Mass Spectrometry – Applications to the Clinical Laboratory of the Future

David Herold, MD, PhD
Professor of Pathology, UCSD
MSACL – Founder and Chair

San Diego, CA

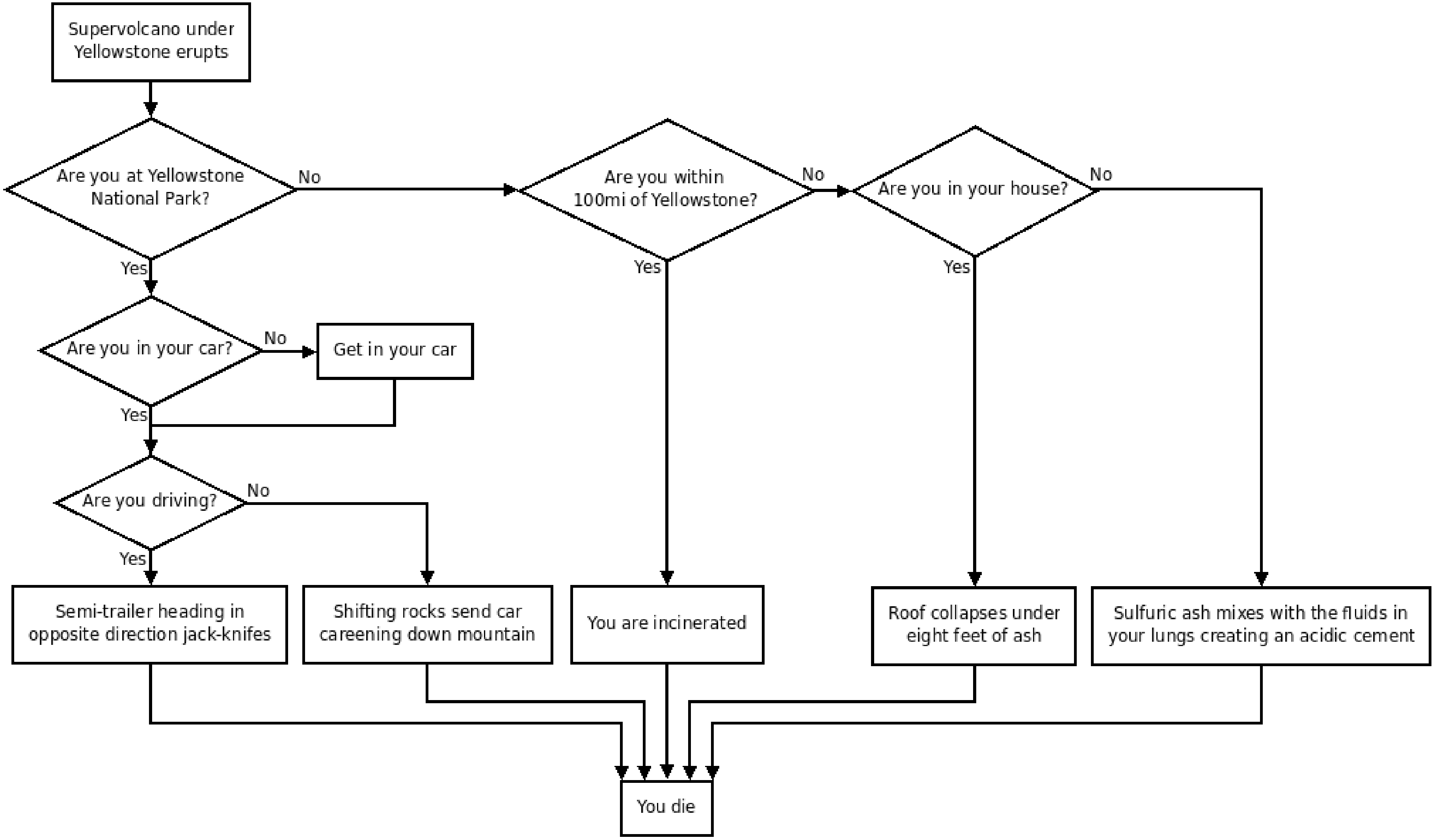
dherold@ucsd.edu

Wham!!!

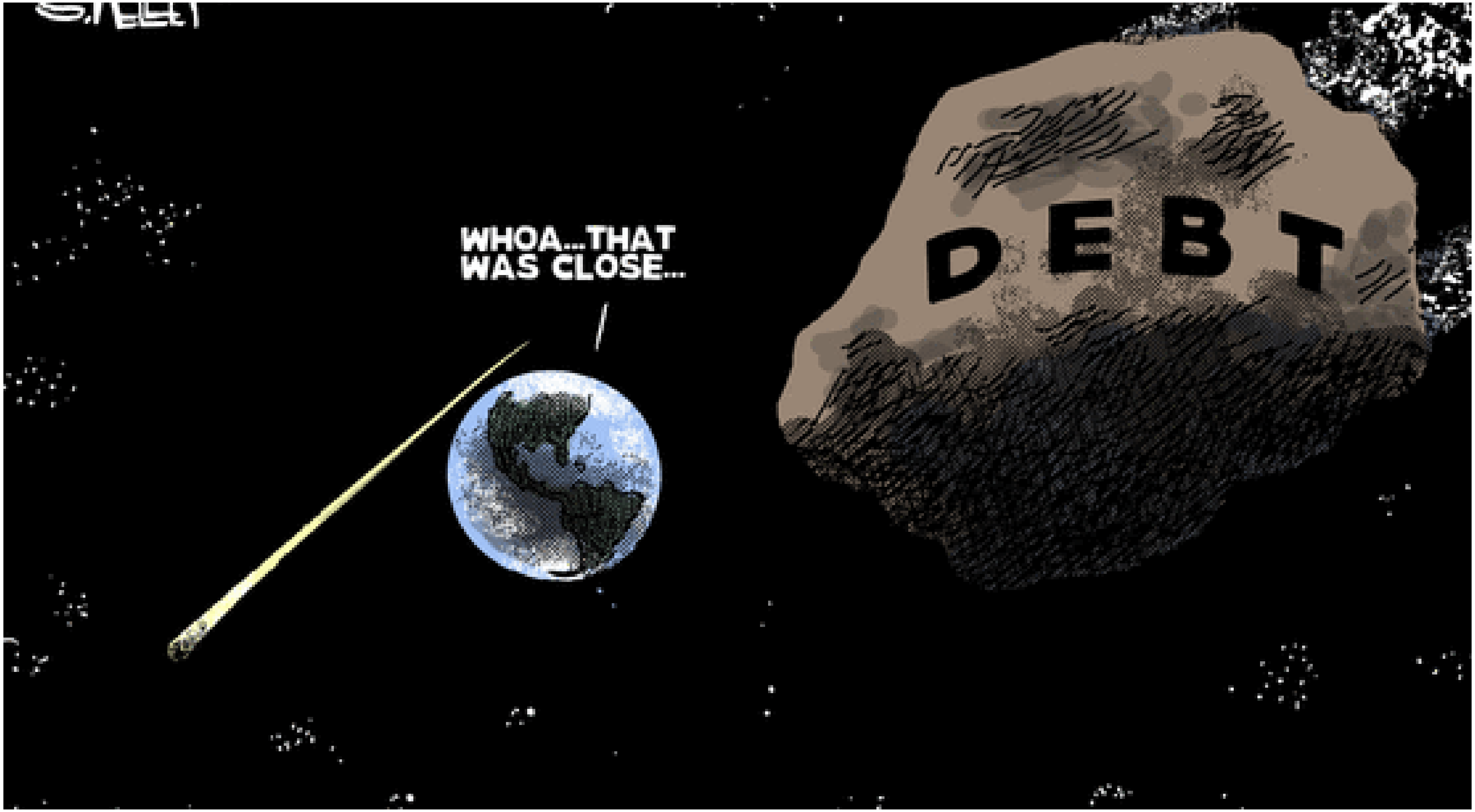
65 Million Years Ago

Where did Kansas go?

Outcome Diagram



Out in the dark – destination Earth



Out of Cash!

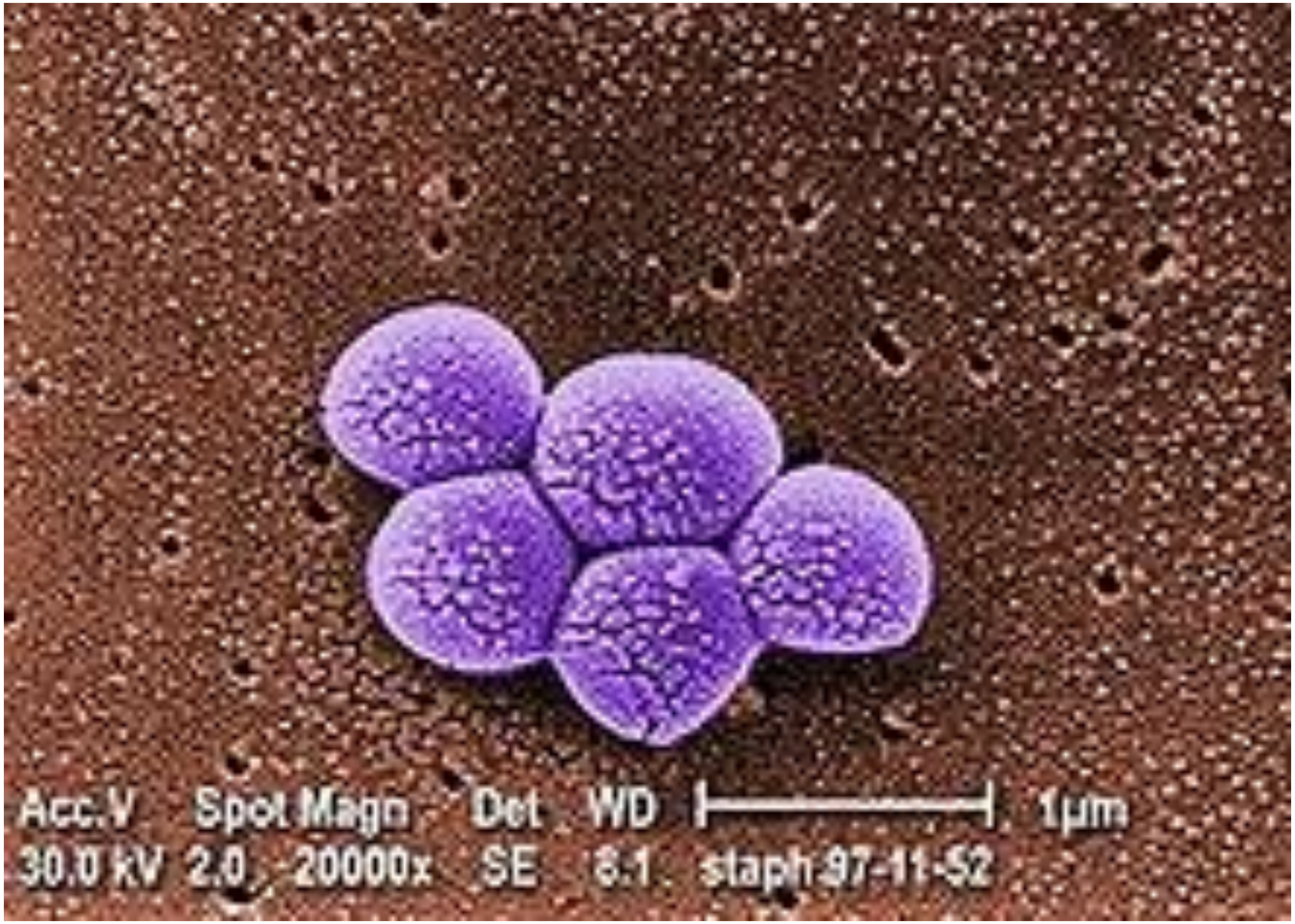
But the scariest,
highest impact threat
are the

Antibiotic Resistant

Microbiota!

Nonresponsive Cellulitis

MRSA



And that is just the tip of the iceberg!

Absence of effective antimicrobial drugs

- "Resistance is said to present a risk that we will fall back into the pre-antibiotic era.”

- "Resistance is not just an infectious disease issue," they say. "It is a surgical issue, a cancer issue, a health system issue."

Resulting in Change in Practice

- Estimate infection rates after hip replacement would increase from about 1% to 40-50%, and that about a third of people with an infection would die. It seems likely that rates of hip replacement would fall, bringing an increased burden of morbidity from hip pain.

- *BMJ* 2013;346:f1663

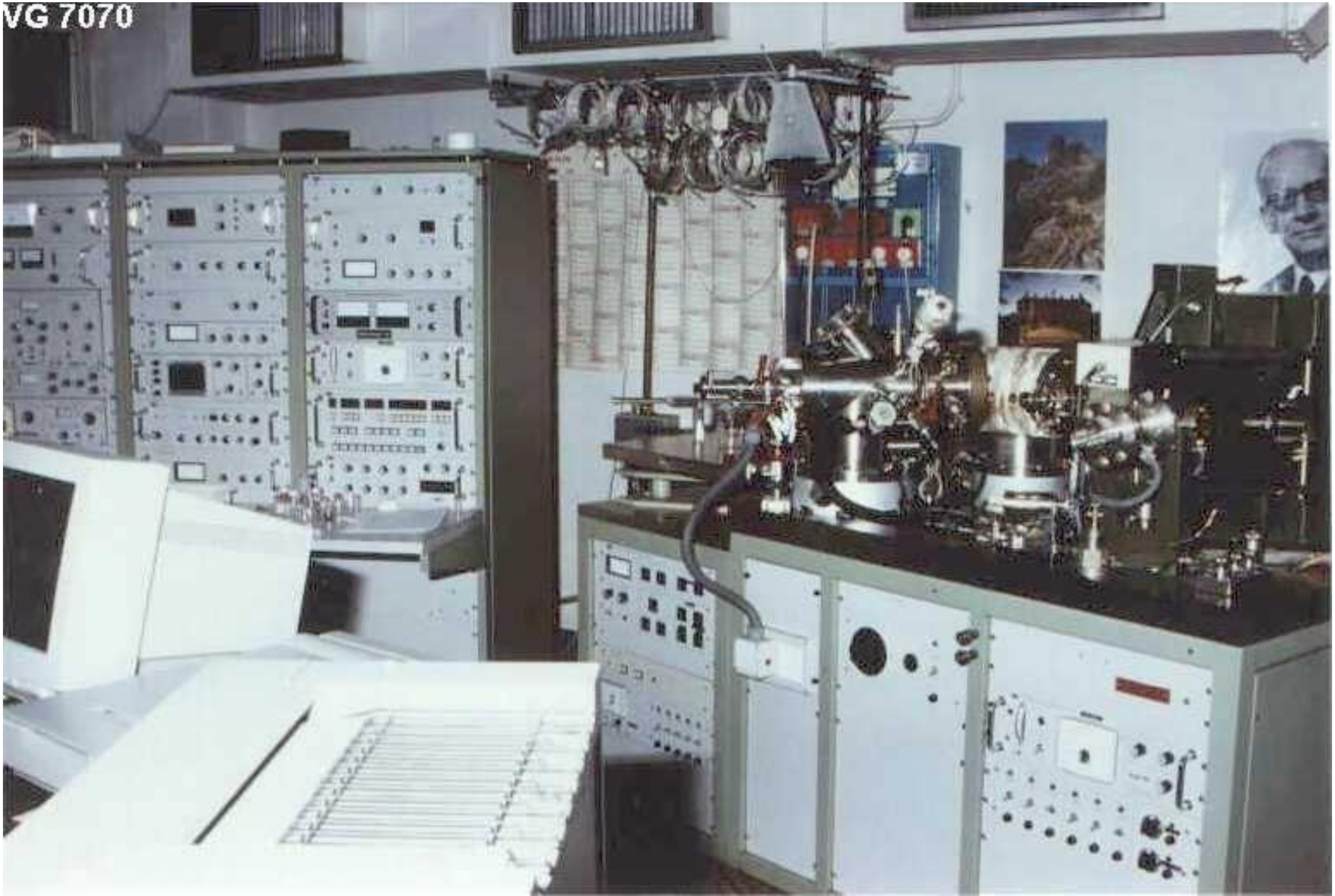
Is Mass Spectrometry an Answer?

- For the Financial Crisis.

- For the Microbial Threat.

- For Other Laboratory Needs.

Your Impression of Mass Spectrometer?



