Validation and Implementation of MALDI-TOF MS in a Public Health Laboratory

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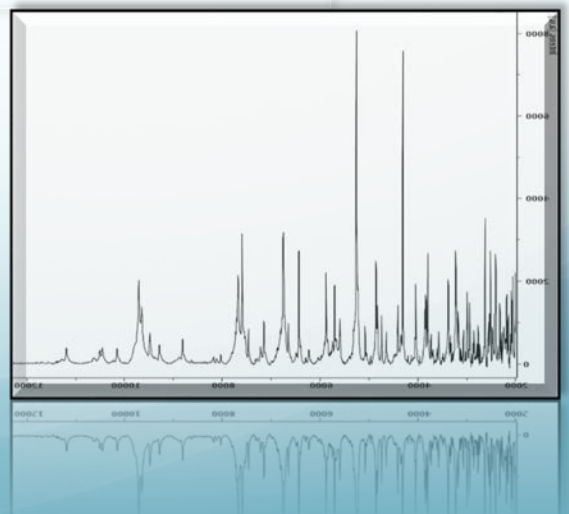
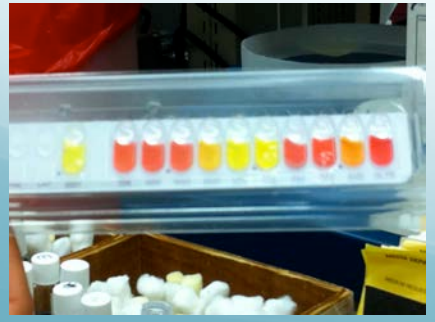
June 2, 2014 1:30 PM

MALDI-TOF MS: Goodbye Biochemicals, Hello Lasers

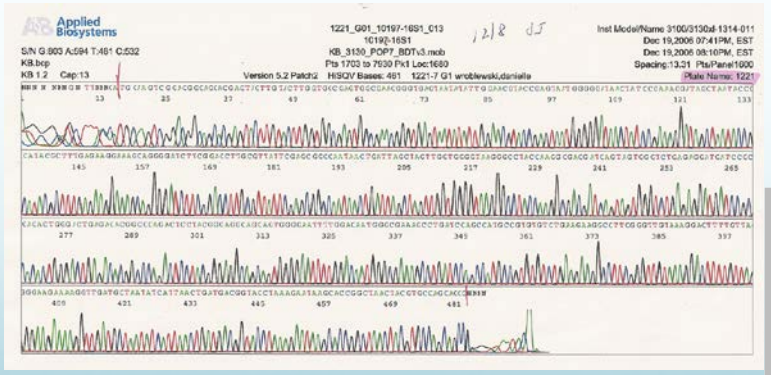
Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH

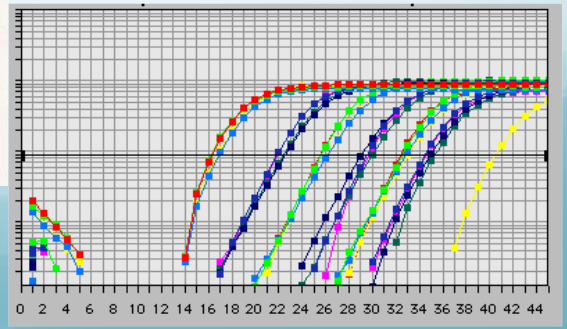


The WC Bacteriology Lab of 2012

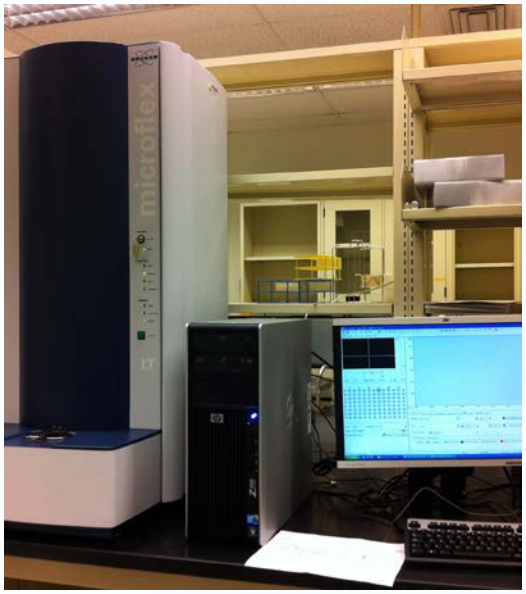


- 6,000 isolates/year cultured
- Biochemical tests, strips, fatty acid analysis
- ~10,000 real-time PCR tests/year
- ~1,000 sequencing reactions/year





