Implementation of MALDI -TOF MS in a Routine Clinical Laboratory

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NHS Trust

Where EVERYONE Matters

Overview

- Why?
- Implementation
  - Planning
  - Validation
    - All bacteria and yeasts
    - Campylobacter
    - Alpha-Haemolytic Streptococci
    - Salmonella ssp.
    - Overall
    - Time to result
- Workflow
- The future and conclusions

About Us

- Large District General Hospital
- c.900 beds (Split across two hospital sites)
- Serves approx 500,000 patients
- Tertiary referral Ophthalmology inc A&E
- Unified microbiology lab on single site
- Current home of BSAC Standardized Method Development Centre. Key interest in A.S.T.

Why MALDI-TOF?

- Efficiency
  - Redesign our workflow
- Reconfiguration of service providers locally
  - 7 other microbiology laboratories within 10 miles
  - 4 within 5 miles!
- Improvement of our service for users.
  - Decrease time to detection
- Standardise our ID methods to a single platform
- Opportunity to get first VITEK – MS in U.K. clinical laboratory.

Why VITEK MS?

- Part way through automation/innovation process
  - Blood Cultures 1994, TB 1998
  - Urines 2007
  - Sensitivity Testing 2009
  - Nucleic Acid Extraction and Detection 2010
  - Specimen Processing 2011

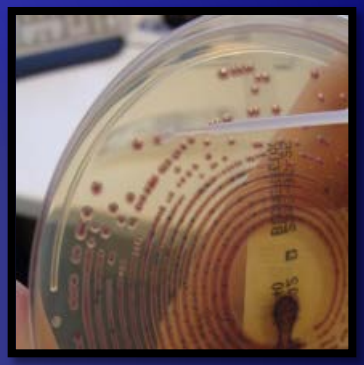
- **Current Vitek 2 User – AST and GN/GP ID**
- **Easy integration to attach ID to sensitivity testing using MYLA middleware solution.**

- **MYLA also has connectivity to our automated sample processing and blood culture system**
  - **Middleware is now a powerful management tool**

- **Very simple ID protocol**
  - **Minimal extraction (Yeasts – done on target)**
  - **Disposable targets**

Why VITEK MS?

- Touch colony(s)
- Inoculate disposable target slide
- Add 0.5µL Formic Acid (yeasts only)



Why VITEK MS?

- Add 1µL CHCA Matrix, Air dry
- Process target in MS
- Validate ID result in MYLA

Pre -implementation

- Planning
  - Project Timeline with milestones
  - Workflow redesign
    - Re-work day 2 culture reading
  - Structural Survey
    - Would floor take the weight!
  - How to validate
    - Parallel testing?
    - Known cultures /Type strains?

Implementation

- Installation & Initial Training
  - Installed End August 2011
    - System set up, fine tuning
  - Training initially provided by bioMerieux on-site
    - Early September
    - 3 Key Users
      - Cascade training
      - Core initial team of users

Implementation - Validation

- 617 well characterized bacteria and yeast isolates
  - NCTC/ATCC/NCPF/Reference Laboratory ID/NEQAS
- 93.8% Species ID Correct
- 97.4% Group/Family Correct
  - E.g. S mitis group/E.cloacae complex
- 98.5% Genus Correct
- 8 Isolates No ID Obtained
- 1 Isolate Incorrect
- Reading et al. ECCMID 2012 P2323

Implementation - Validation

- 98 Enteritis associated Campylobacter spp.