And Related Personnel Problems

However, When I Think Mass Spec:

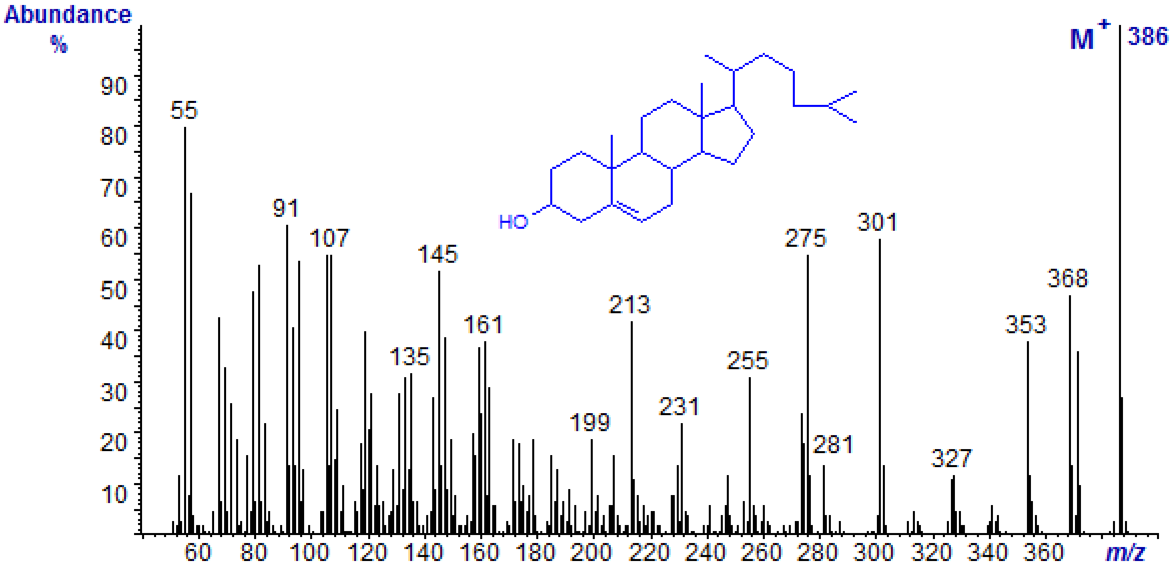

R.K.
R.K.
R.K.
R.K.

Today’s Mass Spectrometer

The Fundamentals of Mass Spec

- carbon has a mass of 12
- hydrogen has a mass of 1
- oxygen has a mass of 16
- nitrogen has a mass of 14

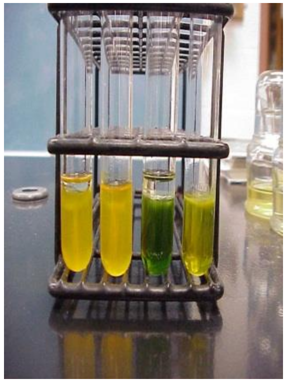
- But this is not strictly true, but close enough



$$C_{27}H_{46}O = (12 X 27) + (1 X 46) + (16 X 1)$$
$$= 324 + 46 + 16 = 386$$

Microbiology Applications

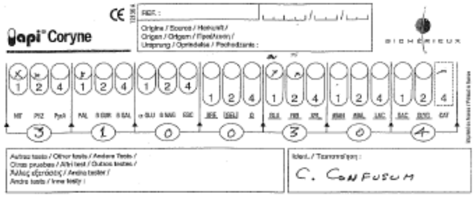
Traditional Microorganism Identification



The tube-method: pattern matching

- The utilization of patterns of reactions that indicate the most likely identification of an unknown organism
- First compendium or “library” were tables that indicated the expected +/- for different reactions
- Depending on the suspected organism – up to 20+ tubes might have been needed

Miniaturized Kits: pattern matching



api Coryne

REF. :

Origine / Source / Herkunft / Origen / Origem / Προέλευση / Ursprung / Oprindelse / Pochodzenie :

3 1 0 0 3 0 4

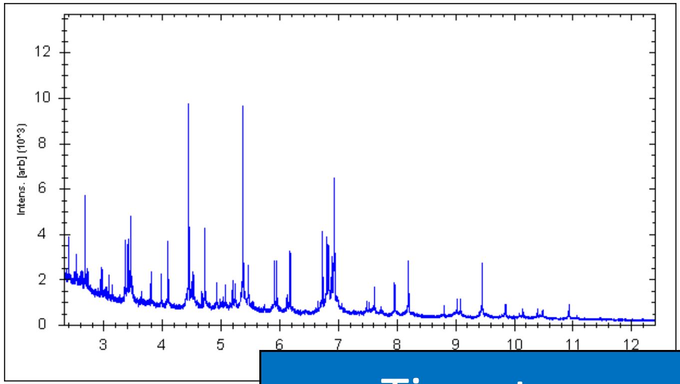
Autres tests / Other tests / Andere Tests / Otras pruebas / Altri test / Outros testes / Άλλες εξετάσεις / Andra tester / Andre tests / Inne testy :

Ident. / Ταυτοποίηση : C. CONFUSUM

API is a registered trademark of bioMerieux

- Biochemical test systems with improved ease of use, cost and reproducibility.
- As with the tube method, one compares a pattern of reactions produced for an unknown to known results contained in a database.
- Scores produced are evaluated in conjunction with other diagnostic information to arrive at an identification.

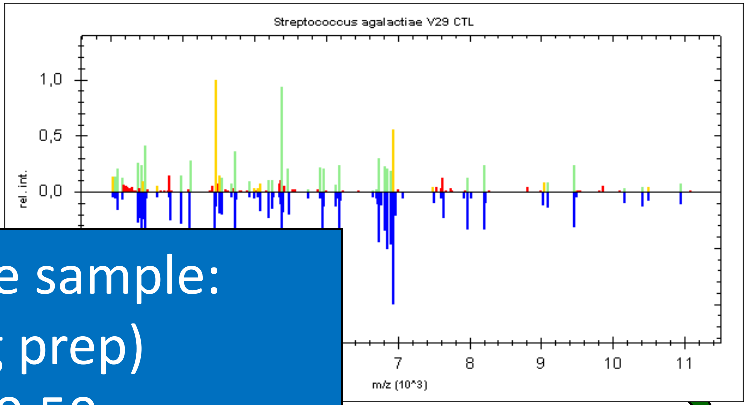
Identification via MALDI-TOF



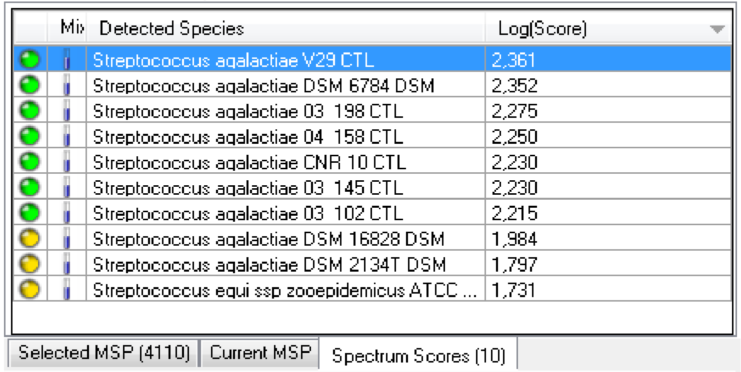
Generate ma

(protein

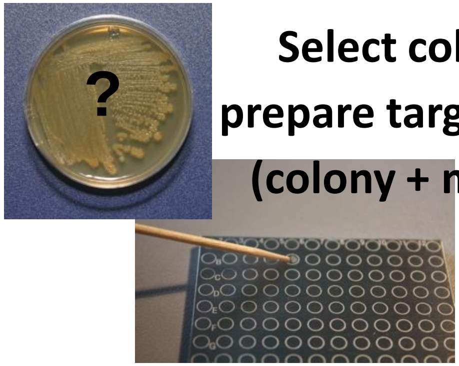
Time to result for one sample:
~ 5 min (including prep)
Cost: ~ $0.30 – 0.50



Match against database, a score is generated, and results are ranked, using thresholds for identification



Mix	Detected Species	Log(Score)
	Streptococcus agalactiae V29 CTL	2,361
	Streptococcus agalactiae DSM 6784 DSM	2,352
	Streptococcus agalactiae 03 198 CTL	2,275
	Streptococcus agalactiae 04 158 CTL	2,250
	Streptococcus agalactiae CNR 10 CTL	2,230
	Streptococcus agalactiae 03 145 CTL	2,230
	Streptococcus agalactiae 03 102 CTL	2,215
	Streptococcus agalactiae DSM 16828 DSM	1,984
	Streptococcus agalactiae DSM 2134T DSM	1,797
	Streptococcus equi ssp zooepidemicus ATCC ...	1,731

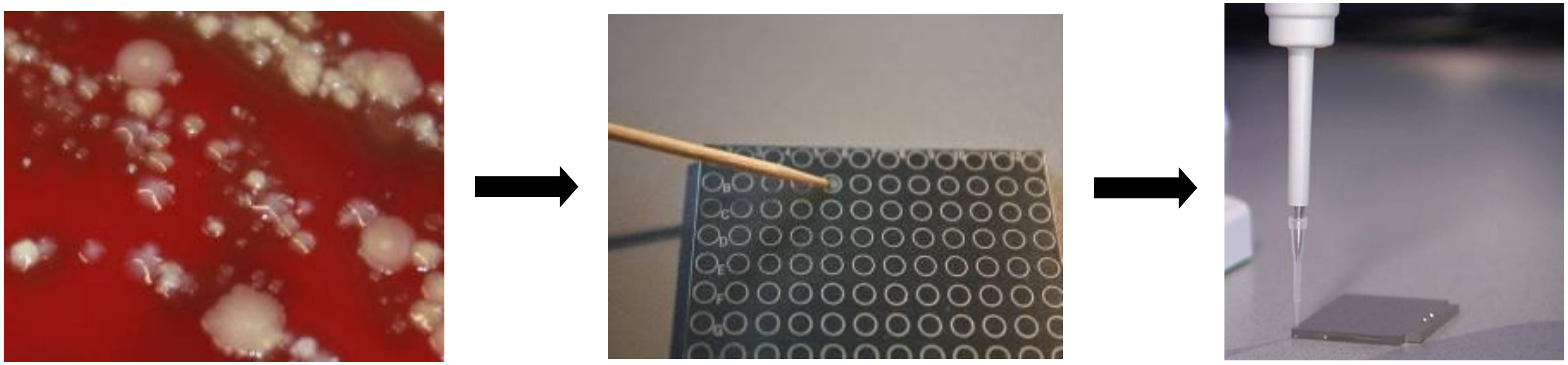


Select colony, prepare target plate (colony + matrix)

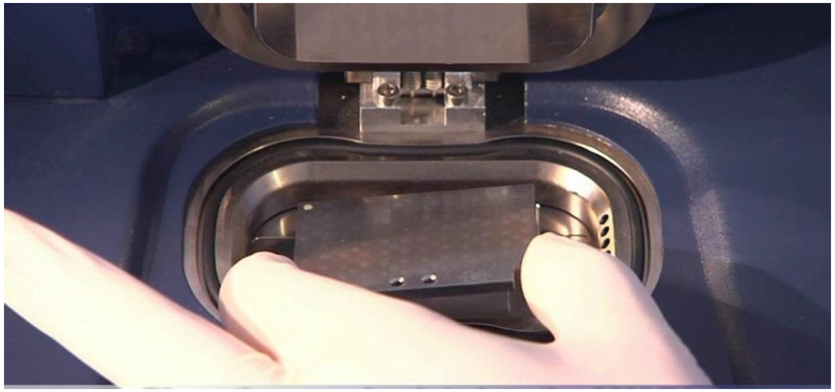
Step 1: Target Preparation

Direct Smear Method:

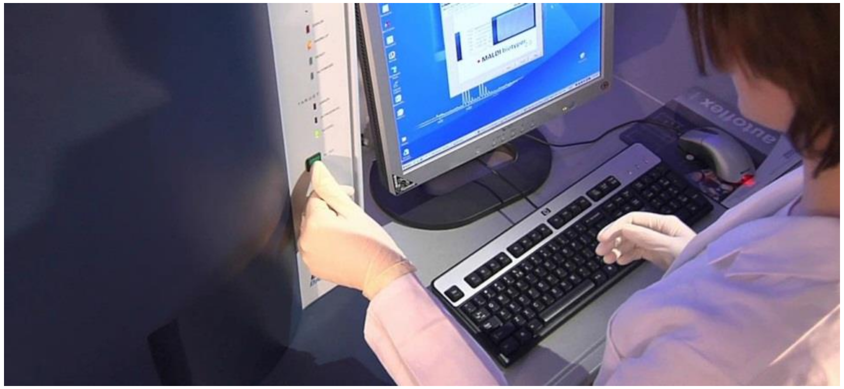
- Touch colony with transfer device, such as toothpick
- Transfer a small amount onto spot
- Let air dry
- Cover with 1 µL of MALDI matrix, let air dry
- Analyze up to 96 samples



Step 2: TOF (Time of Flight) Measurement

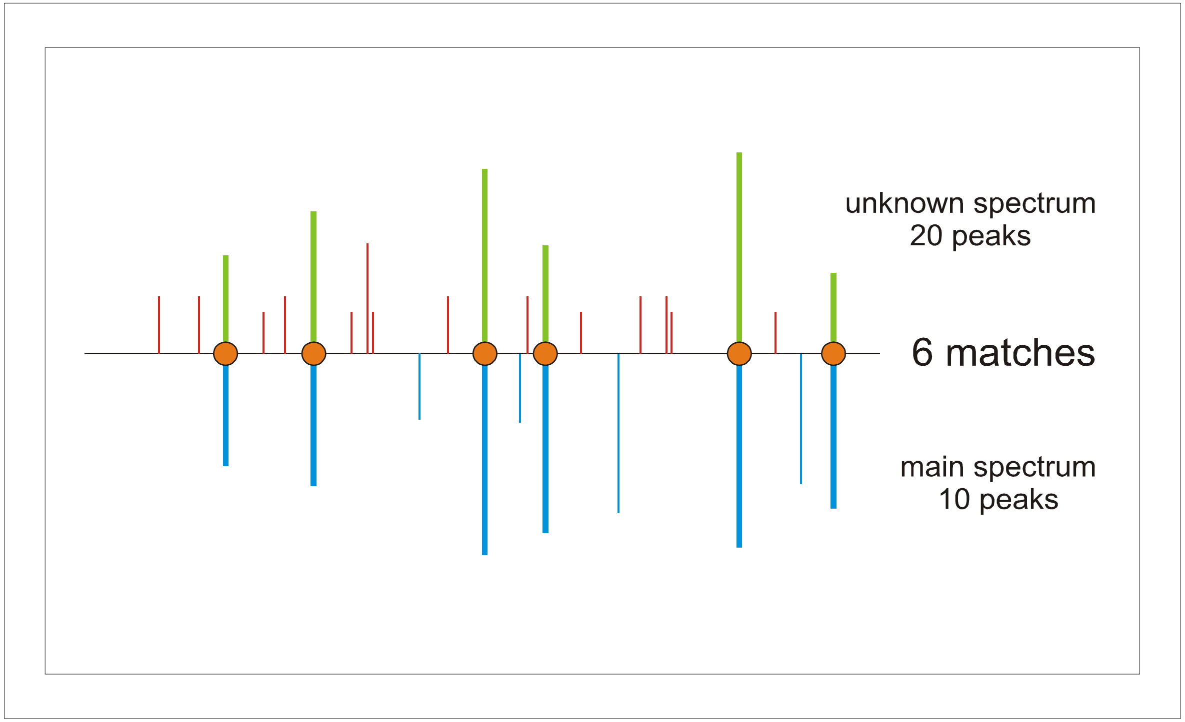


- Insert the dried target plate into the MALDI-TOF



- Close the sample door and start the run

Step 3: Identification



Calculation of a matching score based on:

Rel Score

% matches of the reference spectrum

(e.g. 6 / 10 = 0.6)