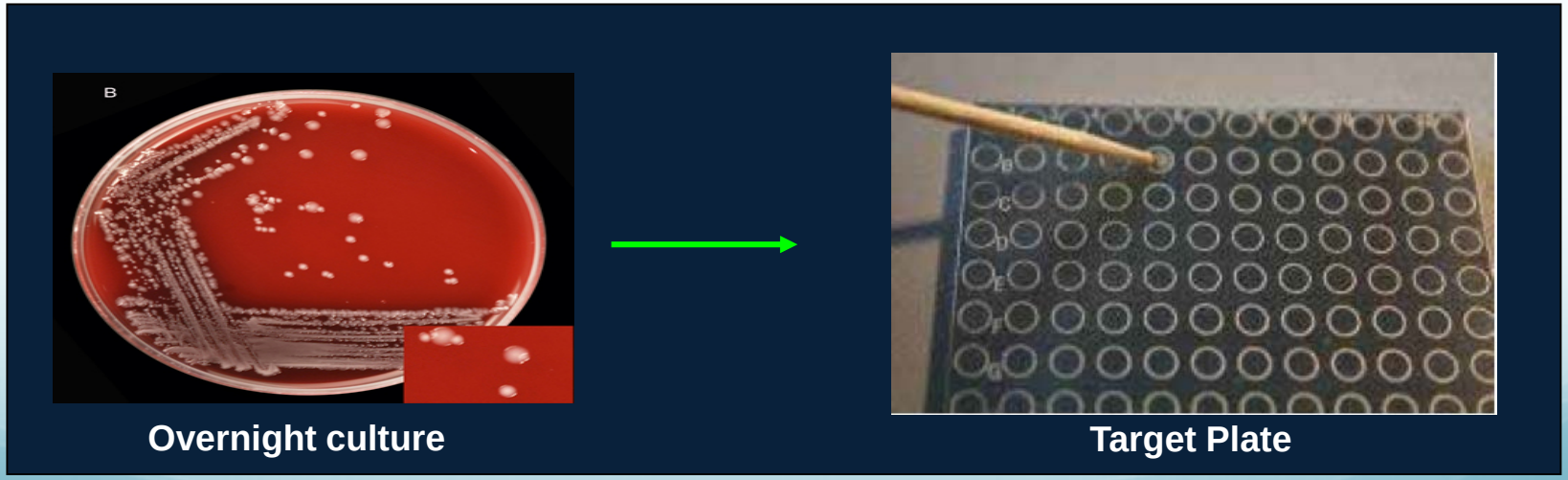
Timeline



- **May 2011:** ASM 2011 General Meeting
- **Aug.-Dec. 2011:** Sales Representatives visits, evaluation of technology
- **Feb. 2012:** Received first MALDI-TOF MS submissions for our NYS CLEP; Decision to evaluate Bruker Daltonics MALDI Biotyper
- **May 2012:** On site demo of instrument, validation of performance in numerous areas
- **Feb. 2013:** Validation application submitted to CLEP, approval granted for identification of bacterial species at the Wadsworth Center

How does MALDI-TOF MS work?

- Individual colony from an overnight culture is smeared (spotted) onto a target plate