- 49 Strains *C. jejuni*
- 49 Strains *C .coli*

- 100% concordance
- Reading et al. ECCMID 2012 P2332

- Important to know ID to utilise EUCAST ECOFF data for Erythromycin susceptibility testing as different ECOFF for each species

Implementation - Validation

- Alpha Haemolytic Streptococci

148 strains, 14 species including 35 *S. pneumoniae*
Reference lab confirmed I.D., Isolates from 2004-2011

83.7% Species Level Correct (97.1% *S.pneumoniae)*

98.6% Group/Family Correct

1 No ID (very mucoid *S.pneumoniae*)

1 Incorrect ID (*S. bovis*)

Symonds et al. ECCMID 2012 Abstract R2637

Implementation - Validation

Sandwell and West Birmingham Hospitals NHS Trust

Identification of *Streptococcus pneumoniae* and Non-pneumococcal Streptococci of the *Streptococcus mitis* Group using the VITEK®MS MALDI-TOF Mass Spectrometry System.

N.P. Reading*, H.M. Kilgariff, N. Ratnaraja

Department of Microbiology, Sandwell and West Birmingham Hospitals NHS Trust, Dudley Road, Birmingham, B18 7QH

Species	Total Strains n=226	Species Correct	Family/ Group Correct	Incorrect Identification	No identification	ID Species	ID Group
S. pneumoniae	141	140	0	0	1	**99.29%**	**99.29%**
S. oralis	44	0	43	1	0	**0%**	**97.73%**
S. mitis	31	0	30	1	0	**0%**	**96.77%**
S. pseudopneumoniae	6	6	0	0	0	**100%**	**100%**
S. cristatus	4	4	0	0	0	**100%**	**100%**

Implementation - Validation

Identification of *Salmonella enterica* ssp *enterica* using the VITEK®MS MALDI-TOF mass spectrometry system.

N. Reading*, H.M. Kilgariff, N Ratnaraja

Department of Microbiology, Sandwell & West Birmingham Hospitals NHS Trust, Birmingham, United Kingdom

Species	Total Strains n=89	Species Correct	Family/ Group Correct	Incorrect Identification	No Identification	ID Species	ID Group
Salmonella typhi	28	27	1	0	0	**99.3%**	**100%**
Salmonella paratyphi A	19	14	5	0	0	**73.7%**	**100%**
Salmonella species	42	40	1	0	1	**95.2%**	**97.6%**

35 (100%) isolates of Salmonella species tested directly for X.L.D. agar correctly identified as Salmonella Group.

When combined, 123/124 (99.2%) isolates correctly identified to Salmonella group using the VITEK MS

Evaluation – Clinical samples

- 400 further isolates
- Parallel testing with current
  - API
  - Vitek 2
  - Phenotypic/Biochemical/Microscopy
- No misidentification when compared to traditional
  - Limitations of database
    - Strains not included
    - Slash line ID?

Evaluation – Time Saving

- When was ID available compared to traditional method?
- Same 400 isolates

	Time to Identification	
Organism	Traditional (mean/range)/hours	MALDI-TOF (mean/range)/hours
Staphylococci	**19/15-29**	**1.5 / 0.3-4**
GNB (Ferm/Non Ferm)	**10 /6-22 , 13/8-36**	**1.5/ 0.3-3 , 2/ 0.5-4**
Streptococci	**8/ 0.1-32**	**1.5/ 0.3-4**
HACEK	**18 / 17-39**	**1.3/ 0.3-3**
Yeasts	**34 / 18-68**	**1/ 0.3-5**
Enterococci	**10 / 9-27**	**1.3/ 0.3-4**

Implementation

- Go live training!
  - Easy process to manage using standard bench rotation. (In theory)
  - However……
    - Weekend working!
    - Extended day!
    - Bank Holidays!
    - Sickness!
    - Annual leave
  - **Management of rota's & staffing is key!**
    - **And very stressful……..**

Implementation – Considerations

- Taxonomy!

- Streptococci
  - Species name!
  - S.dysgalactiae
    - Clinical significance?
    - Previous lancefield grouping…. G/C
    - Now no differentiation – so what?
  - S.”milleri”
    - Now species level

- End users – confusion?