Rel P-Num

% matches of the unknown spectrum

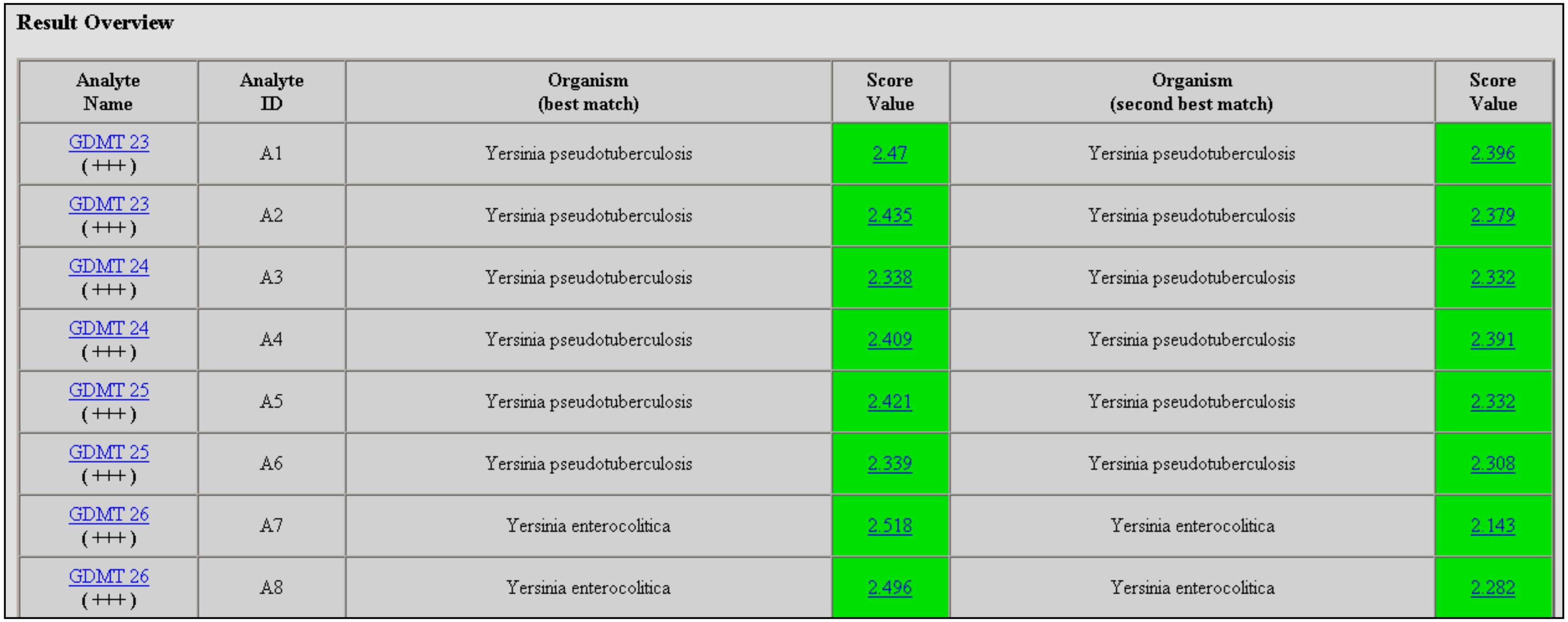
(e.g. 6 / 20 = 0.3)

I-Corr

value of intensity correlation

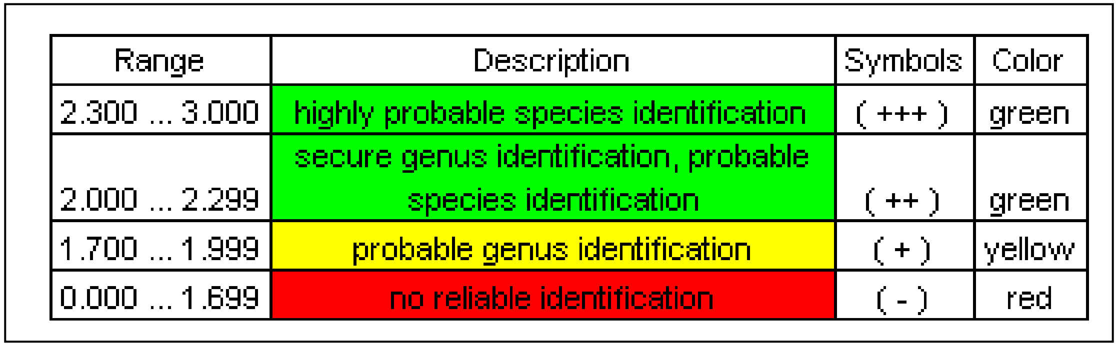
- **Unknown microorganism is matched against each main spectrum in the library**
- **Ranking according to matching score and threshold for ID**

Step 3: Identification - Results table



Result Overview

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
GDMT 23 (+++)	A1	Yersinia pseudotuberculosis	2.47	Yersinia pseudotuberculosis	2.396
GDMT 23 (+++)	A2	Yersinia pseudotuberculosis	2.435	Yersinia pseudotuberculosis	2.379
GDMT 24 (+++)	A3	Yersinia pseudotuberculosis	2.338	Yersinia pseudotuberculosis	2.332
GDMT 24 (+++)	A4	Yersinia pseudotuberculosis	2.409	Yersinia pseudotuberculosis	2.391
GDMT 25 (+++)	A5	Yersinia pseudotuberculosis	2.421	Yersinia pseudotuberculosis	2.332
GDMT 25 (+++)	A6	Yersinia pseudotuberculosis	2.339	Yersinia pseudotuberculosis	2.308
GDMT 26 (+++)	A7	Yersinia enterocolitica	2.518	Yersinia enterocolitica	2.143
GDMT 26 (+++)	A8	Yersinia enterocolitica	2.496	Yersinia enterocolitica	2.282

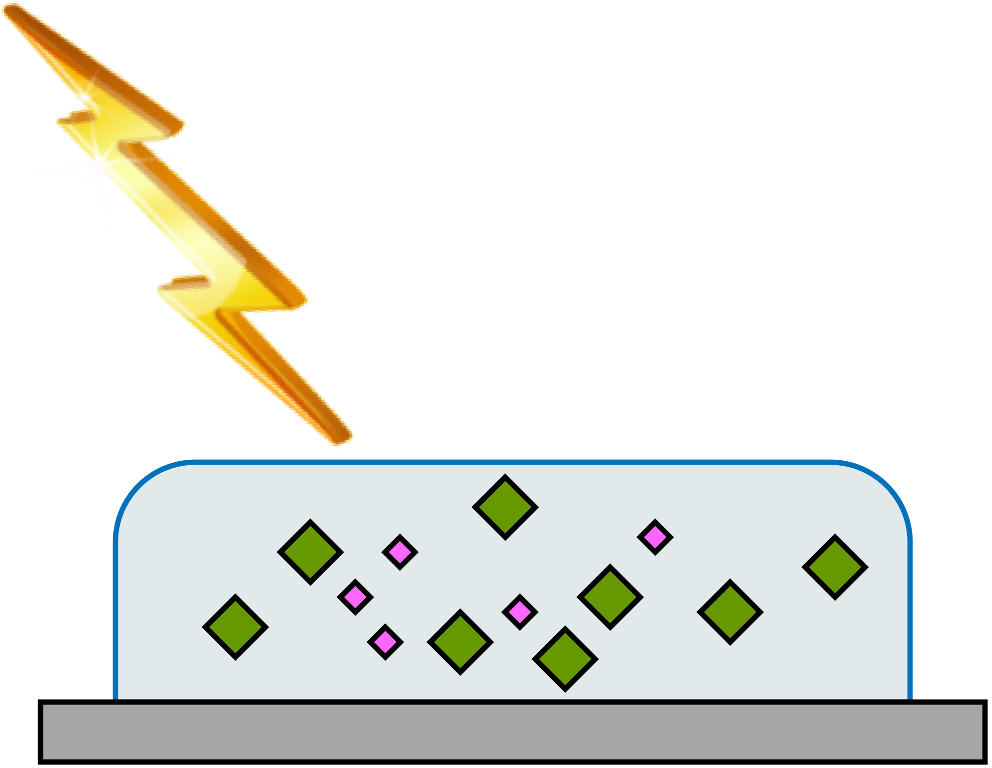


Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	no reliable identification	(-)	red

How does it work?

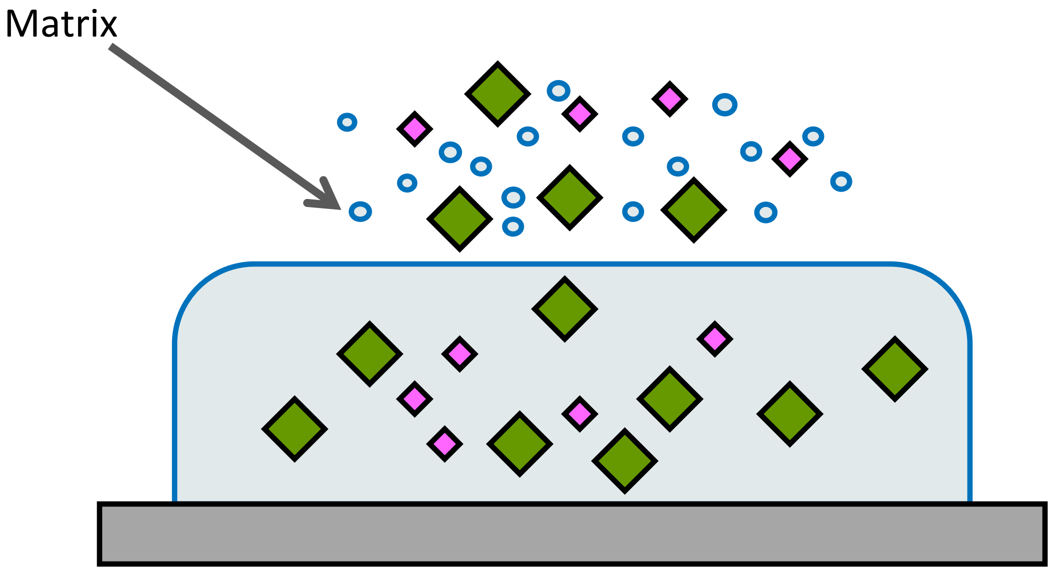
Matrix Assisted Laser Desorption/Ionization

- Matrix: alpha-cyano-4-hydroxycinnamic acid (1 uL)
- Matrix molecules readily absorb laser light (photon energy)
- The matrix is acidic, and donates positive charge to the analytes



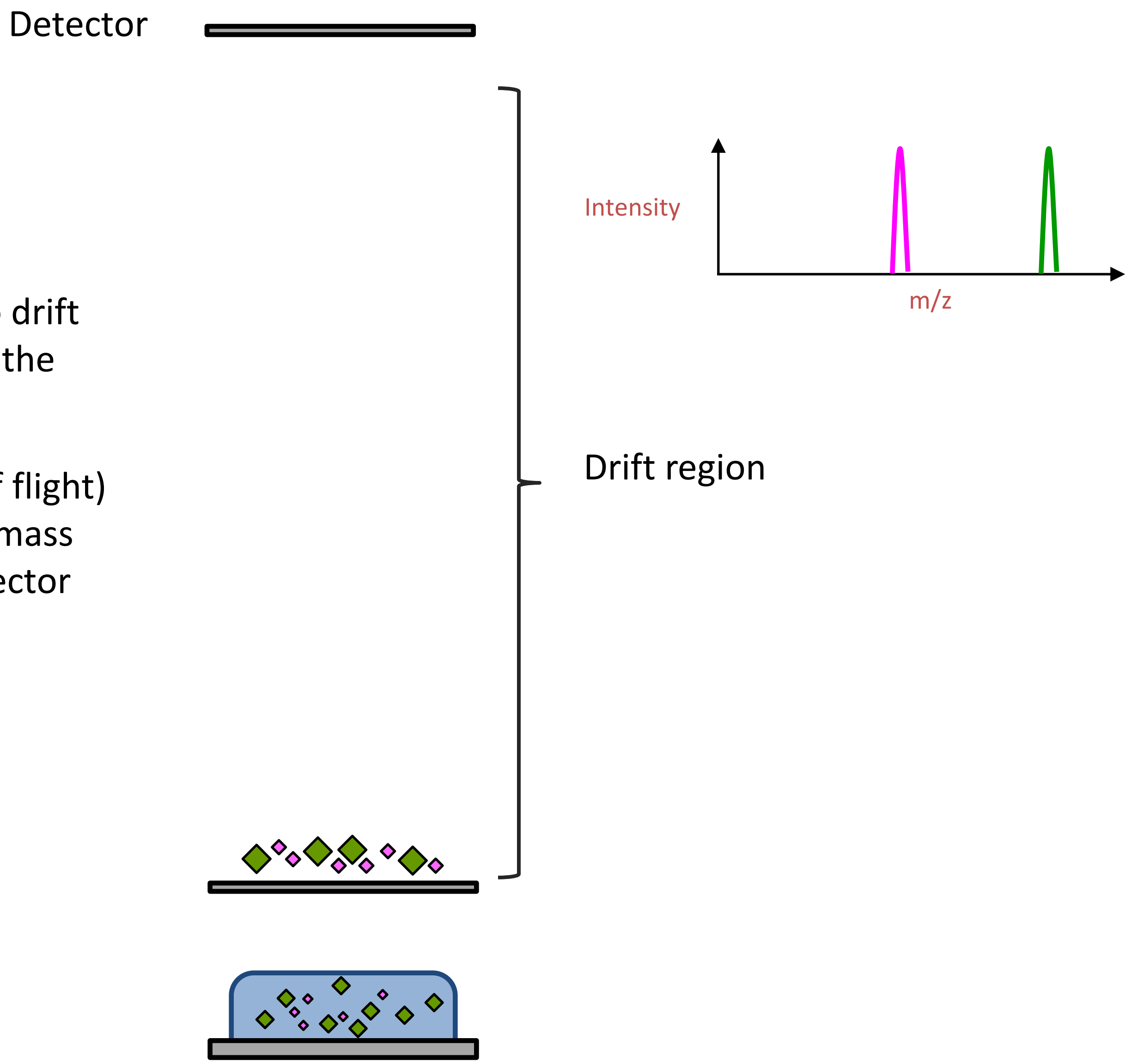
Matrix Assisted Laser Desorption/Ionization

- Localized heating causes micro-explosion of material
- Ions “desorb” from the target surface in the gas phase



TOF – Time of Flight

- Following acceleration, the charged ions are allowed to drift through a free field toward the detector
- The speed of travel (time of flight) is proportional to the ion’s mass (smaller ions reach the detector first)



Utility of MALDI-TOF Mass Spectrometry Following Introduction for Routine Laboratory Identification

Neville S, LeCordier A, Ziochos H, Chater M, Gosbell I, Maley, M and van Hal J

- Study was to determine the utility of MALDI-TOF in a routine diagnostic laboratory.
- One months of isolates run (N=927 run in triplicate)
- Cost study Run

Organism Group	Genus ID %	Species ID %
Anaerobes	97	64
Enterobacteriaceae	96	87
Gram + Rods	91	57
Gram + Cocci	95	83
Misc Gram -	100	92
NFGNR	100	89
TOTAL	96	84

Based on 927 isolates (1 month)
Current Method Cost= $10,354 (AUD$)
MALDI-TOF MS = $1,958 (AUD$)
Savings = $8,395 (AUD$)

JCM, August 2011

MALDI-TOF – Blood Culture Analysis

Positive blood culture bottle

Harvest 1 mL blood culture liquid in an Eppendorf tube
1 min

Add Lysis Buffer and mix
30 sec

Centrifuge (1 min, 13,000 rpm), discard supernatant
1 min

Add Washing Buffer and mix
1.5 min

Centrifuge (1 min, 13,000 rpm), discard supernatant
1 min

Suspend pellet in 300 µl water

***Schubert et al.*, ECCMID 2010**

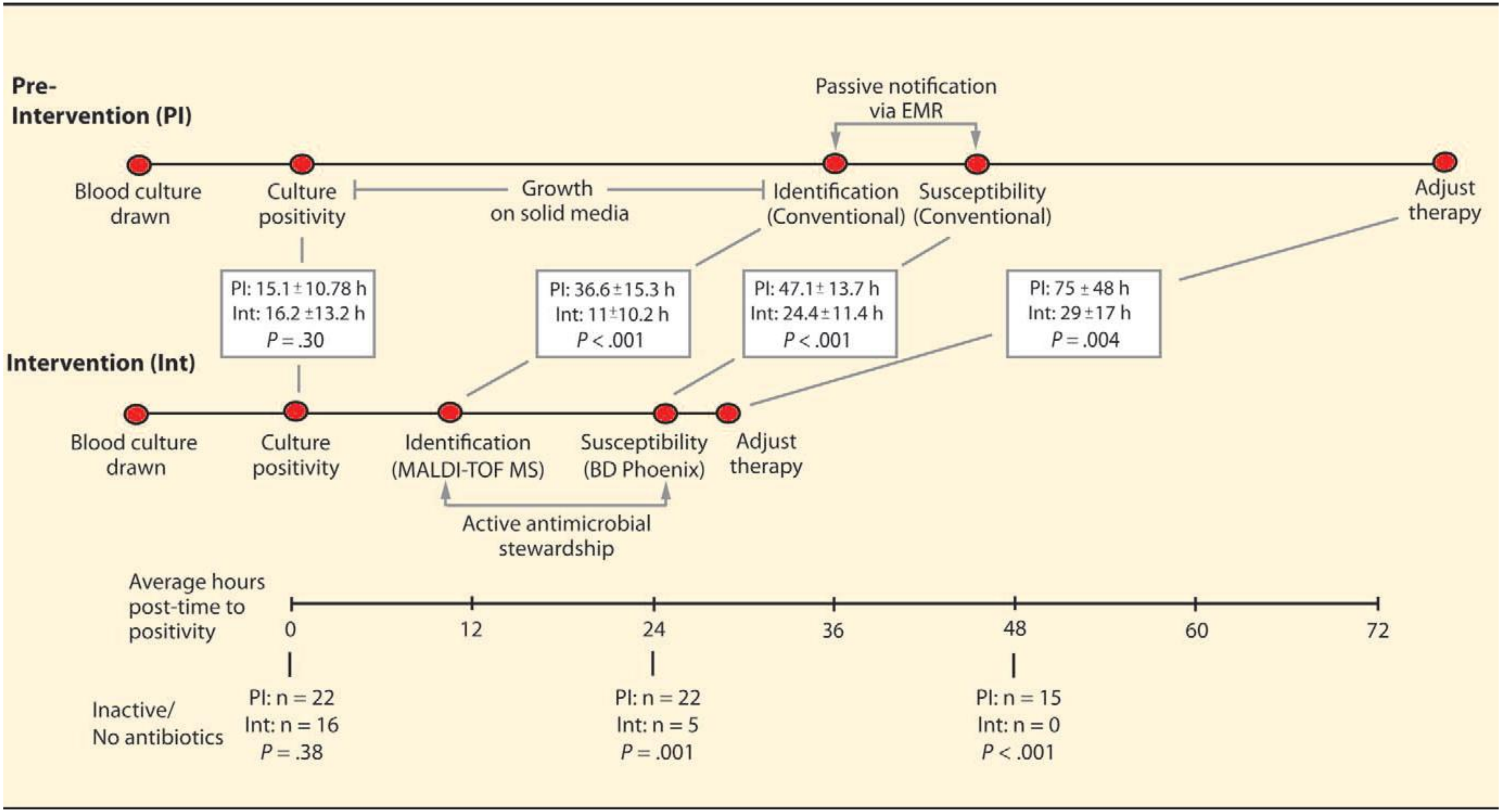
Total time for bacterial isolation
~5 min