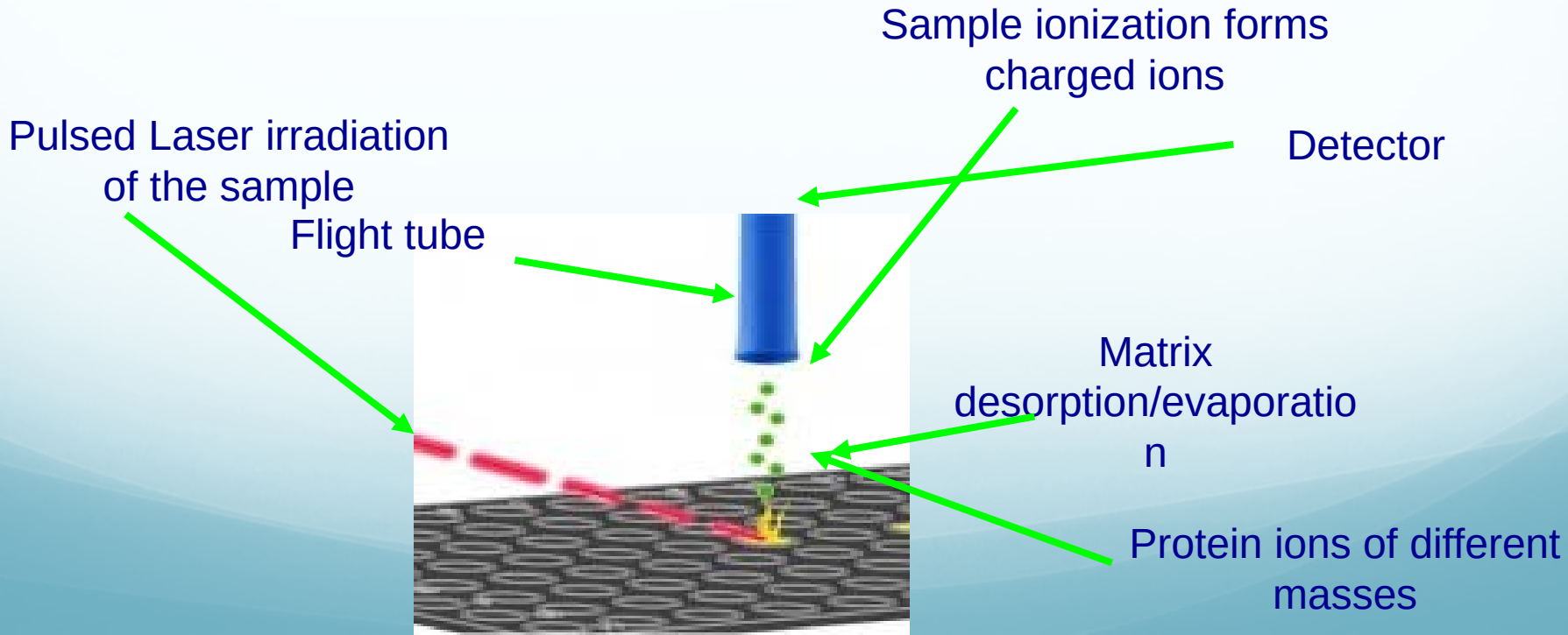


*Extended direct smear

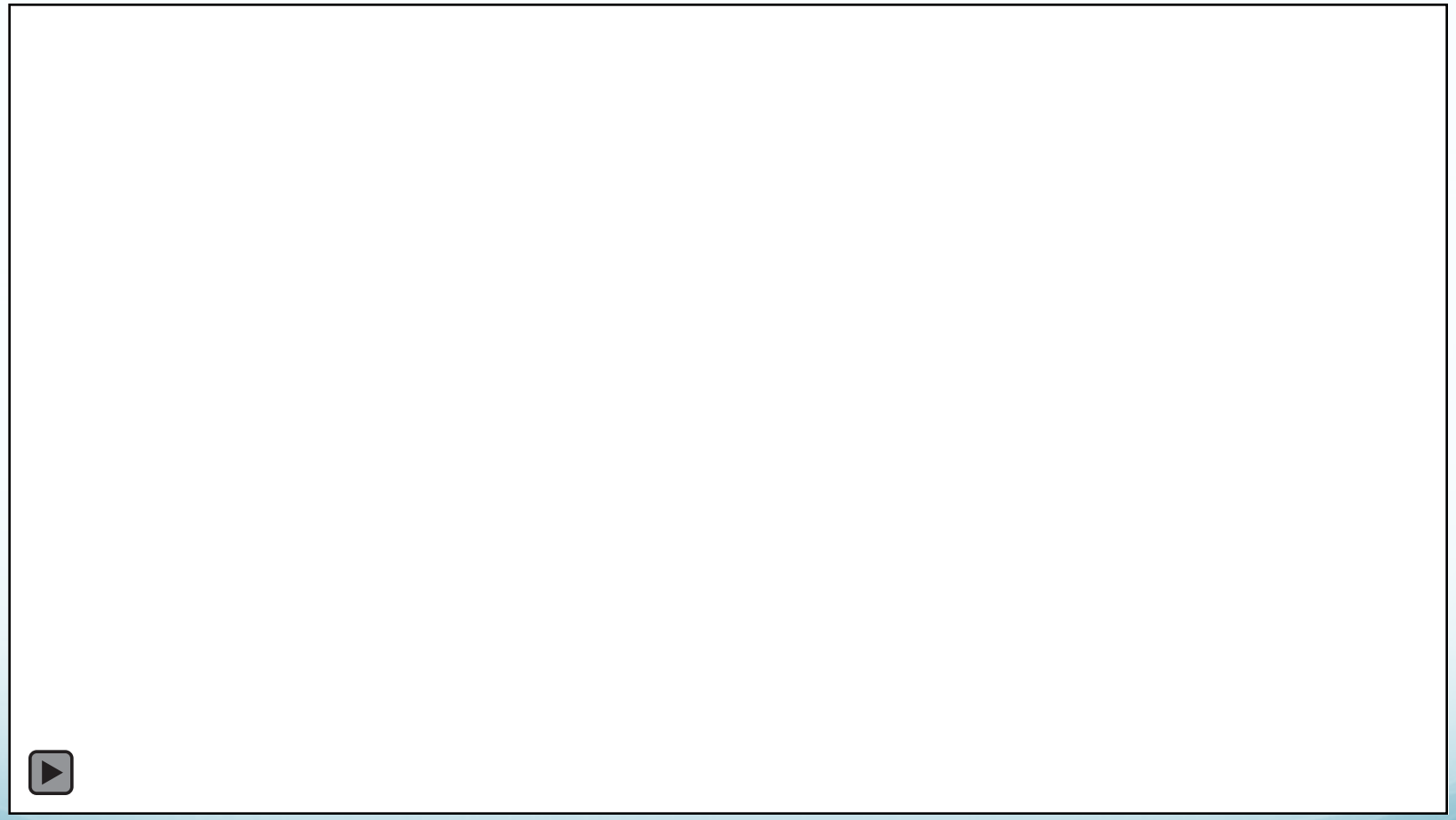
MALDI-TOF MS

Matrix-**a**ssisted **L**aser **D**esorption/**I**onization-

Time-**o**f-**F**light **M**ass **S**pectrometry



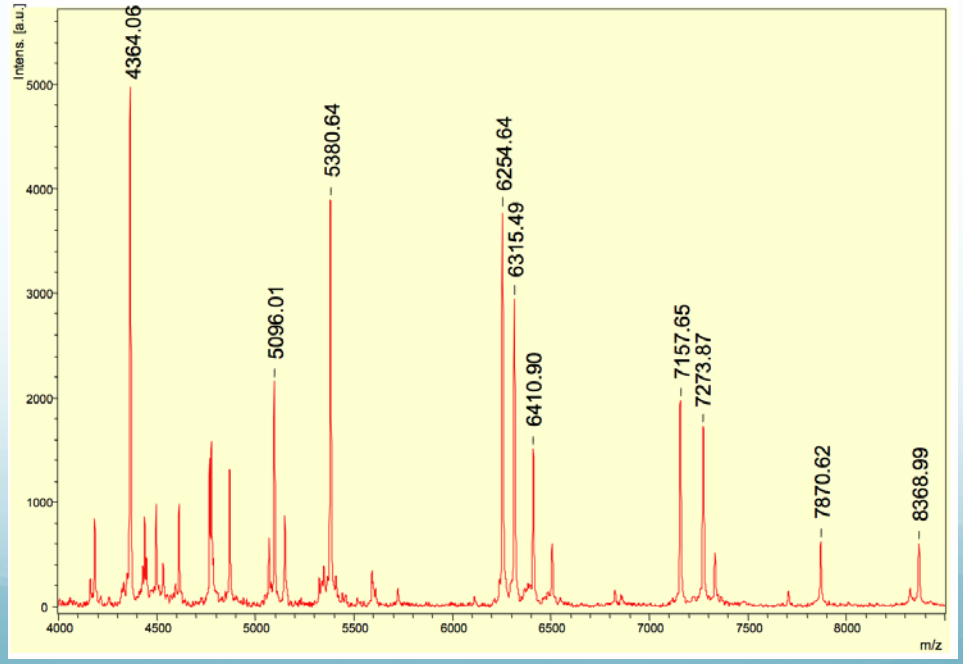
MALD-TOF MS in action



MALDI-TOF MS

Protein Profile

The result is a mass-spectrum of mainly ribosomal proteins that are species-specific for a large number of microorganisms. These spectra are compared to a reference database.



Bruker Daltonik MALDI Biotyper Classification Results

Project Info

Project Name:	**MD013013**
Project Description:	
Project Owner:	Administrator@FLEX-PC
Project Creation Date/Time:	2013-01-30T11:00:44.187
Project Analyte Count:	5
Project Type:	Development
Validation:	not present
Validation Position:	

Result Overview

AnalyteName	AnalyteID	Organism(best match)	ScoreValue	Organism(second best match)	ScoreValue
A1(-) (C)	BTS	**no peaks found**	< 0	**no peaks found**	< 0
A2(+++) (A)	IDR13-2899	Enterococcus faecalis	2.347	Enterococcus faecalis	2.312
A3(+++) (A)	IDR13-2899	Enterococcus faecalis	2.406	Enterococcus faecalis	2.322
A4(+++) (A)	IDR13-2899	Enterococcus faecalis	2.513	Enterococcus faecalis	2.39
A5(+++) (A)	IDR13-2899	Enterococcus faecalis	2.426	Enterococcus faecalis	2.401

Matching Hints

Matched Pattern	Comment
Pseudomonas mucidolens LMG 2223T HAM	is a member of Pseudomonas fluorescens group

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)