Workflow

- 8am -7pm = normal laboratory hours
- 9am- 5pm = core hours

- Preanalytical
  - Specimen sort, label, processing etc

- Culture reading
  - Divided into similar samples
    - Resp,Gastro, Swabs etc etc

Workflow – 6 months ago…..

- **Combined ID/AST section**
  - **Sensitivity only (ID from Chromogenic Agar)**
    - E.coli from Urines
      - Data from evaluation showed this to be accurate and compared very well with MS
  - **ID and Sensitivity (on Vitek 2)**
    - Systemic coliforms
    - Enterococci
    - Staphylococci (pre screened with latex agglutination)
  - **ID and Sensitivity (Manual – discs/gradient)**
    - All with EUCAST/BSAC interpretation
      - Antibiotic Discs or Gradient Method
  - **ID only**

Workflow as of 26/11/12

- MS is now 'de-facto' first line ID
  - No latex for staphylococci
  - No streptococcal grouping
- ID set up and result review by bench readers as cultures read
  - Processed in groups of 16 isolates
  - Organism ID ready for choice of sensitivity testing
    - Minimise waste of false positive latex etc
    - Ensure correct isolate picked

Workflow

- Previous
  - 1 Band 7 Senior Biomedical Scientist WTE
  - 1.5 Band 6 Biomedical Scientist WTE
  - 0.5 Band 2 Laboratory Assistant WTE

Now

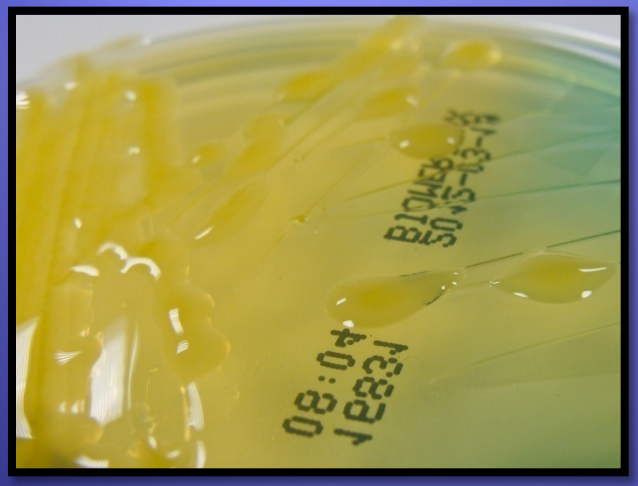
- **0.6 Band 7 Senior BMS WTE**
- **1.25 Band 6 BMS WTE**
- **0.25 Band 2 MLA WTE**

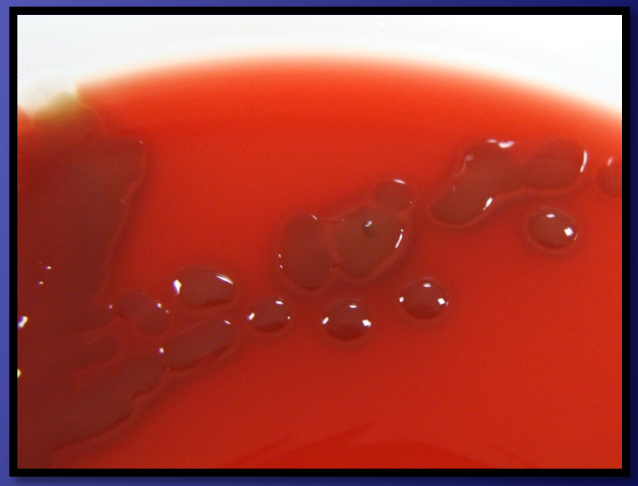
Summary of experience so far…..

- Simple, easy to use.
- Accurate
  - All odd isolates from significant sites
    - Reference lab ID
    - 100% concordance with isolates in MS database so far
    - 100% NEQAS samples , correct ID
- Very Rapid
- Excellent field support from Biomerieux
- Enabling technology for workflow improvements
- Standardised / Reproducible ID method

But….When it all goes wrong……

- Need Plan B
  - Some isolates don't behave!
    - Mucoid isolates (Vitek MS has no extraction protocol)





    - Isolates not in database
      - Actinomyces poorly represented
      - Few rapid growing Mycobacteria
      - No Brucella……

But.....