Performance: >80% correct ID
no false-positive ID

The promise of MALDI-TOF Mass Spectrometry:

- **Time-to-result -> Faster**
- **Analytical capabilities -> Better**
- **Cost/sample -> Cheaper**

Archives of Pathology and Laboratory Medicine - K. Perez et al., epublished Dec 2012



Savings over Conventional Methods

- Cost savings for the more rapid id = $19,457/pt
- Methodist Houston is 1000 bed hospital
- Expect savings of $19 M/year
- USA has ~1,000,000 hospital beds
- Therefore, expected annual US savings = $19 B/year
- This is gram negative blood cultures only!
- Does not include gram positive, mycobacteria, mold and fungi - this will increase savings
- But we are leaving $26,162 on the table, thus…..


R.L.K.

Problem Addressed by Ibis PLEX-ID Biosensor Technology

- Over 1,000 infectious microbes known to cause disease in humans*
  - 217 viral species
  - 538 bacterial species
  - 307 fungi
  - 66 parasitic protozoa

- Numerous strain variations of each species (i.e., >100 strains of *Streptococcus pyogenes*)

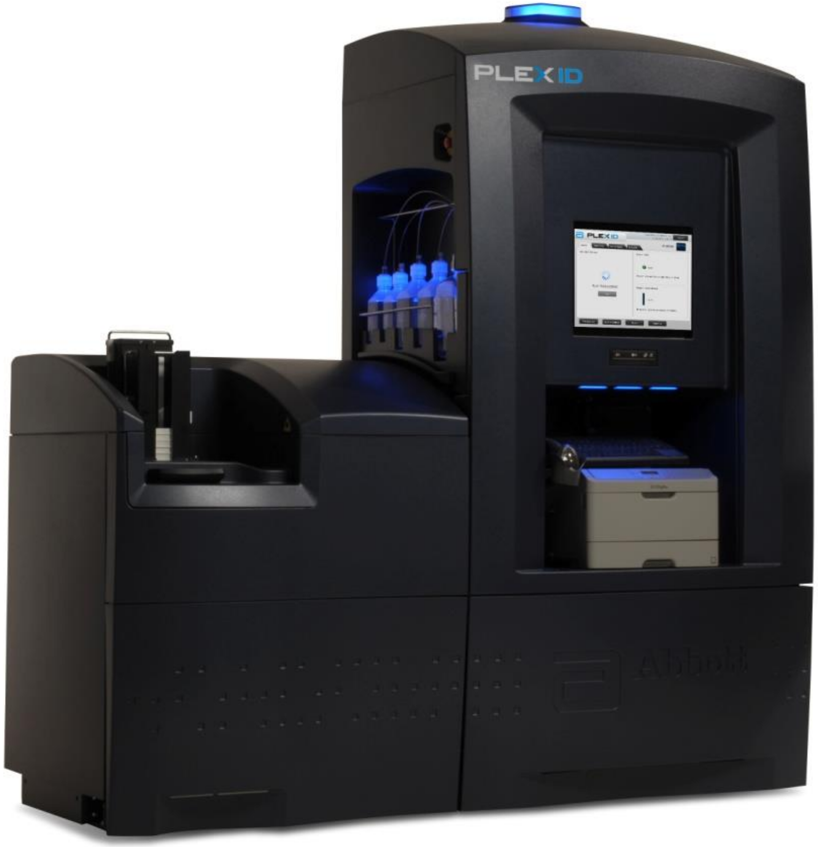
  - Emergence of multi-drug resistant and highly pathogenic strain types

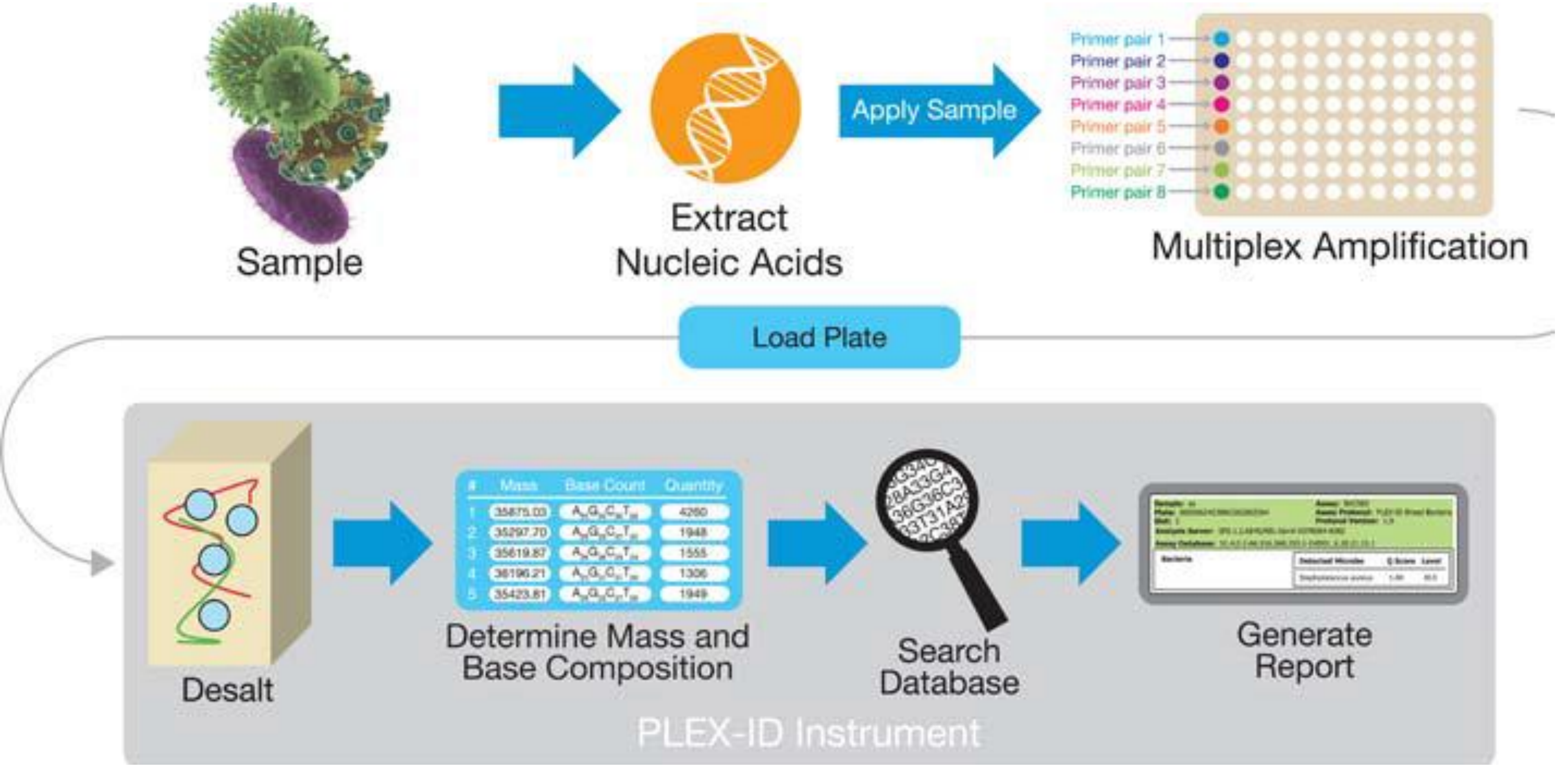
- Unknown and unculturable pathogens

*Taylor et al., Phil. Trans. R. Soc. Lond. B (2001) 356, 983-989.

Ibis PLEX-ID Biosensor

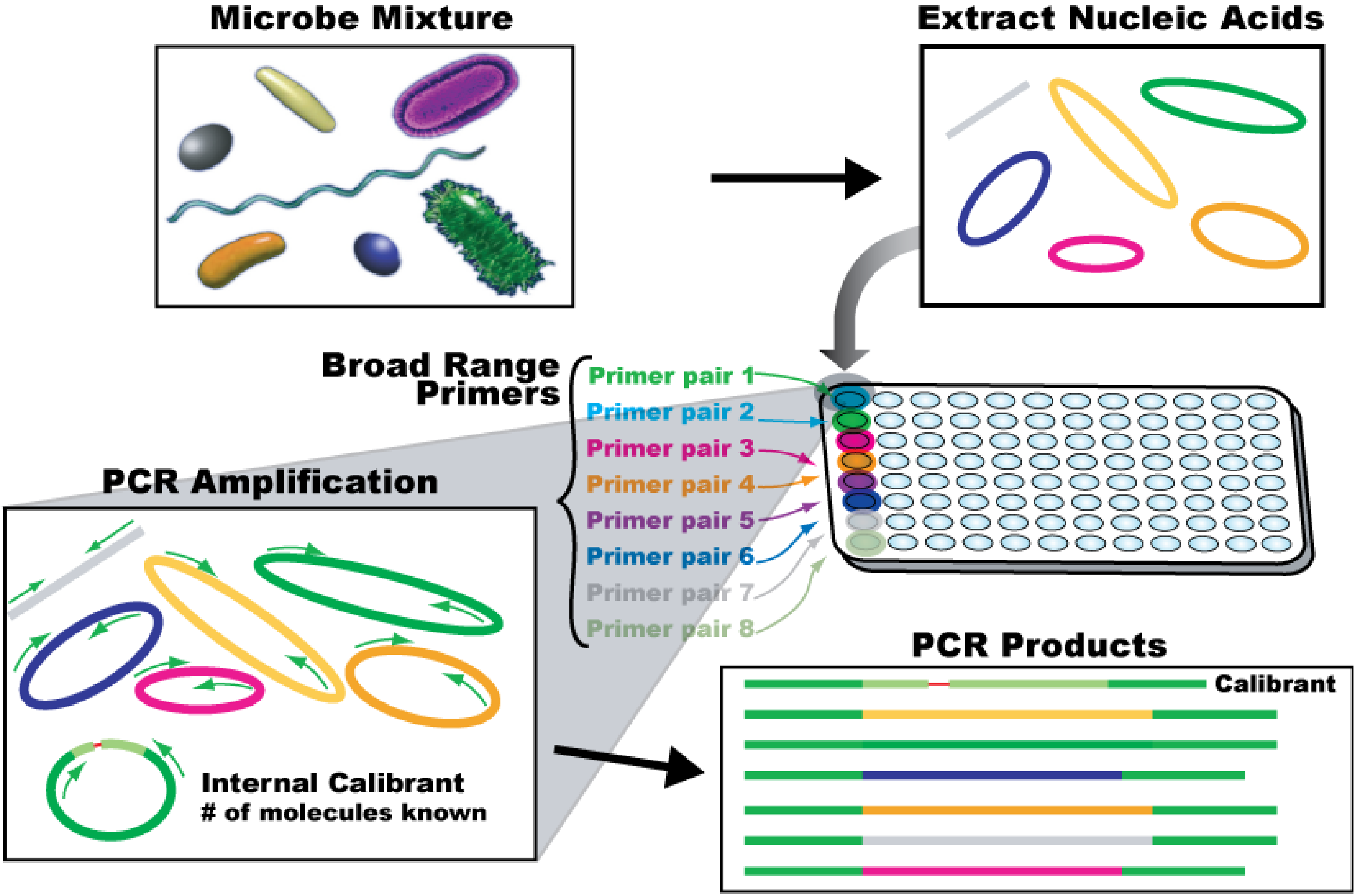
- Broad identification of all microbes
  - Bacteria, Viruses, Fungi, Parasites
- No culturing
- Detects mixtures of microbes
- High resolution genotyping and strain identification
- Drug resistance testing
- Identify emerging agents
- Rapid, high throughput, cost effective





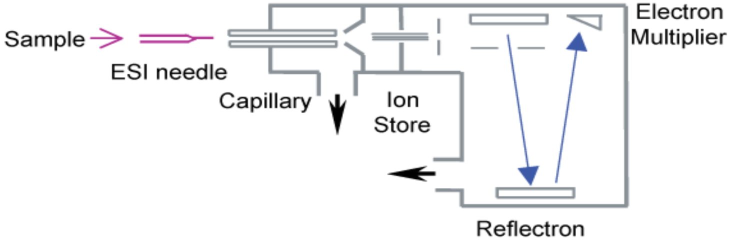
#	Mass	Base Count	Quantity
1	35875.03	[illegible]	4260
2	35297.70	[illegible]	1948
3	35619.87	[illegible]	1555
4	36196.21	[illegible]	1306
5	35423.81	[illegible]	1949