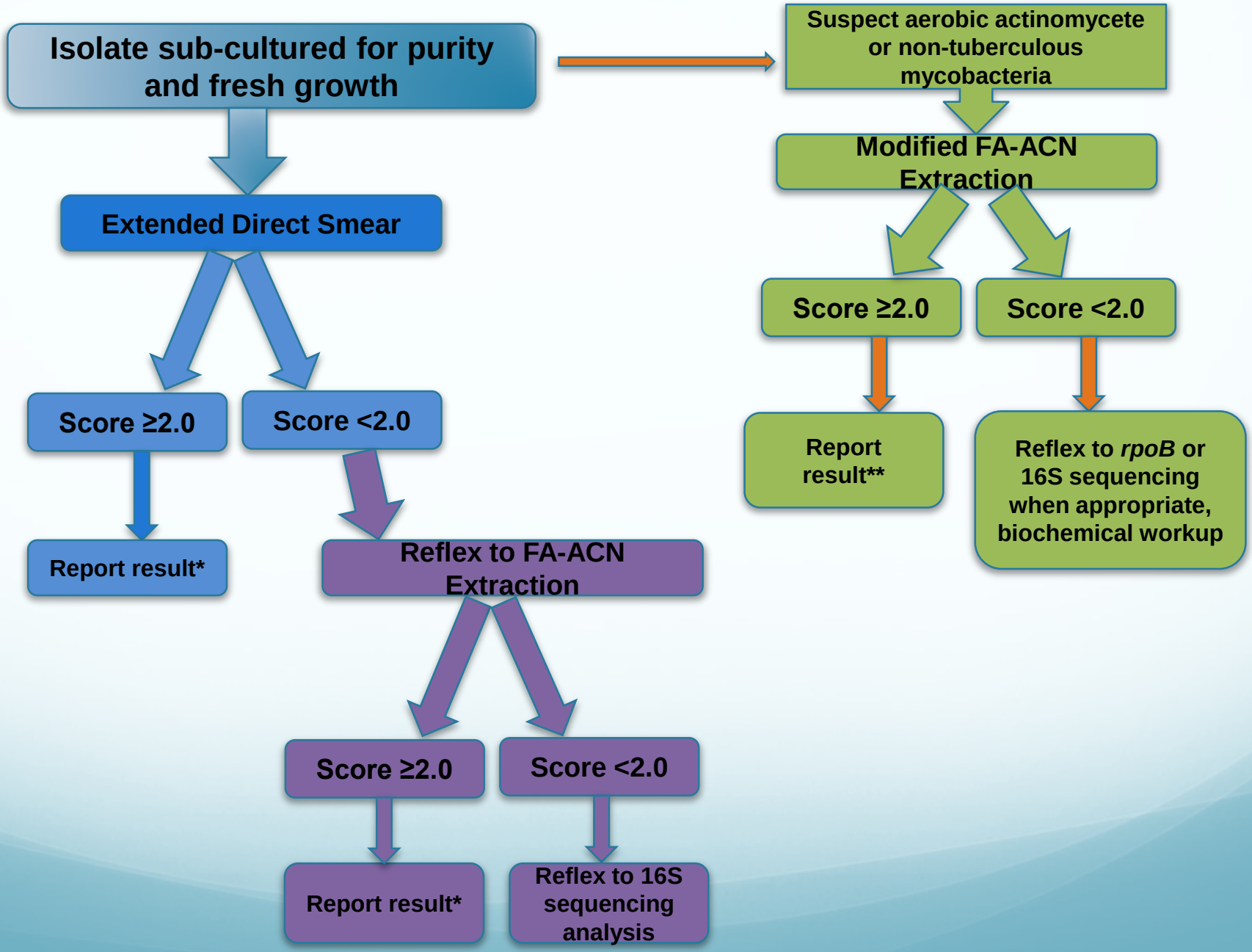
Category	Description
A	**Species Consistency:** The best match was classified as 'green' (see above). Further 'green' matches are of the same species as the first one. Further 'yellow' matches are at least of the same genus as the first one.
B	**Genus Consistency:** The best match was classified as 'green' 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 microbial mixture).

Is it that easy?

sometimes...

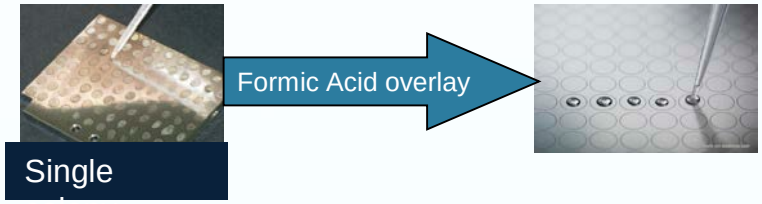
But there is a back-up plan

Testing Algorithm

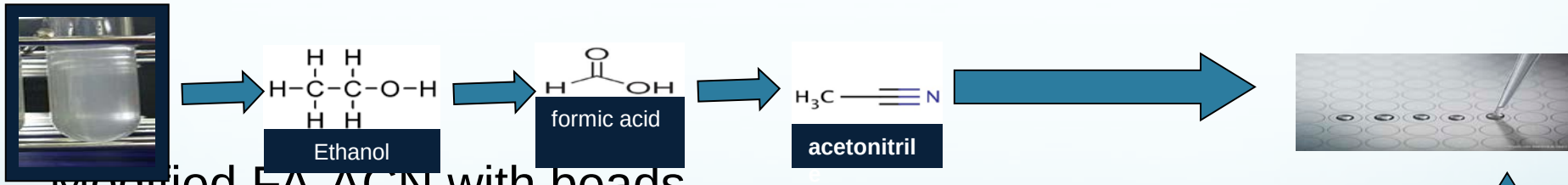


Three extraction methods routinely run according to a testing algorithm:

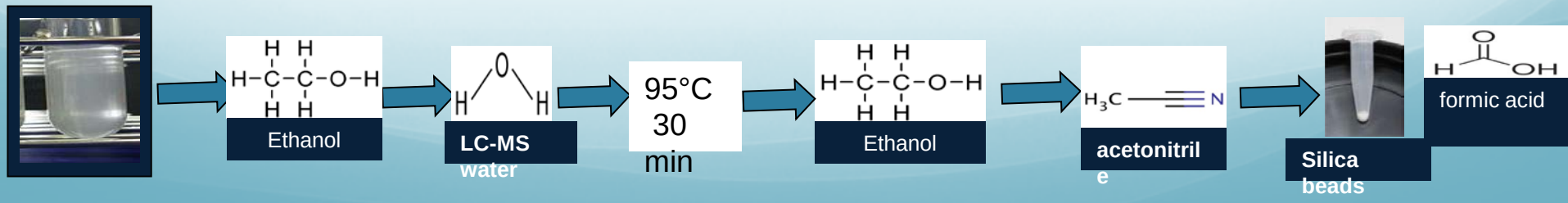
1. Extended direct smear (direct smear with 70% Formic Acid overlay)



