species (? Typing)

Conclusion

Is it Clinically useful?

Time! – Investigate oddities and need to believe

Will it replace traditional diagnostic and Biochemical tests?

Biomerieux!!!!!

‘Charcoal’??

BD Diagnostics and Bruker Collaborate to Improve Microbial Identification and Antimicrobial Susceptibility Testing

Collaboration Aims to Improve Speed, Accuracy and Efficiency in the Microbiology Laboratory

(September 29, 2010)

With thanks to all NGH staff especially

The END!!!!!