Ibis Process Part 1:
Sample Prep and Broad Range PCR

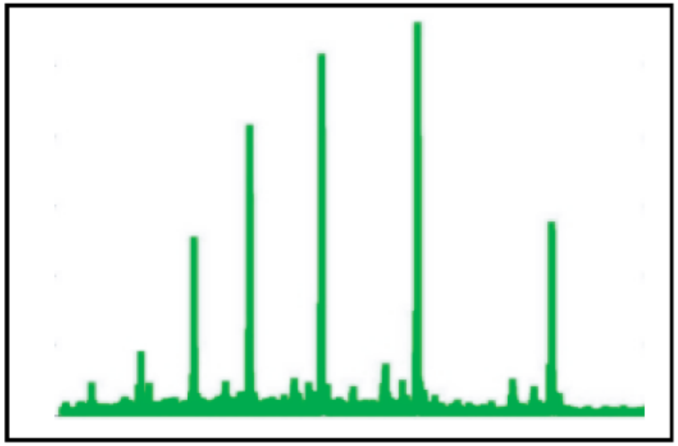


Ibis Process Part 2:
MS Analysis and Signal Processing

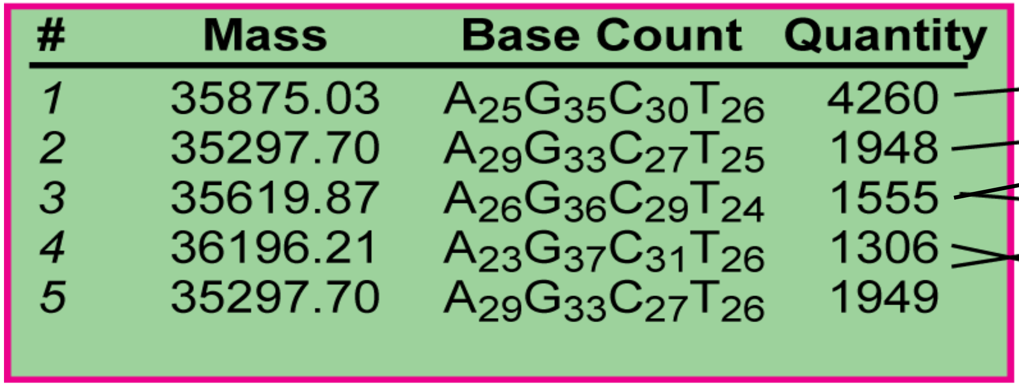
Mass Spectrometer



Spectral Signal

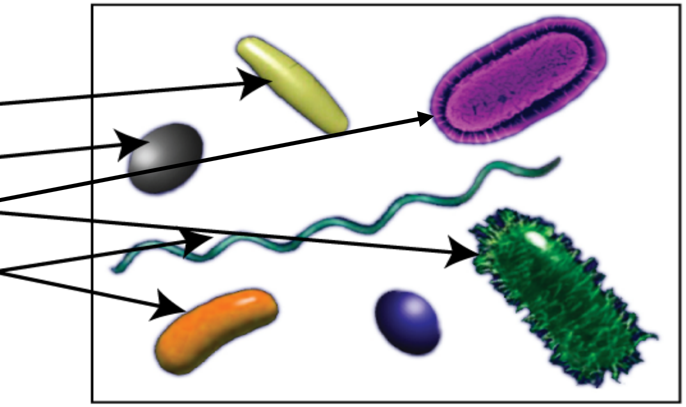


Signal Processing
Masses to Base Compositions

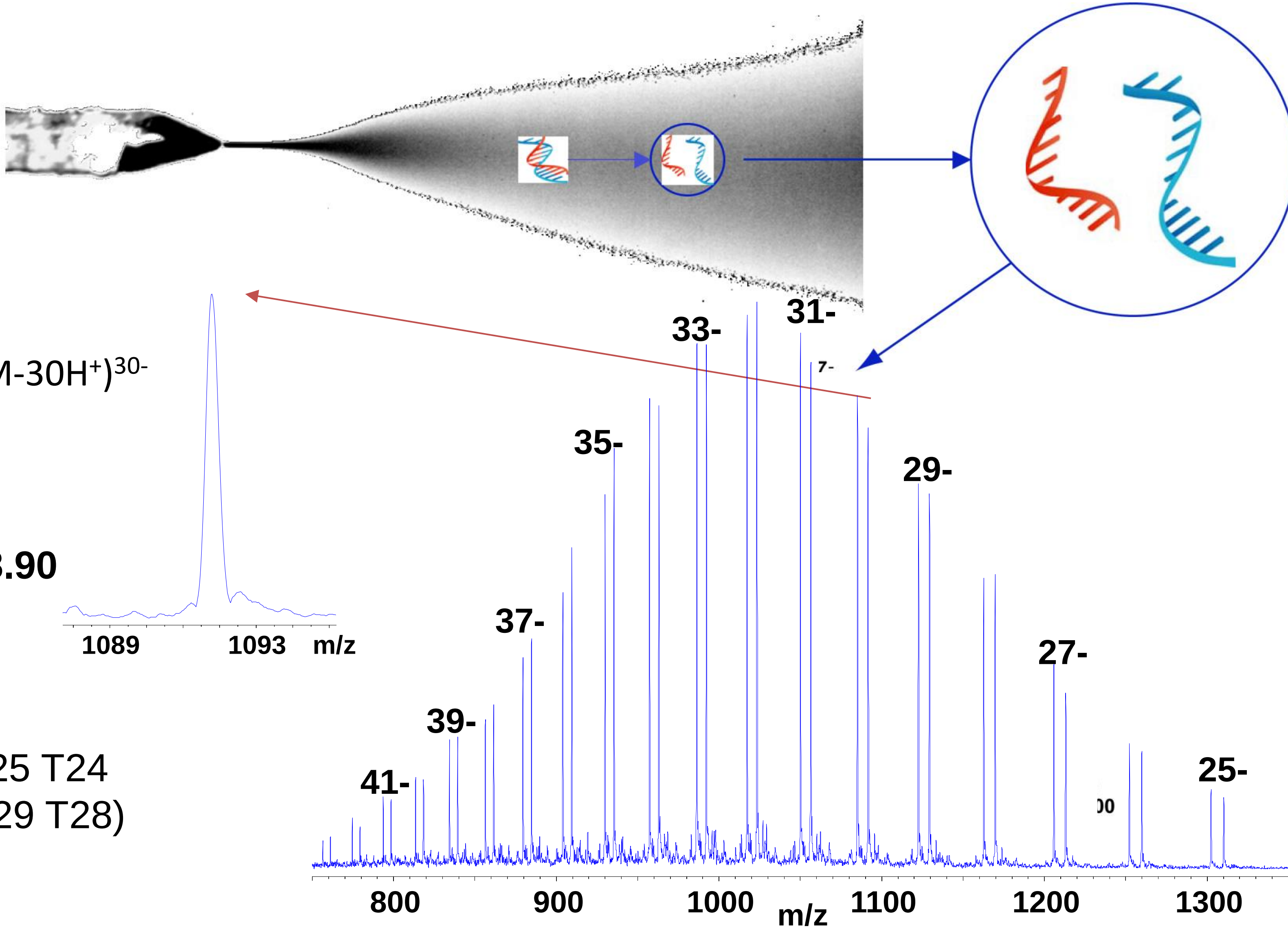


#	Mass	Base Count	Quantity
1	35875.03	$A_{25}G_{35}C_{30}T_{26}$	4260
2	35297.70	$A_{29}G_{33}C_{27}T_{25}$	1948
3	35619.87	$A_{26}G_{36}C_{29}T_{24}$	1555
4	36196.21	$A_{23}G_{37}C_{31}T_{26}$	1306
5	35297.70	$A_{29}G_{33}C_{27}T_{26}$	1949

Base Compositions Map to Microbes



Electrospray Ionization does not break DNA

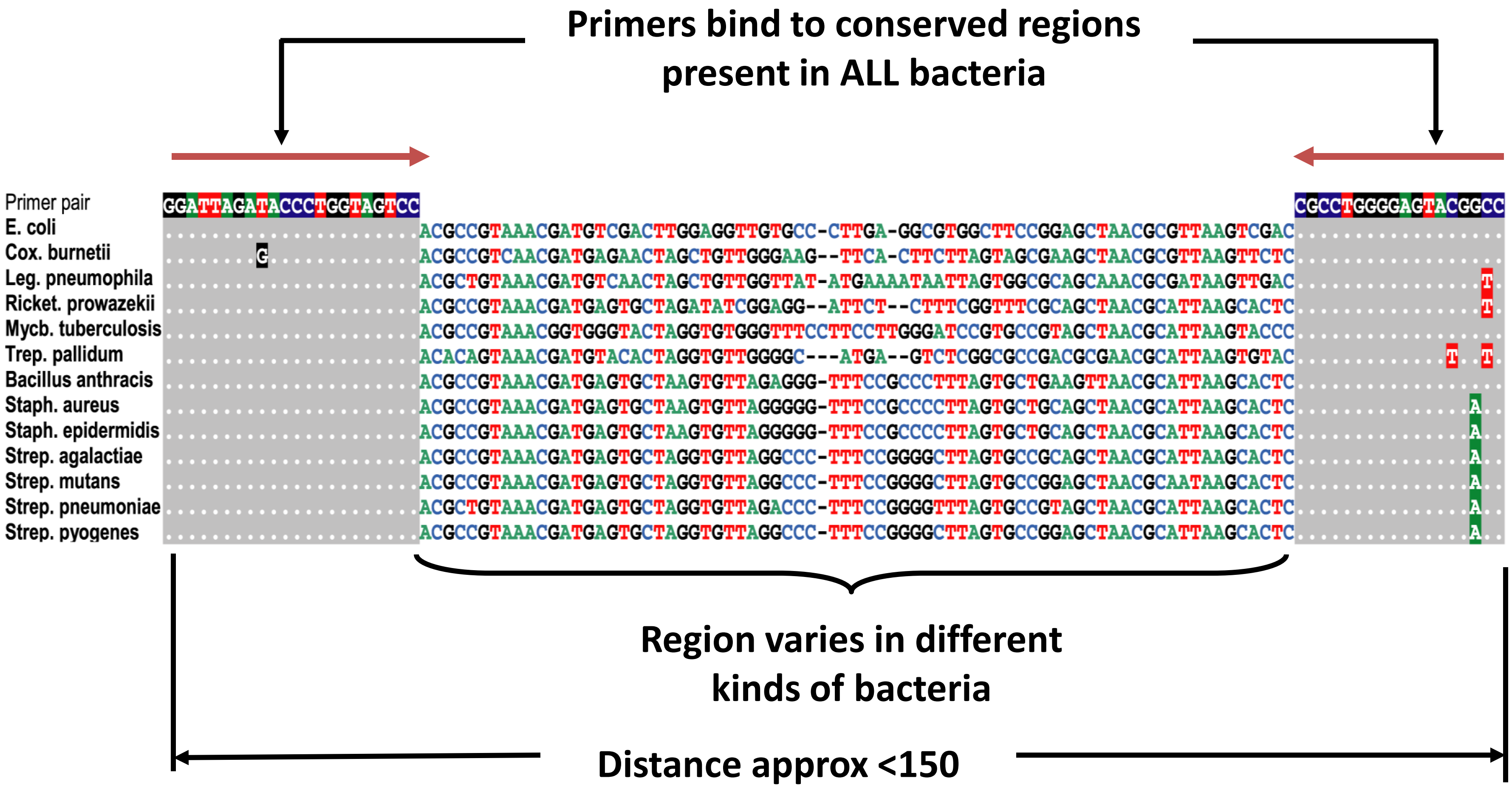


$(M\text{-}30H^{+})^{30-}$

MW = **32,588.90**

A28 G29 C25 T24
(A24 G25 C29 T28)

Typical Primer-Amplified Region in Bacteria



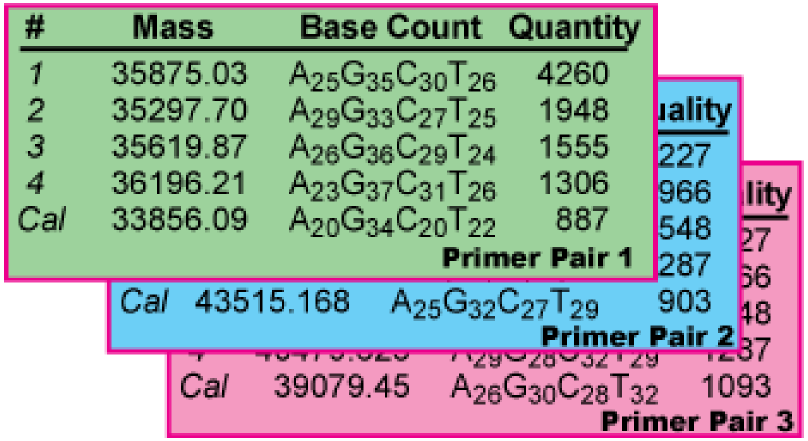
Primers bind to conserved regions present in ALL bacteria

```
Primer pair         GGATTAGATACCCTGGTAGTCC                                                                           CGCCTGGGGAGTACGGCC
E. coli             ......................ACGCCGTAAACGATGTCGACTTGGAGGTTGTGCC-CTTGA-GGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGAC..................
Cox. burnetii       ........G.............ACGCCGTCAACGATGAGAACTAGCTGTTGGGAAG--TTCA-CTTCTTAGTAGCGAAGCTAACGCGTTAAGTTCTC..................
Leg. pneumophila    ......................ACGCTGTAAACGATGTCAACTAGCTGTTGGTTAT-ATGAAAATAATTAGTGGCGCAGCAAACGCGATAAGTTGAC................T.
Ricket. prowazekii  ......................ACGCCGTAAACGATGAGTGCTAGATATCGGAGG--ATTCT--CTTTCGGTTTCGCAGCTAACGCATTAAGCACTC................T.
Mycb. tuberculosis  ......................ACGCCGTAAACGGTGGGTACTAGGTGTGGGTTTCCTTCCTTGGGATCCGTGCCGTAGCTAACGCATTAAGTACCC..................
Trep. pallidum      ......................ACACAGTAAACGATGTACACTAGGTGTTGGGGC---ATGA--GTCTCGGCGCCGACGCGAACGCATTAAGTGTAC.............T..T.
Bacillus anthracis  ......................ACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGG-TTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTC..................
Staph. aureus       ......................ACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGG-TTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTC...............A..
Staph. epidermidis  ......................ACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGG-TTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTC...............A..
Strep. agalactiae   ......................ACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCC-TTTCCGGGGCTTAGTGCCGCAGCTAACGCATTAAGCACTC...............A..
Strep. mutans       ......................ACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCC-TTTCCGGGGCTTAGTGCCGGAGCTAACGCAATAAGCACTC...............A..
Strep. pneumoniae   ......................ACGCTGTAAACGATGAGTGCTAGGTGTTAGACCC-TTTCCGGGGTTTAGTGCCGTAGCTAACGCATTAAGCACTC...............A..
Strep. pyogenes     ......................ACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCC-TTTCCGGGGCTTAGTGCCGGAGCTAACGCATTAAGCACTC...............A..
```

Region varies in different kinds of bacteria

Distance approx <150

Ibis Process Part 3: Triangulation Using Multiple Primer Pairs



#	Mass	Base Count	Quantity
1	35875.03	$A_{25}G_{35}C_{30}T_{26}$	4260
2	35297.70	$A_{29}G_{33}C_{27}T_{25}$	1948
3	35619.87	$A_{26}G_{36}C_{29}T_{24}$	1555
4	36196.21	$A_{23}G_{37}C_{31}T_{26}$	1306
Cal	33856.09	$A_{20}G_{34}C_{20}T_{22}$	887

Primer Pair 1

uality
227
966
548
287

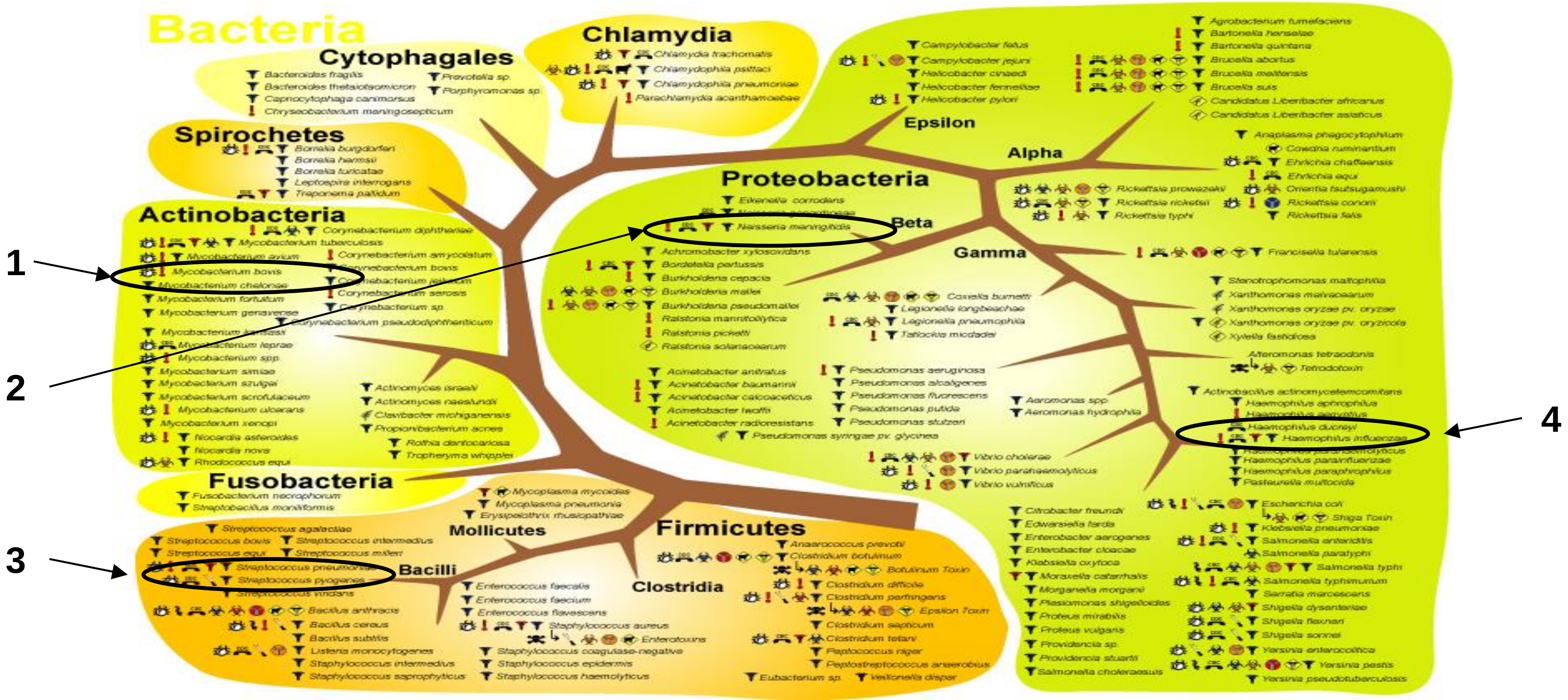
Cal 43515.168 $A_{25}G_{32}C_{27}T_{29}$ 903