2. Formic Acid-Acetonitrile Extraction (FA-ACN)



3. Modified FA-ACN with beads



Sometimes it is that easy, 10 min of prep and 10 min run

Reporting Algorithm

Score Value	Consistency Category	Extraction Method	Next closest species match	Result Report/Interpretation
≥2.2	A	Extended Direct Smear or Formic Acid/Acetonitrile Extraction	Not applicable	Report ID of top match
≥2.0 and <2.2	A	Extended Direct Smear or Formic Acid/Acetonitrile Extraction	>10% lower than top score	Report ID of top match
≥2.0 and <2.2	A	Extended Direct Smear or Formic Acid/Acetonitrile Extraction	Within 10% of top score	Report as *Genus*. Additional testing may be required
≥2.0	B or C	Extended Direct Smear or Formic Acid/Acetonitrile Extraction	>10% lower than top score	Report ID of top match
≥2.0	B or C	Extended Direct Smear or Formic Acid/Acetonitrile Extraction	Within 10% of top score	Report as *Genus*. Additional testing may be required
<2.0	B or C	Extended Direct Smear	Not applicable	Repeat using Formic Acid/Acetonitrile Extraction method.
≥1.9 and <2.0	B or C	Formic Acid/Acetonitrile Extraction	>10% lower than top score	Report as *Genus*. Include note: "Most closely resembles *Genus species*" of top match.
≥1.9 and <2.0	B or C	Formic Acid/Acetonitrile Extraction	Within 10% of top score	Report as *Genus*. Additional testing may be required
≥1.7 and <1.9	B or C	Formic Acid/Acetonitrile Extraction	Not applicable	Report as *Genus*.
<1.7	C	Formic Acid/Acetonitrile Extraction	Not applicable	Additional testing is required. Reflex to appropriate lab as determined by specimen source and

Our Plan

- **Step 1- Evaluation (4 months)**
  - Isolates sent for testing
  - Age of culture
  - Preliminary testing
- **Step 2- Validation (6months)**
  - 16S
  - General Bacteriology
  - Anaerobic Bacteriology
  - Enteric Bacteriology

NYS approval to test

- **Step 3- Implementation (11 months)**

Overview of Implementation of MALDI-TOF MS

In the 11 months since implementation:

- **>1700 clinical isolates from >1400 unique specimens**
- **This testing has resulted in the identification of >100 genera comprising >200 species.**

Reportable score (>2.0) by Extraction Method

Extended Direct Smear	FA-ACN Extraction	No Reliable Identification
76%	15%	9%

Testing Required for Final Identification

MALDI-TOF MS only	MALDI-TOF MS + Conventional Biochemical Analysis	MALDI-TOF MS + 16S rDNA sequence analysis	MALDI-TOF MS + Conventional Biochemical Analysis + 16S rDNA sequence analysis
50%	37%	1%	12%

NTM testing

Identification of NTM isolates with MALDI Implementation Data			
Final ID by *rpoB*/16S sequencing	Total isolates received	Reportable with score ≥ 2.0	Score ≥ 1.8 and < 2.0
M. gordonae	24	10	3
M. abscessus	11	9	0
M. fortuitum	16	11	0
M. chelonae	6	4	2
M. xenopi	9	2	4
M. marinum	3	1	0
M. mageritense	2	0	1
M. gastri	1	0	1
M. neoaurum	2	1	1
M. paragordonae	1	0	0
M. peregrinum	1	0	0
M. salmoniphilum[#]	1	0	1[#]
M. septicum	1	0	0
M. triviale	1	0	0
M. arupense	1	0	0
M. parafinicum	1	0	0
M. terrae complex	1	0	0
*M. phocaicum**	2	1	1
*M. porcinum***	1	0	0
Mycobacteria spp.[+]	4	0	0
total	89	39(44%)	14(16%)[++]

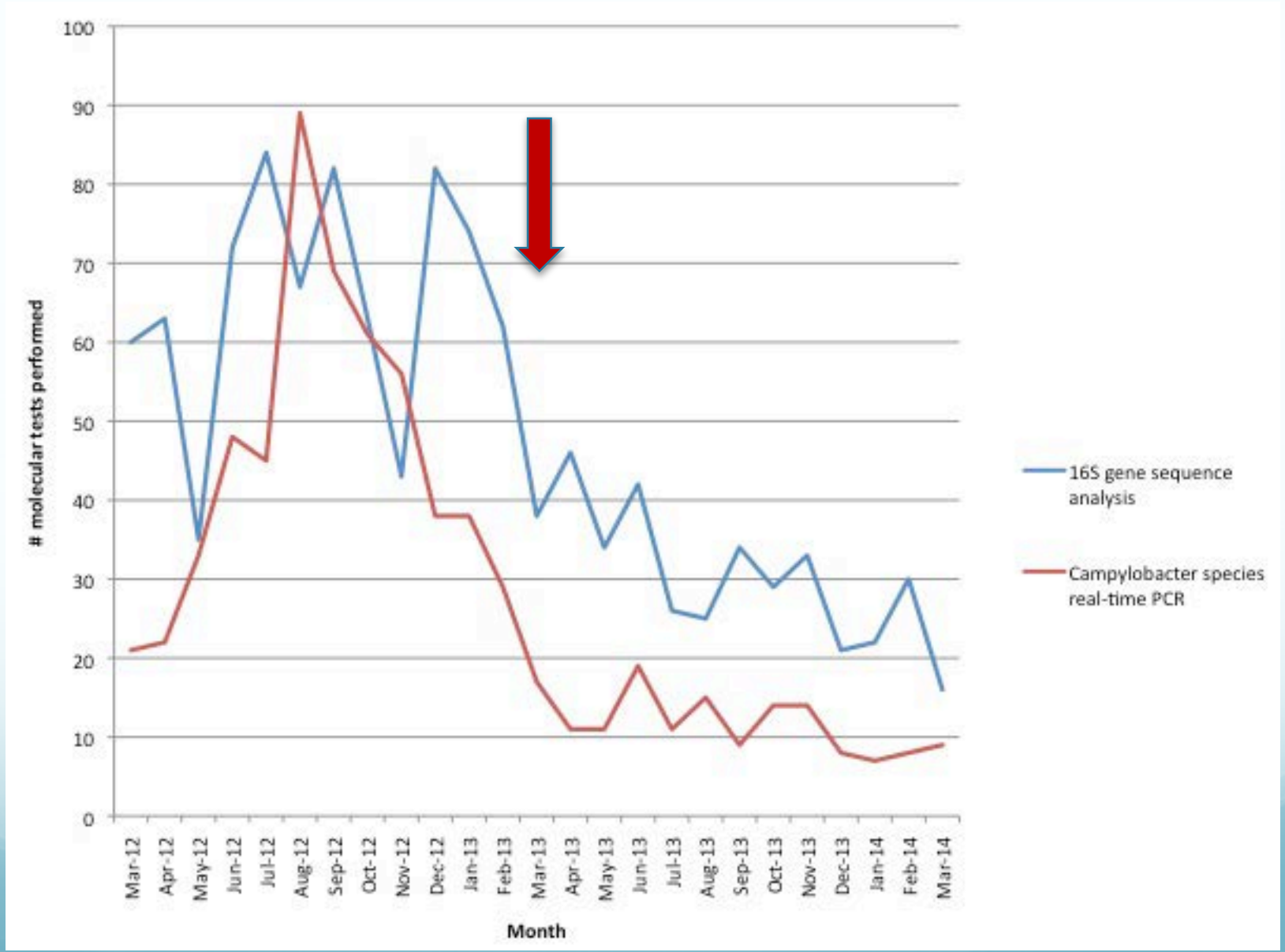
Results

- **MALDI-TOF MS implementation has resulted in a significant in esting volume**
  - **46 % reduction in the # of biochemical tests needed**
  - **52 % reduction in 16S rDNA sequence analysis**
  - **72 % reduction in *Campylobacter* species real-time PCR**