- Machine downtime
  - Planned – e.g. Preventative maintenance or servicing
    - Catch up or alternative methods
    - Stock can be planned.
  - Unplanned
    - Management of workflow
      - Very Large labs – 2nd system? Redundant capacity?
      - Partnerships with other users
    - Stock – how much to hold, shelf life, staff competency etc.
    - Users unhappy
      - **Consultants complain no ID available after 4hrs!**
      - **Previously managed with 12-18hrs!!!!**

But….

- Lazy Microbiology!
  - MS instead of Gram's Stain!
  - Just because you can does not mean you should!
  - Basics!

Benefit to patient – Case Study

- Friday – Doesn't this always happen…….!
- 4 y.o. Female
- Vaginal swab taken at G.P. Surgery
- Grey Colonies growing on Chocolate Agar at 18hrs
  - (with commensal flora)
    - Gram Negative Cocci
    - Oxidase positive
    - Insufficient colonies for biochemical ID
- To MS……

Benefit to patient – Case Study

- MS = N. gonorrhoea
- Immuno-chromatographic method
  - N. gonorrhoea
- No Secure Evidence trail……

So……

Sample reprocessed – ensure no lab error

New samples taken with evidence trail same day via Consultant Community Paediatrician / GP

Case study

Sample reprocessed = same 24 hrs later

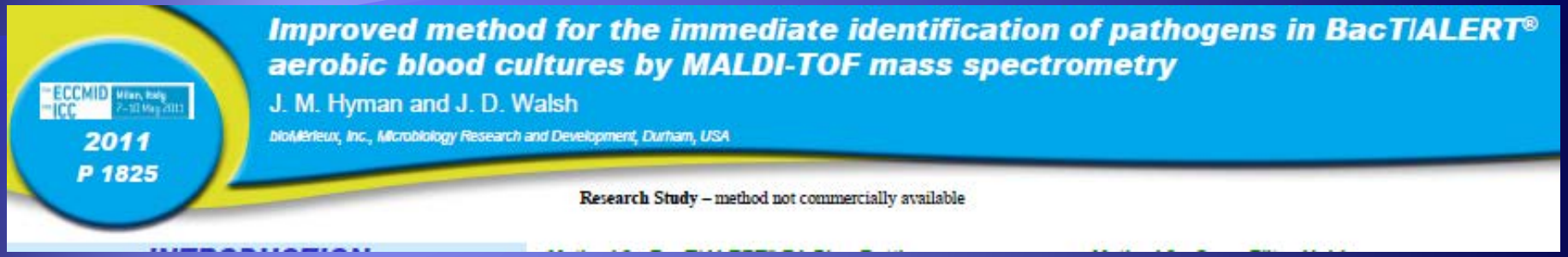
New samples also positive – MS ID same

Patient into care within 20 hrs of initial result.

Sad, but excellent outcome

Where are we going next?

- Direct from blood culture identification



Lysis – Filtration method

Our own evaluation with clinical samples suggests excellent performance

- 2013 – Look to implement into routine workflow

Where are we going next?

- Some Aspergillus sp. in Vitek MS database
  - Not validated by us yet
  - ECCMID 2012 – challenge of sampling of colony and age of colony to produce reference spectra.
- No Mucoraceous moulds
- No Dermatophytes
- Await database expansion

Where are we going next?

- Longer term goals
  - Pathogenicity markers
    - ?PVL
      - Some conjecture with regards feasibility and which peaks associated with PVL

Int J Antimicrob Agents. 2009 Nov;34(5):467-70. Epub 2009 May 5.