Primer Pair 2

lity
27
66
48
37

Cal 39079.45 $A_{26}G_{30}C_{28}T_{32}$ 1093

Primer Pair 3

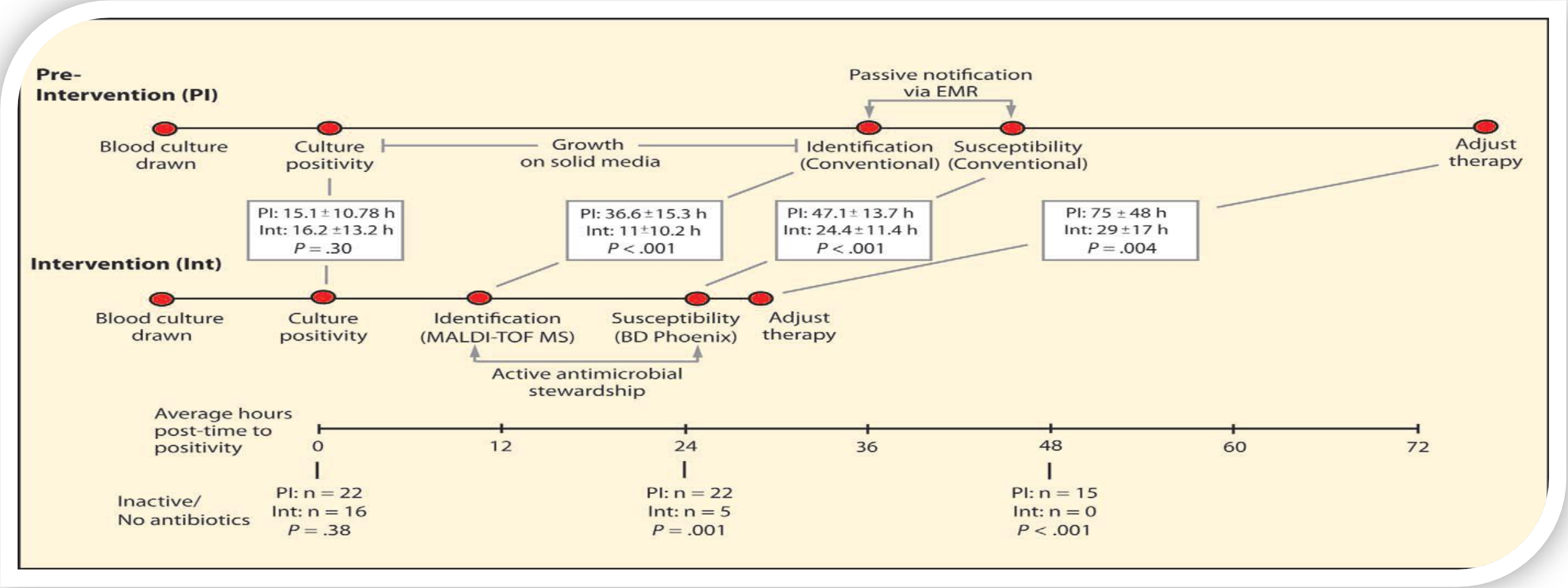


What is the expected outcome?

Expect $500 - $1,000/hr cost for delay in information for patient treatment

We have to attack 4 points:

- Early Diagnostic Indication
- Microbial Identification
- Microbial Susceptibility
- Medical Intervention



- Now that we have saved the world from deadly resistant infections –well ok, we have helped!
- Helped reduce the cost of heath care.
- Provided better clinical outcomes.

Your boss will say that's history!

What have you done lately?

Better Clinical Chemistry

- **Fast is fine, but accuracy is everything!**

  – Wyatt Earp (1849 – 1929),
    - American gambler, gunfighter and lawman

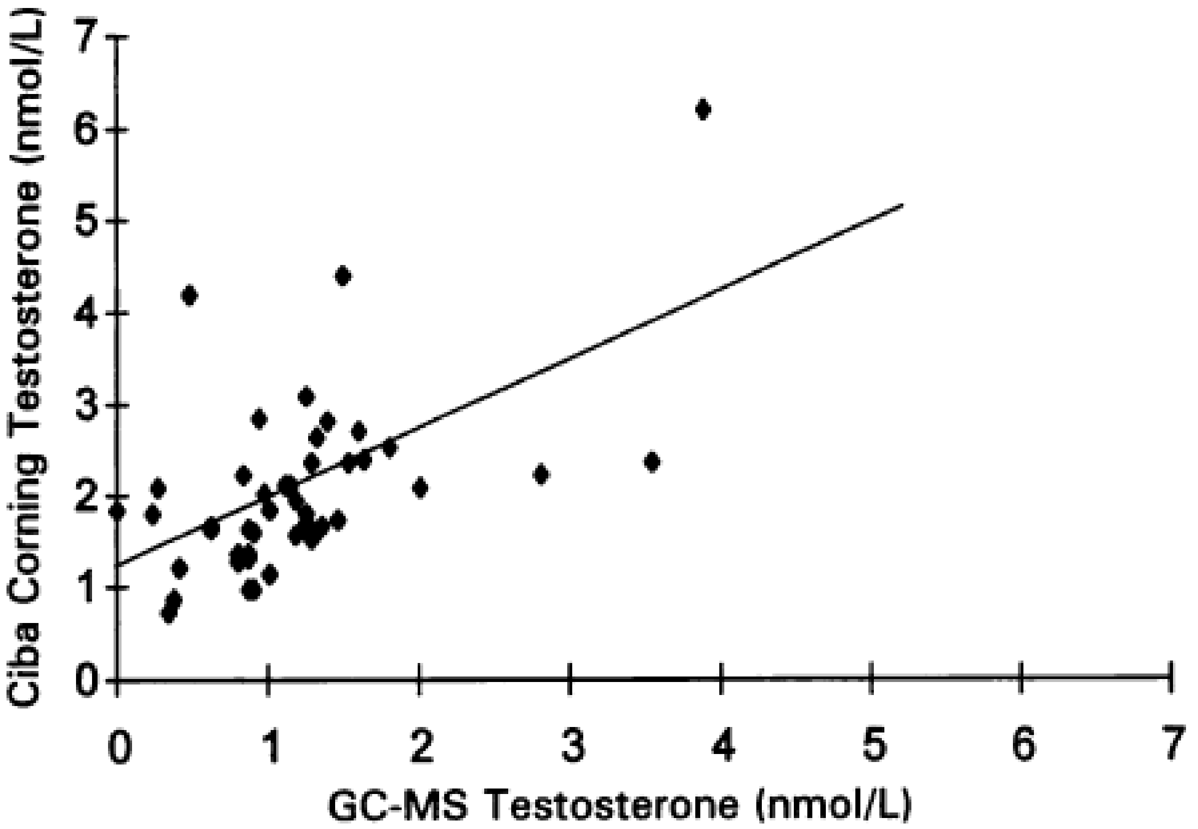
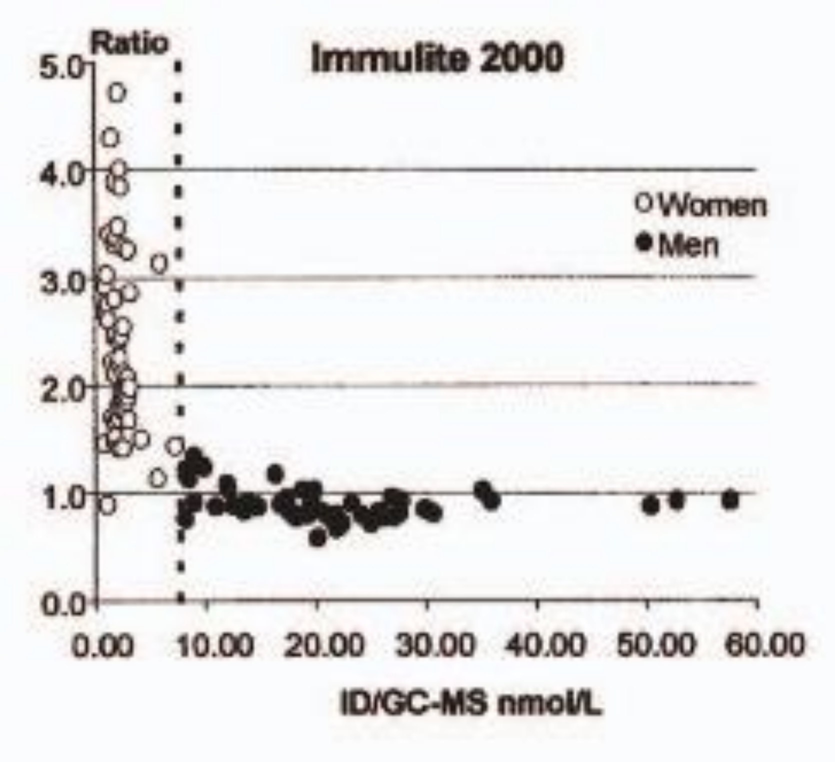
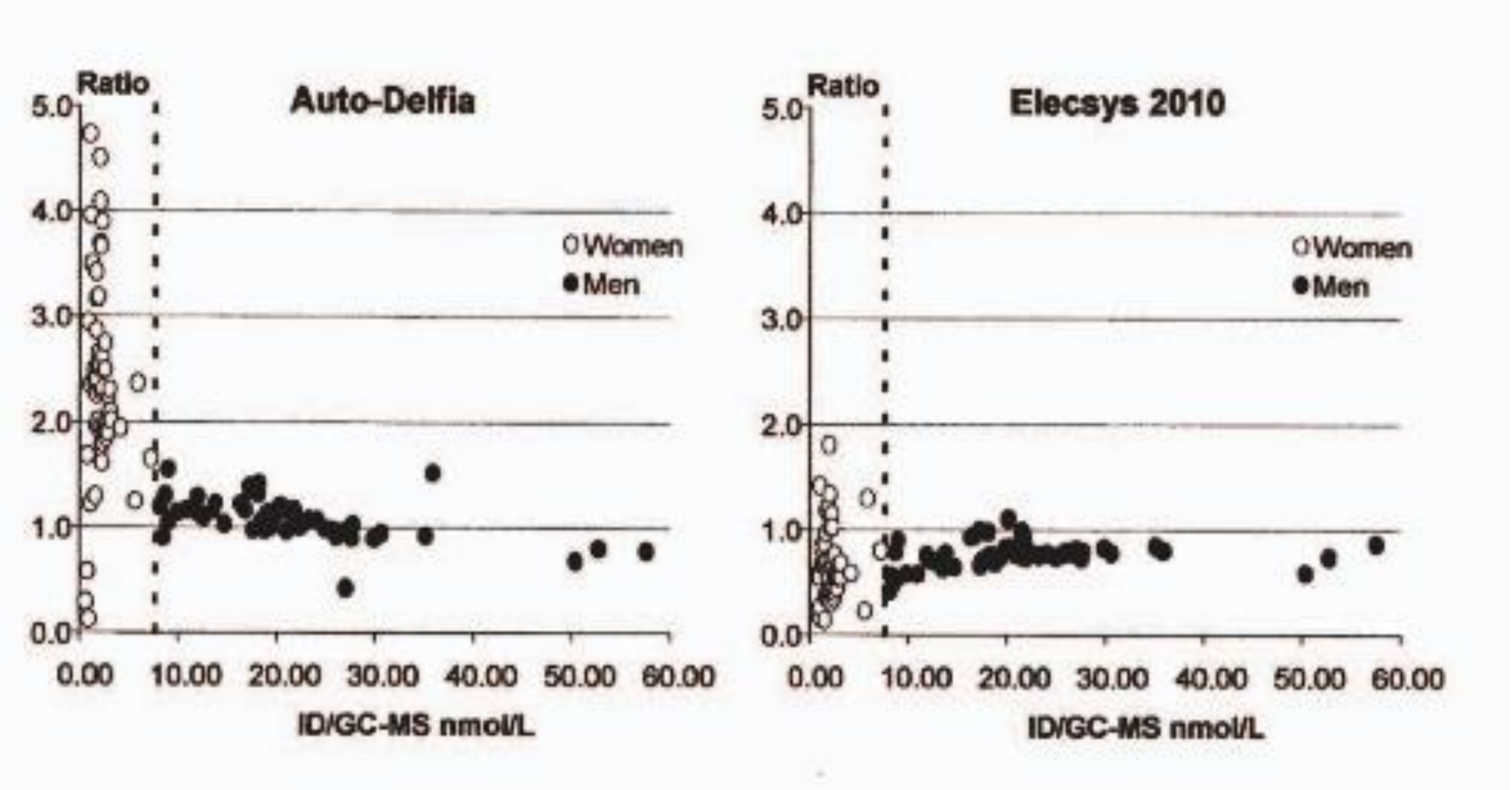


Fig. 5. Comparison of GC-MS and ACS testosterone assays for female specimens (ACS testosterone = 0.72 GC-MS + 1.2 nmol/L, r^2 = 0.31).

R.L. Fitzgerald, D.A. Herold; Clin Chem 42:5:749-755(1996)



J.Taieb et al; Clin Chem 49:8:1381-1395(2003)



J.Taieb et al; Clin Chem 49:8:1381-1395(2003)

Immunoassays for Testosterone in Women: Better than a Guess?

“Laboratory professionals should not be associated with a test where an educated guess would provide an equivalent or better result.”

Clinical Chemistry Editorial
D.A. Herold and R.L. Fitzgerald
Clin. Chem. 49:8 1250 - 1251(2003)

Why Vitamin D?

- Cancer - 50 to 80% decreased risk
- Multiple Sclerosis - 62% decreased
- Type I DM - 80% decreased
- Stroke and MI - decreased about 40%
- Rickets - decreased by ~100%
- Antimicrobial effect - TB

Why Vitamin D?

- Multiple Sclerosis
  - Extra Medical Cost ~ $2 M over last 20 years or $100,000 year
  - 320 M in US population
  - Incidence = 1 in a 1000 thus 320,000 cases
  - Thus, $100,000 X 320,000 = $32 B/year

Comments on Vitamin D

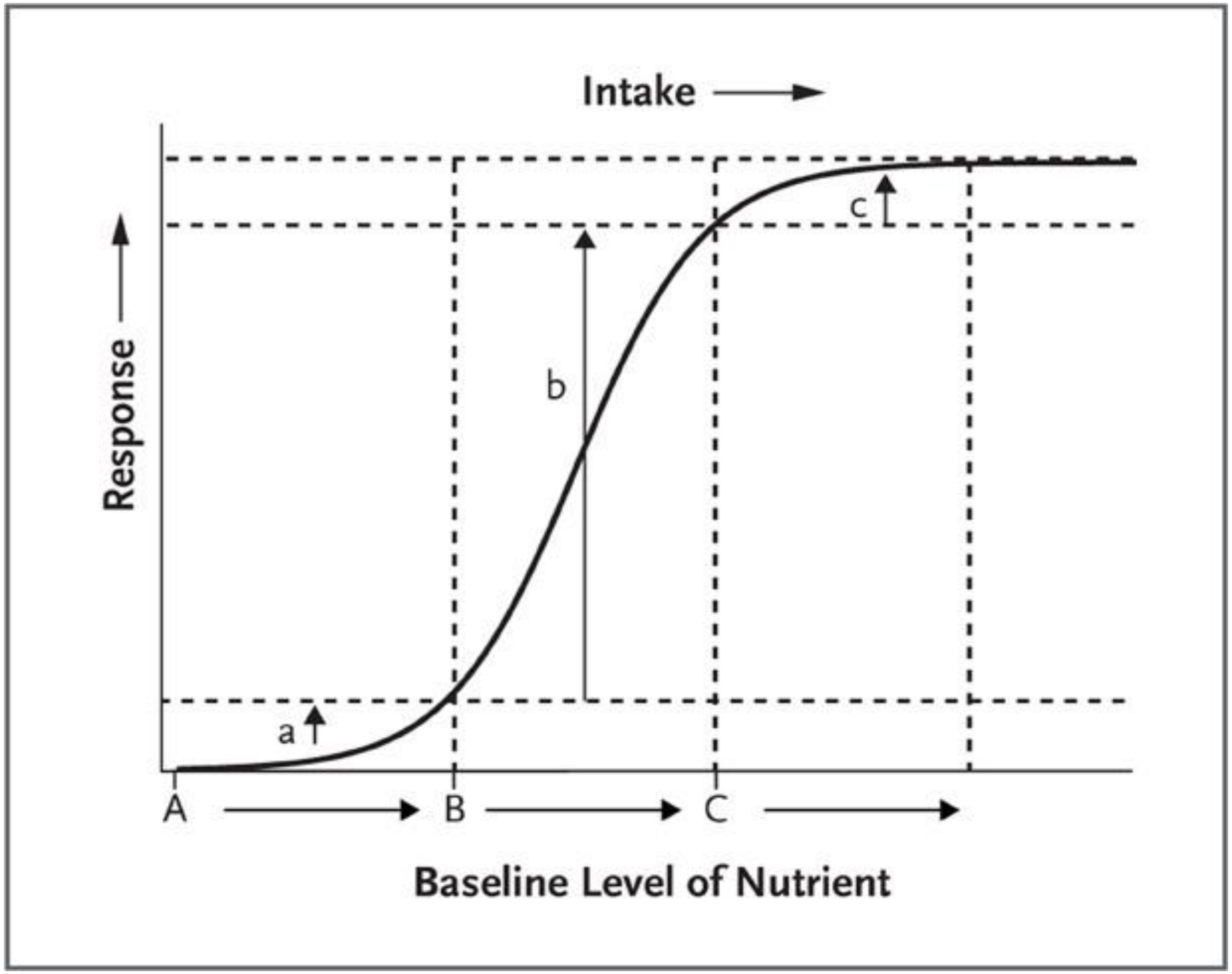
- What level
  - – IOM says >20 ng/mL
  - - Endocrine Society says >30 ng/mL
  - - Dave’s Vitamin D is 50 – 60 ng/mL on 10,000 IU/d

  Cost?