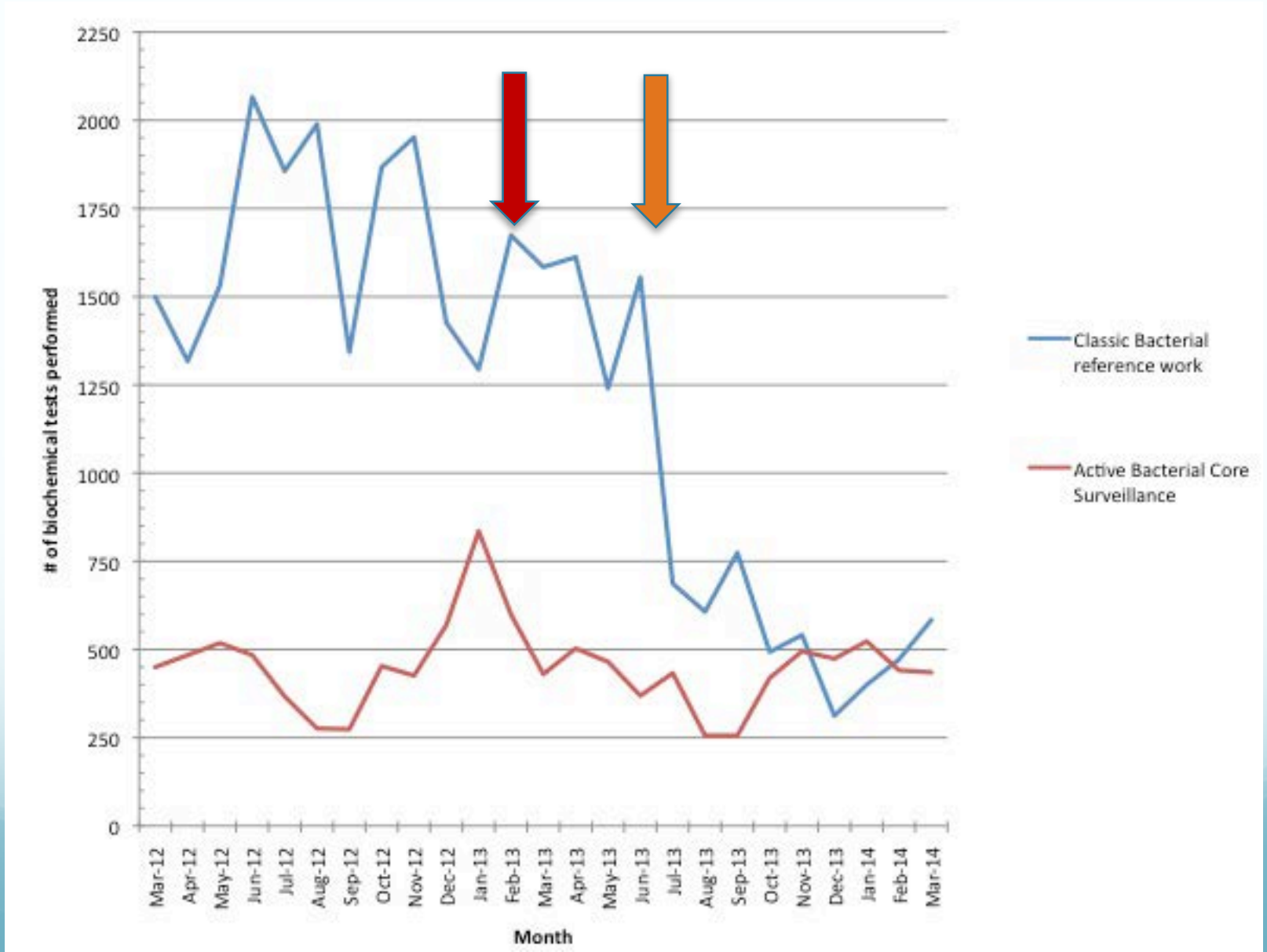
- **TAT to final identification has 13.2 net work days pre-implementation to 8.2 net work days post-implementation, a time savings of 5 full days on average**

- **Average TAT by genus also for nearly every genus analyzed**
  - **2-4 days for *Campylobacter, Clostridium, Corynebacterium,* and *Enterococcus***
  - **6-9 days for *Actinomyces, Burkholderia, Nocardia* and *Pseudomonas***
  - **18-24 days for *Achromobacter, Moraxella* and *Staphylococcus***
  - **32-37 days for *Acinetobacter, Enterobacter* and *Streptococcus* (excluding *S. pneumoniae, S. pyogenes* and *S. agalactiae*)**