MALDI-TOF-MS for rapid detection of staphylococcal Panton-Valentine leukocidin.

Bittar F, Ouchenane Z, Smati F, Raoult D, Rolain JM.

Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, CNRS-IRD, UMR 6236, Faculté de Médecine et de Pharmacie, Université de la Méditerranée, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France.

Int J Antimicrob Agents. 2010 Aug;36(2):193-4. Epub 2010 May 31.

Detection of Panton-Valentine toxin in Staphylococcus aureus by mass spectrometry directly from colony: time has not yet come.

Dauwalder O, Carbonnelle E, Benito Y, Lina G, Nassif X, Vandenesch F, Laurent F.

Where are we going next?

- Antibiotic resistance
  - MRSA
    - ?Possible

J. Med. Microbiol. — Vol. 49 (2000), 295–300
© 2000 The Pathological Society of Great Britain and Ireland
ISSN 0022-2615

TECHNICAL NOTE

Rapid discrimination between methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* by intact cell mass spectrometry

VALERIE EDWARDS-JONES*, M. A. CLAYDON*, D. J. EVASON*, J. WALKER†, A. J. FOX*† and D. B. GORDON*

Where are we going next?

- Carbapenem hydrolysis assay(s)
  - Lots of posters/presentations at ECCMID 2011 & 2012

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2011, p. 3222–3227 Vol. 49, No. 9
0095-1137/11/$12.00 doi:10.1128/JCM.00984-11
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Carbapenemase Activity Detection by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry▽

Jaroslav Hrabák,* Radka Walková, Vendula Študentová, Eva Chudáčková, and Tamara Bergerová

Department of Microbiology, Faculty of Medicine and University Hospital in Plzen, Charles University in Prague, Plzen, Czech Republic

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MALDI-TOF MS: detection of carbapenem resistance within one hour

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[1] Department for Infectious Diseases, Microbiology and Hygiene, University of Heidelberg, Heidelberg, Germany

*corresponding author: irene.burckhardt@med.uni-heidelberg.de

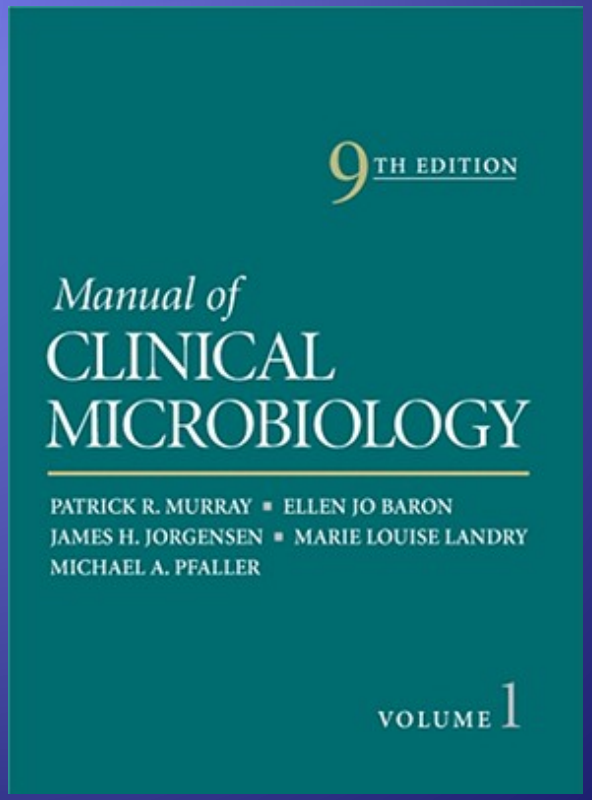
- Other resistance markers?

In conclusion

- Simple technique
- Accurate
- Enabling technology for workflow re-design
- Rapid
- Cost effective

  Including capital – approx cost neutral

  Excluding capital approx 80% saving on reagents

In conclusion

- The best tools required when using and implementing MS in a routine lab!