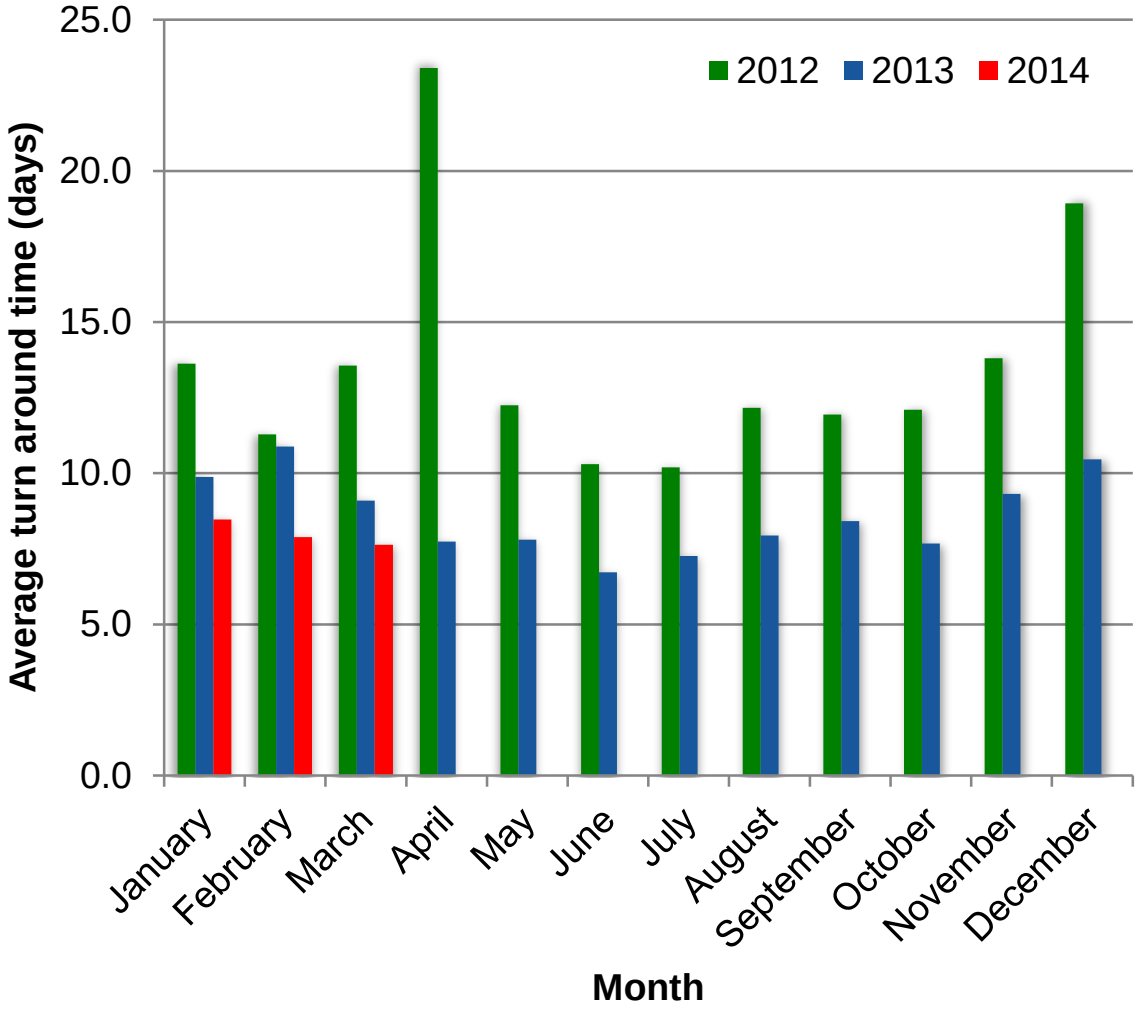
Decrease in 16S and Real-time PCR testing volume due to implementation of MALDI-TOF MS



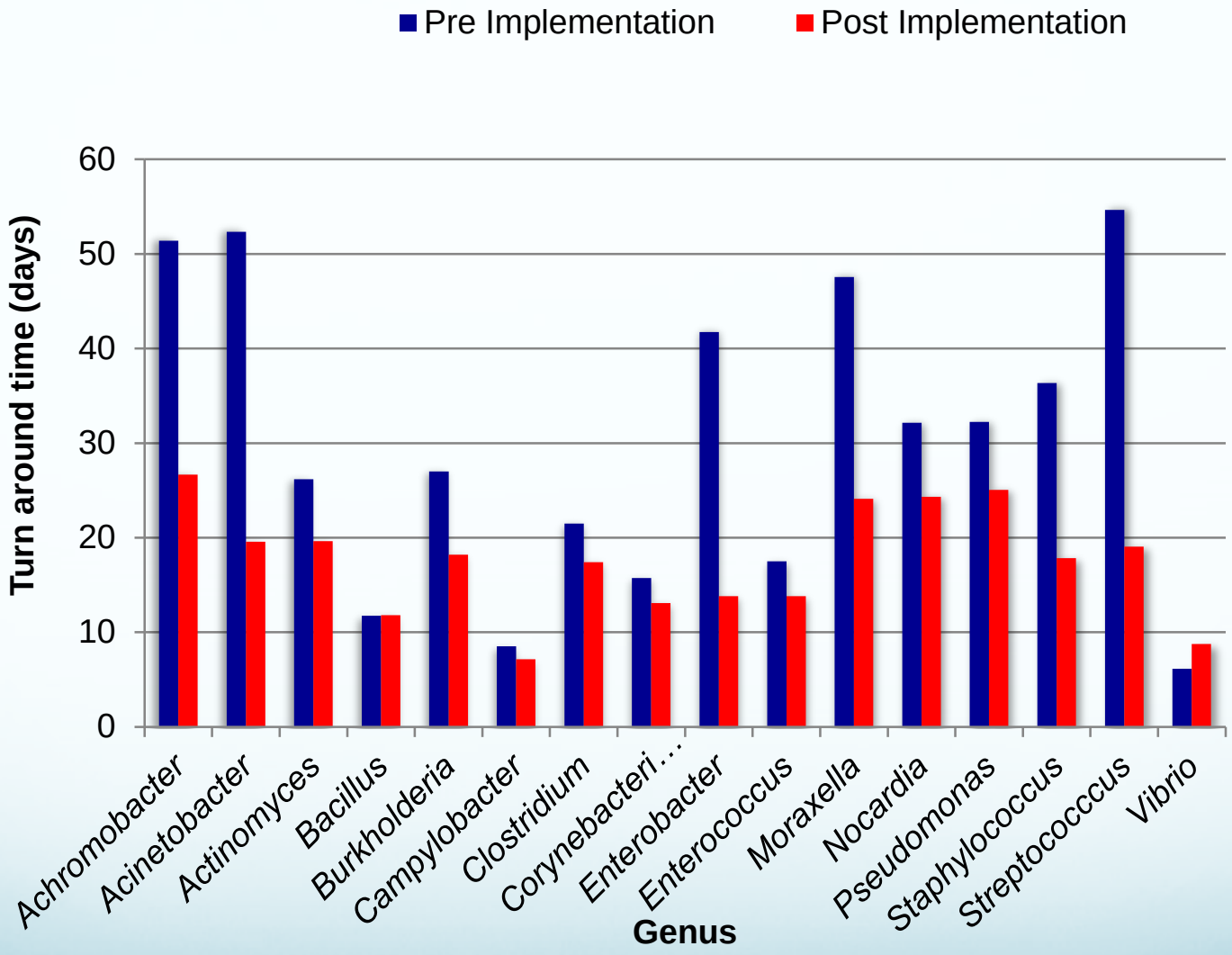
Decrease in biochemical testing volume due to implementation of MALDI-TOF MS



Decrease in TAT by month as a result of implementation of MALDI-TOF MS



Decrease in TAT by genus due to implementation of MALDI-TOF MS



*** The average TAT remained the same or decreased, by as much as 36 days.**

Estimated Cost Savings Analysis Resulting from MALDI-TOF MS Implementation

Test	Cost of test (reagents only)	Estimated staff time needed to perform test	Hourly rate including benefits	Estimated cost in staff time per test	Total cost per test (staff and reagents)
16S rDNA Sequence Analysis	$12.00	3 hours	$73	$219.00	**$231.00**
Campylobacter Real-time PCR*	$5.00	0.5 hours	$73	$36.50	**$41.50**
Individual Biochemical Test	$1.00	0.15 hours	$73	$10.95	**$11.95****
MALDI-TOF MS	$0.50	0.5 hours	$73	$36.50	**$37.00**

Total cost per test calculation of four unique tests performed by the WC. The table above calculates the total cost per test including reagent cost and staff time of 4 tests performed for bacterial identification.

*This real-time PCR test represents one of many that are impacted by this implementation.

**This represents one biochemical test not a panel of tests utilized.

Total Savings

Test	Total cost per test (staff and reagents)	Pre-implementation		Post-implementation		Predicted cost savings per month	Estimated cost savings since implementation
		Average # of tests/ month	Cost per month	Average # of tests/month	Cost per month		
16S rDNA Sequence Analysis	$231.00	66	$15,246.00	32	$7,392.00	$7,854.00	$109,956.00
Campylobacter Real-time PCR	$41.50	47	$1,950.50	13	$539.50	$1,411.00	$19,754.00
Individual Biochemical Test	$11.95	1649	$19,705.55	895	$10,695.25	$9,010.30	$126,144.20
MALDI-TOF MS	$37.00	0	$0.00	140	$5,180.00	-$5,180.00	-$72,520.00
Total			**$36,902.05**		**$23,806.75**	**$13,095.30**	**$183,334.20**

Considerations

- **Importing to in-house LIMs system**
- **Many algorithms**
  - **Deciding where to implement**
  - **Reflex testing**
  - **Decision making**
  - **Requisition test request**
- **Determining which staff will run, interpret, review results**
- **Gram- negative rods- rule out *Brucella* spp.**
- **Controls, SOP cleaning plates, remote laser tuning**

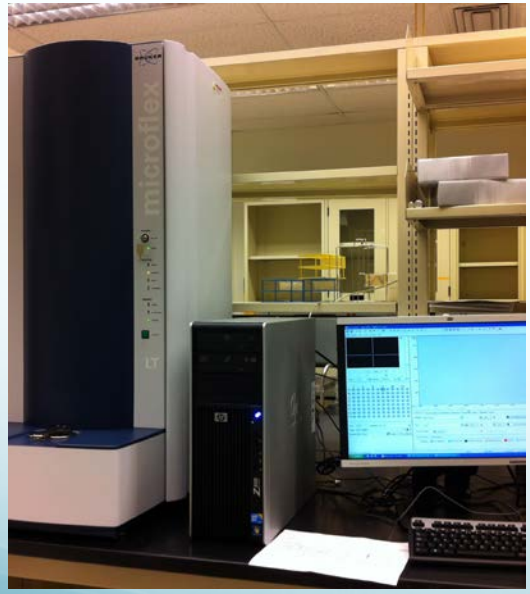
Future applications

- **Upcoming projects involving:**
  - **Assessment specimen viability**
  - **Other bacterial genus not examined in evaluation**
  - **Typing, species differentiation**
  - **Antibiotic resistance detection**
  - **Identification from clinical specimens (blood culture, urine, CSF)**

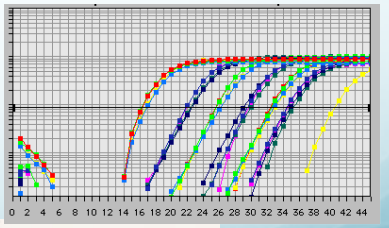
Conclusions

- **MALDI-TOF MS is a fast, reliable, inexpensive technique for bacterial identification in our laboratory.**
- **Extended direct smear is adequate for most bacterial isolates.**
- **FA-ACN extraction is a faster, more cost effective alternative to 16S rDNA sequence analysis and biochemical analysis for a subset of isolates.**
- **Implementation of MALDI-TOF MS has decreased workload, TAT and costs for WC.**
- **We estimate at least 22 days in staff time is saved each month.**
- **It is estimated that use of MALDI-TOF MS has resulted in over $180,000.00 in reagent and staff savings since February 2013.**
- **We predict that we can save nearly $13,000 a month in reagents and staff time with MALDI-TOF MS in our testing algorithm.**

The WC Bacteriology Lab of Today



- 6,000 isolates/year sub-cultured
- MALDI-TOF MS
- ½ amt. Biochemical tests
- ~4000 real-time PCR tests/year
- ~350 sequencing reactions/year



Acknowledgements

- **Lisa Mingle**
- **Elizabeth Nazarian**
- **Kara Mitchell**

- **Bacteriology Lab**
  - **Linda Gebhardt**
  - **Michelle Dickinson**
  - **Teresa Passaretti**
  - **Anna Kidney**
  - **Nellie Dumas**
  - **Donna Kohlerschmidt**
  - **Geetha Nattanmai**
  - **Sherly Jose**
  - **Andrea Carpenter**
  - **Jill Hayes**
  - **Tim Root**
  - **Charles MacGowan**
  - **Leeanna Armstrong**
  - **George Hannett**
  - **Jocelyn Cole**
  - **Yan Zhu**
  - **Jana McGinnis**
  - **Erin Parks**
  - **Dominic Centurioni**

- Mycobacteriology Lab
  - Vincent Escuyer
  - Michelle Isabelle
  - Susan Wolfe
- Joseph Schwendemann
- Bruker Daltonics
  - Gary Kruppa
  - George Godesky
  - Markita Weaver
  - Justin Clark
- NYS CLIMS Group
  - Alok Mehta
  - Colleen Walsh
- Applied Genomic Technologies Core
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  - Lisa Mingle
  - Elizabeth Nazarian
  - Sudha Chaturvedi
  - Mike Perry
  - Nellie Dumas