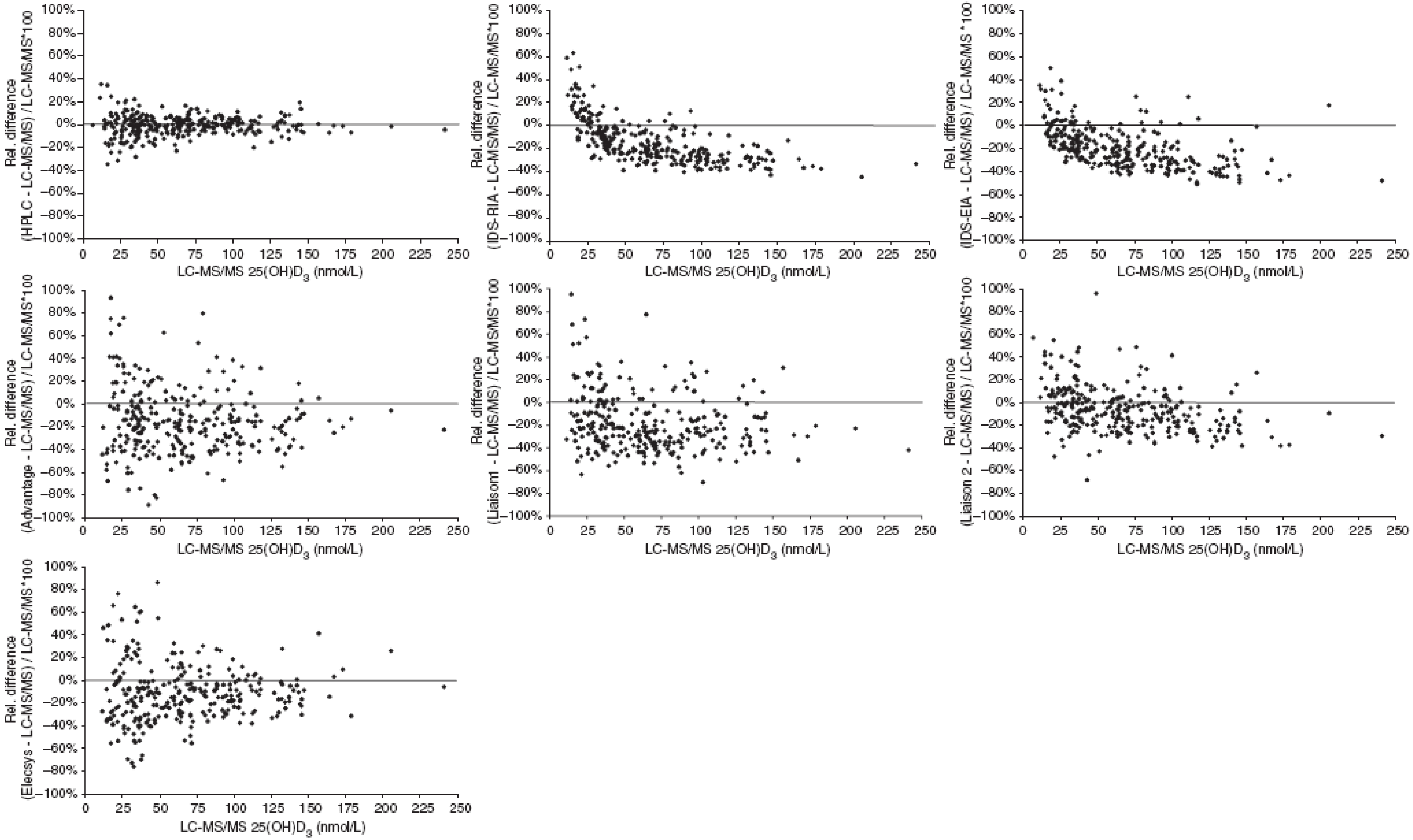
  Toxicity?

  Benefits – Huge long term

Response Curve for a Typical Nutrient



Comparison of 25(OH)D_3 Levels Measured in 7 Methods



Roth, et al. *Ann Clin Biochem*. 2008;45:153-159. Used with permission.

VA San Diego Cost Justification

- Assume a single analyte with a 10 sec wide peak
- Have a 2 minute total UPLC run time
- Connect to one MS/MS
- Total instrument cost is about $350,000
- Number of specimens analyzed per day 250
- Savings per specimen is $14
- Daily income is $3,500
- ROI of instruments 100 days….
- Requires high sample volume

“Big Lab” Cost Justification

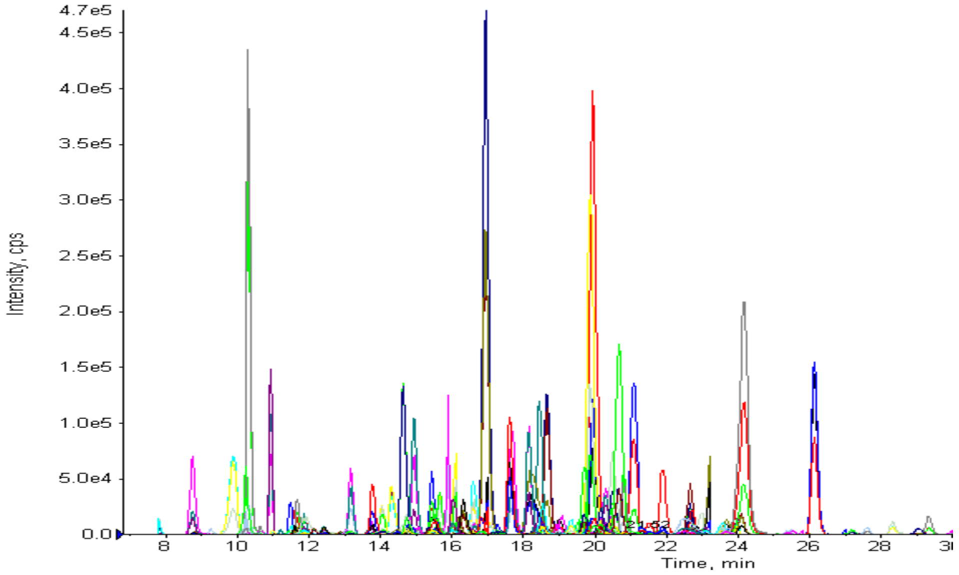
- Assume a single analyte with a 30 sec wide peak
- Have a 4 minute total LC run time
- Connect 4 HPLC systems to one MS/MS
- Total instrument cost is about $600,000
- Number of specimens analyzed per day 1200
- Reimbursement per specimen is $40
- Daily income is $48,000
- ROI of instruments 13 days!
- Requires very high sample volume

What about panels?

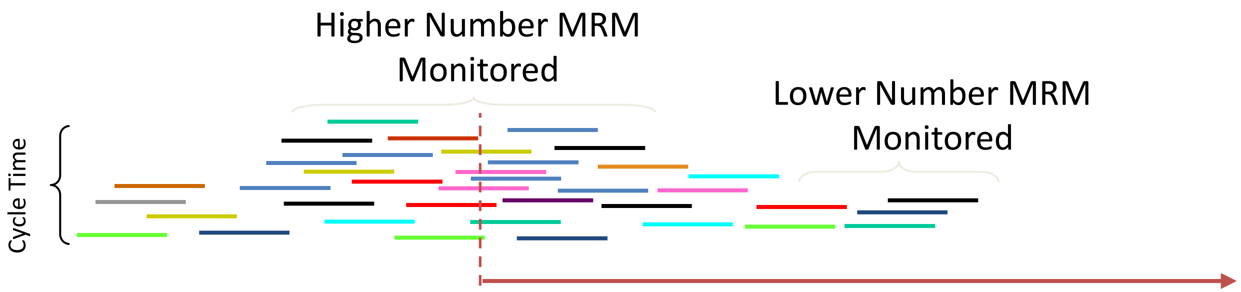
- Steroids (Cushing’s, Conn’s, Addison’s, CAH)
- Drugs of Abuse (Bath Salts, Spice, JWH series)
- Pain Profiles (Big business)
- Thyroid Hormones (fT4, fT3 and frT3)

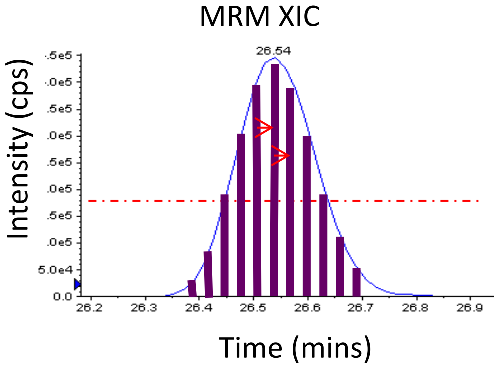
Scheduled MRM™ Algorithm

- Improving MRM Method Efficiency by Maximizing Analyte Utilization

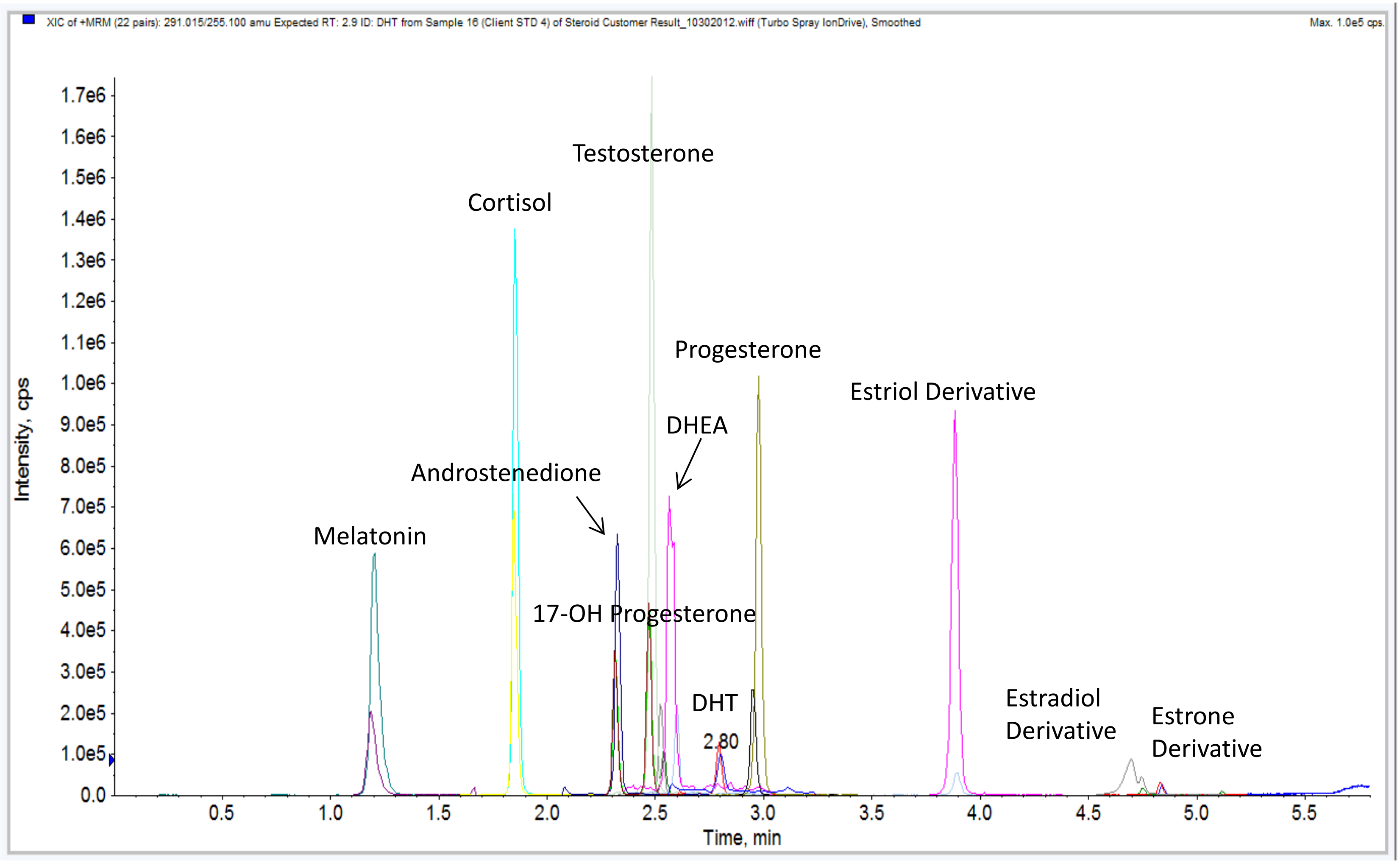


- Each MRM monitored only across its expected elution time
- ↓ concurrent MRMs
- Maintain cycle time and dwell time
- ↑ effective duty cycle for every compound
- Maintain analytical precision





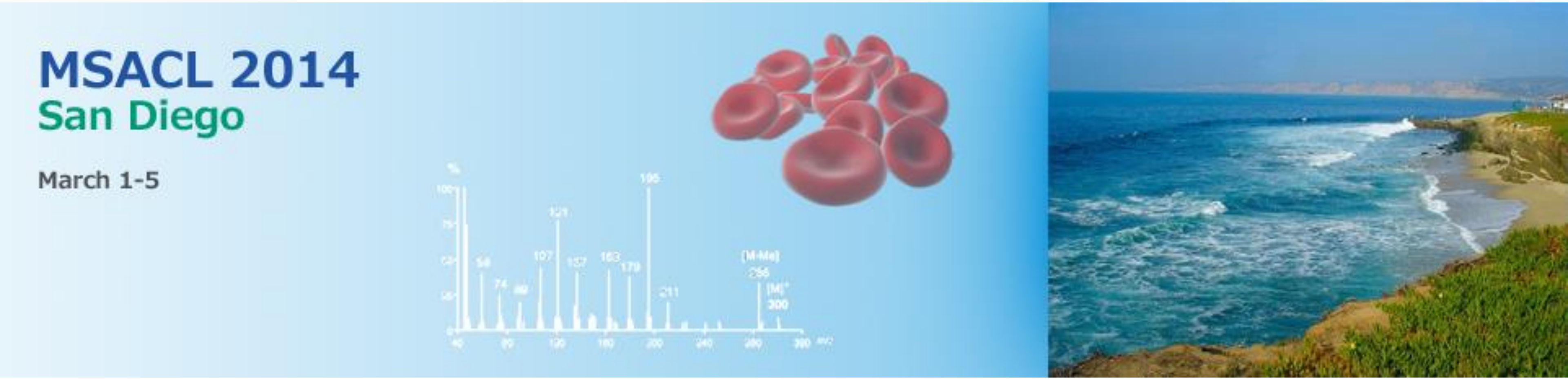
A Representative Chromatogram



The 6th Annual MSACL Conference

March 1-5, 2014

Sheraton San Diego Hotel & Marina



www.msacl.org

Presented by:

The Association for Mass Spectrometry: Applications to the Clinical Lab

MSACL is a 501(c)(3) non-profit California corporation

Advanced Technologies

- High Resolution Mass Spectrometry

The Fundamentals of Exact Mass

carbon	has a mass of 12.0000
hydrogen	has a mass of 1.0078
oxygen	has a mass of 15.9949
nitrogen	has a mass of 14.0031

- It is possible to have combinations of atoms which have the same nominal (or integer) mass but different accurate mass
- If such compounds can be mass measured with sufficient accuracy it is possible to determine elemental composition

Simple Examples

- CO = 27.9949
- N_2 = 28.0061
- C_2H_4 = 28.0313

- These elemental combinations have the same nominal mass but different exact mass
- A nominal mass measurement cannot distinguish
- If compounds differ in elemental compositions then the exact mass measurement may be useful

Unknown Screening and Compound Identification

Unknown Compound in Equine Urine Extracted Using Comparative Screening

Accurate Mass and High Resolution MS and MS/MS of 337 at 2.76 min

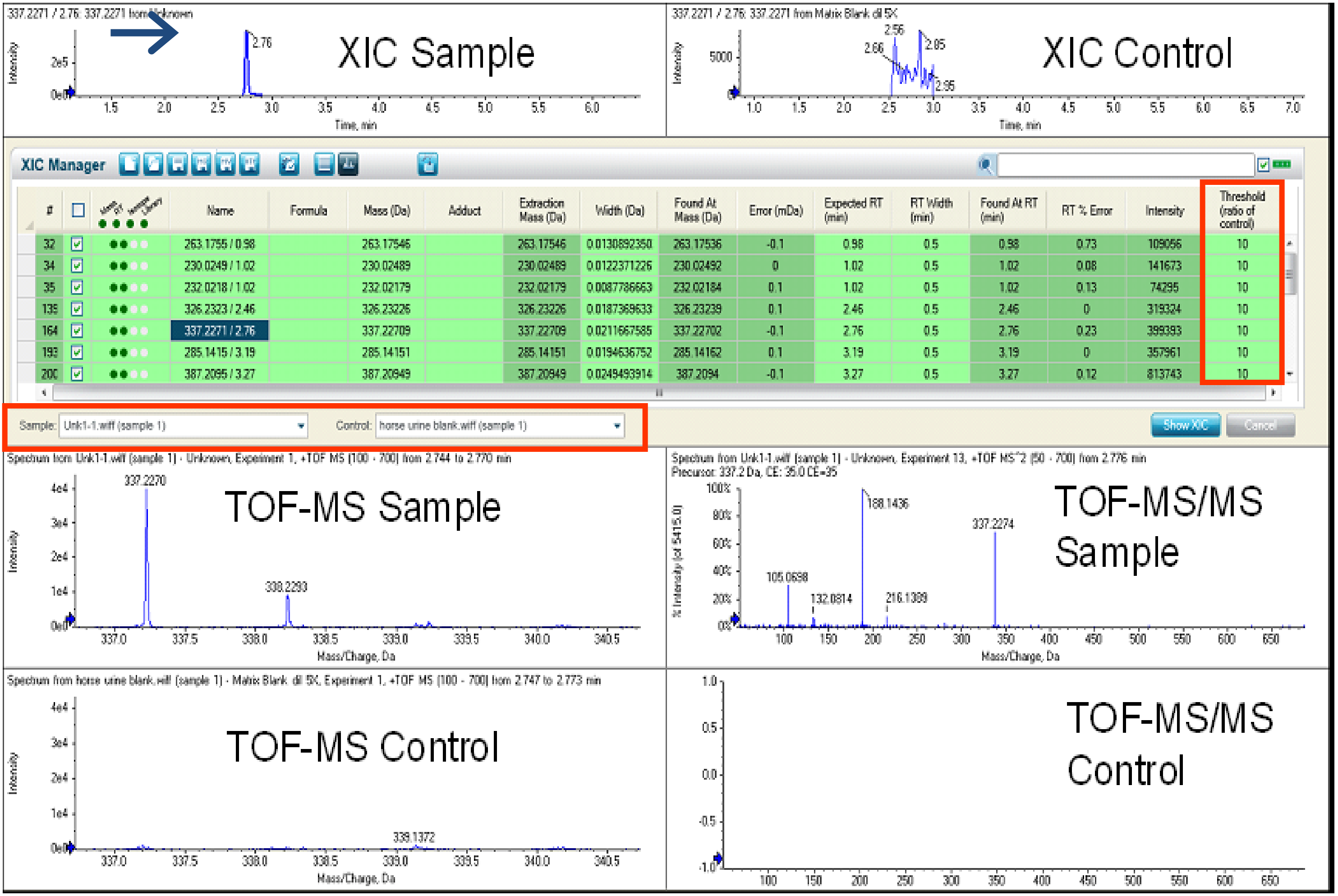
Step 1

Find Unknown

Step 2

Step 3

Step 4



337.2271 / 2.76: 337.2271 from Unknown

337.2271 / 2.76: 337.2271 from Matrix Blank dil 5X

XIC Manager

#	☐	● ● ● ●	Name	Formula	Mass (Da)	Adduct	Extraction Mass (Da)	Width (Da)	Found At Mass (Da)	Error (mDa)	Expected RT (min)	RT Width (min)	Found At RT (min)	RT % Error	Intensity	Threshold (ratio of control)
32	☑	●●○○	263.1755 / 0.98		263.17546		263.17546	0.0130892350	263.17536	-0.1	0.98	0.5	0.98	0.73	109056	10
34	☑	●●○○	230.0249 / 1.02		230.02489		230.02489	0.0122371226	230.02492	0	1.02	0.5	1.02	0.08	141673	10
35	☑	●●○○	232.0218 / 1.02		232.02179		232.02179	0.0087786663	232.02184	0.1	1.02	0.5	1.02	0.13	74295	10
139	☑	●●○○	326.2323 / 2.46		326.23226		326.23226	0.0187369633	326.23239	0.1	2.46	0.5	2.46	0	319324	10
164	☑	●●○○	337.2271 / 2.76		337.22709		337.22709	0.0211667585	337.22702	-0.1	2.76	0.5	2.76	0.23	399393	10
193	☑	●●○○	285.1415 / 3.19		285.14151		285.14151	0.0194636752	285.14162	0.1	3.19	0.5	3.19	0	357961	10
200	☑	●●○○	387.2095 / 3.27		387.20949		387.20949	0.0249493914	387.2094	-0.1	3.27	0.5	3.27	0.12	813743	10

Sample: Unk1-1.wiff (sample 1)

Control: horse urine blank.wiff (sample 1)

Spectrum from Unk1-1.wiff (sample 1) - Unknown, Experiment 1, +TOF MS (100 - 700) from 2.744 to 2.770 min

Spectrum from Unk1-1.wiff (sample 1) - Unknown, Experiment 13, +TOF MS^2 (50 - 700) from 2.776 min
Precursor: 337.2 Da, CE: 35.0 CE=35

Spectrum from horse urine blank.wiff (sample 1) - Matrix Blank dil 5X, Experiment 1, +TOF MS (100 - 700) from 2.747 to 2.773 min

Unknown Screening and Identification

Empirical Formula Calculation Combining MS and MS/MS Information

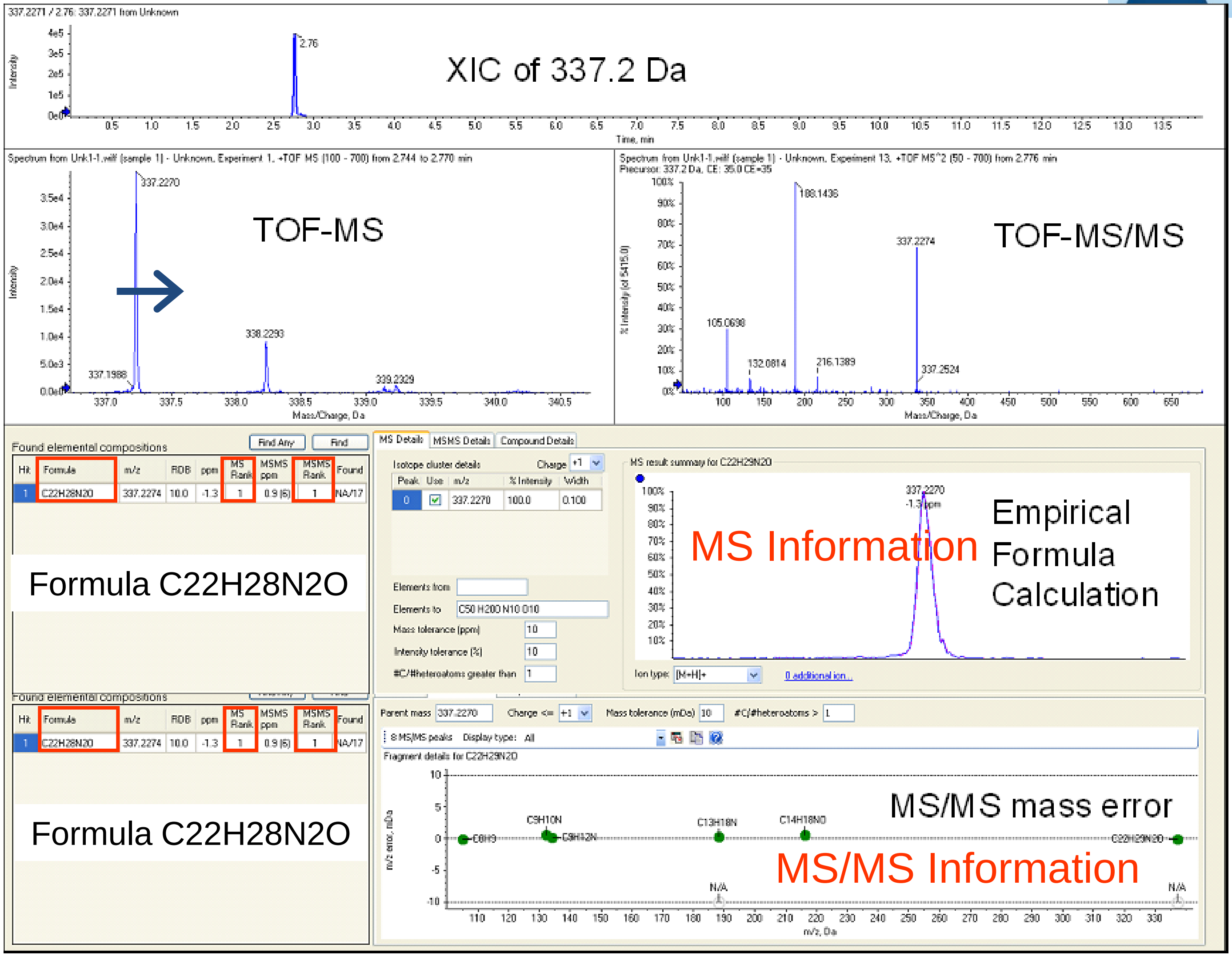
Step 1

Step 2

Formula Finder

Step 3

Step 4



Found elemental compositions

Hit	Formula	m/z	RDB	ppm	MS Rank	MSMS ppm	MSMS Rank	Found
1	C22H28N2O	337.2274	10.0	-1.3	1	0.9 (6)	1	NA/17

Formula C22H28N2O

Isotope cluster details Charge +1

Peak	Use	m/z	% Intensity	Width
0	☑	337.2270	100.0	0.100

Elements from

Elements to C50 H200 N10 O10

Mass tolerance (ppm) 10

Intensity tolerance (%) 10

#C/#heteroatoms greater than 1

Ion type: [M+H]+

0 additional ion...

Found elemental compositions

Hit	Formula	m/z	RDB	ppm	MS Rank	MSMS ppm	MSMS Rank	Found
1	C22H28N2O	337.2274	10.0	-1.3	1	0.9 (6)	1	NA/17

Formula C22H28N2O

Parent mass 337.2270 Charge <= +1 Mass tolerance (mDa) 10 #C/#heteroatoms > 1

8 MS/MS peaks Display type: All

Unknown Screening and Identification

Automatic Search of Found Formulas Against Online Databases

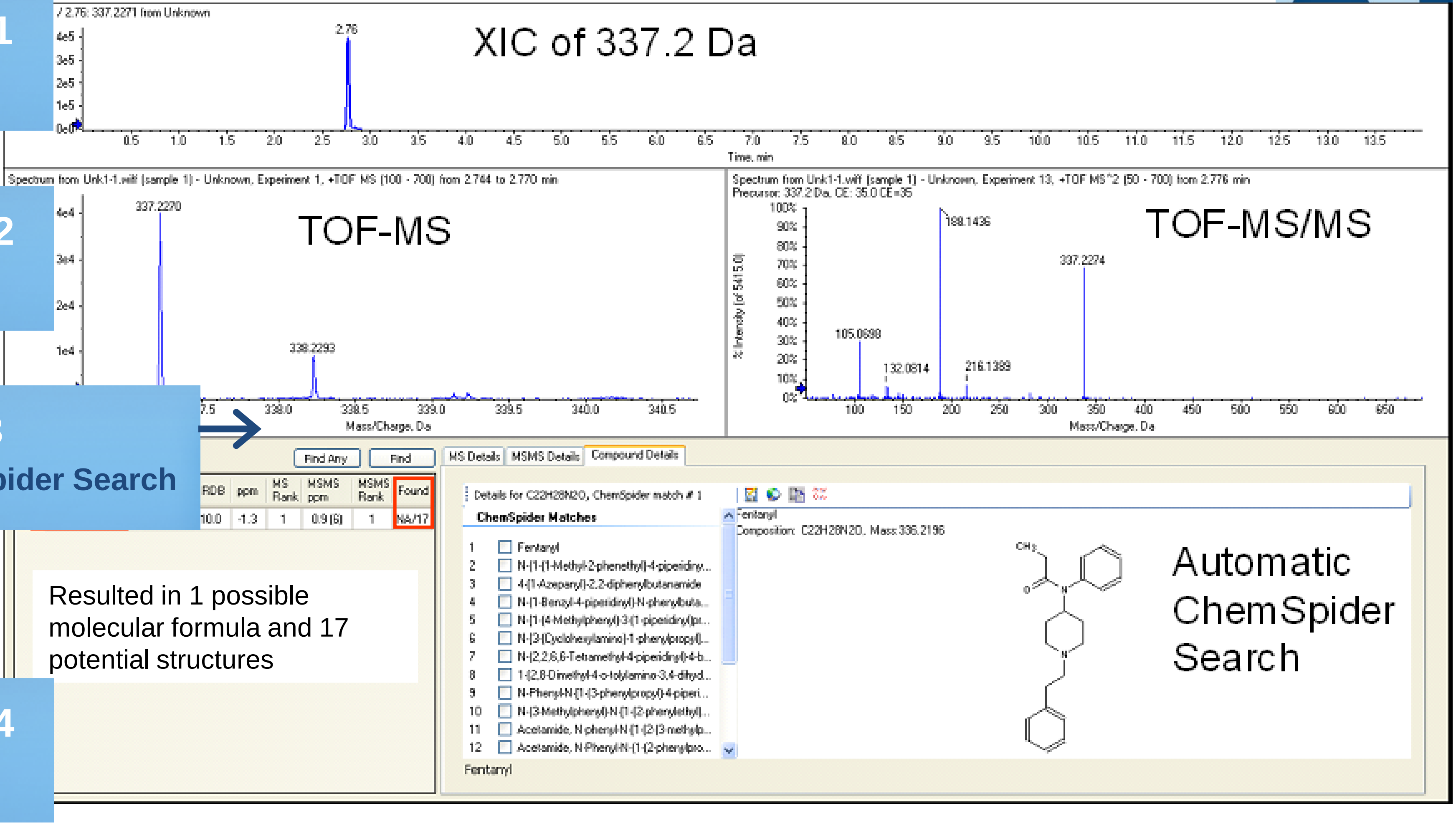
Step 1

Step 2

Step 3

ChemSpider Search

Step 4



XIC of 337.2 Da

TOF-MS

TOF-MS/MS

Automatic ChemSpider Search

Resulted in 1 possible molecular formula and 17 potential structures

XIC of +MS2 (337.2 / 2.76: 337.2271 from Unknown

Spectrum from Unk1-1.wiff (sample 1) - Unknown, Experiment 1, +TOF MS (100 - 700) from 2.744 to 2.770 min

Spectrum from Unk1-1.wiff (sample 1) - Unknown, Experiment 13, +TOF MS^2 (50 - 700) from 2.776 min
Precursor: 337.2 Da, CE: 35.0 CE=35

RDB	ppm	MS Rank	MSMS ppm	MSMS Rank	Found
10.0	-1.3	1	0.9 (6)	1	NA/17

Details for C22H28N2O, ChemSpider match # 1

ChemSpider Matches

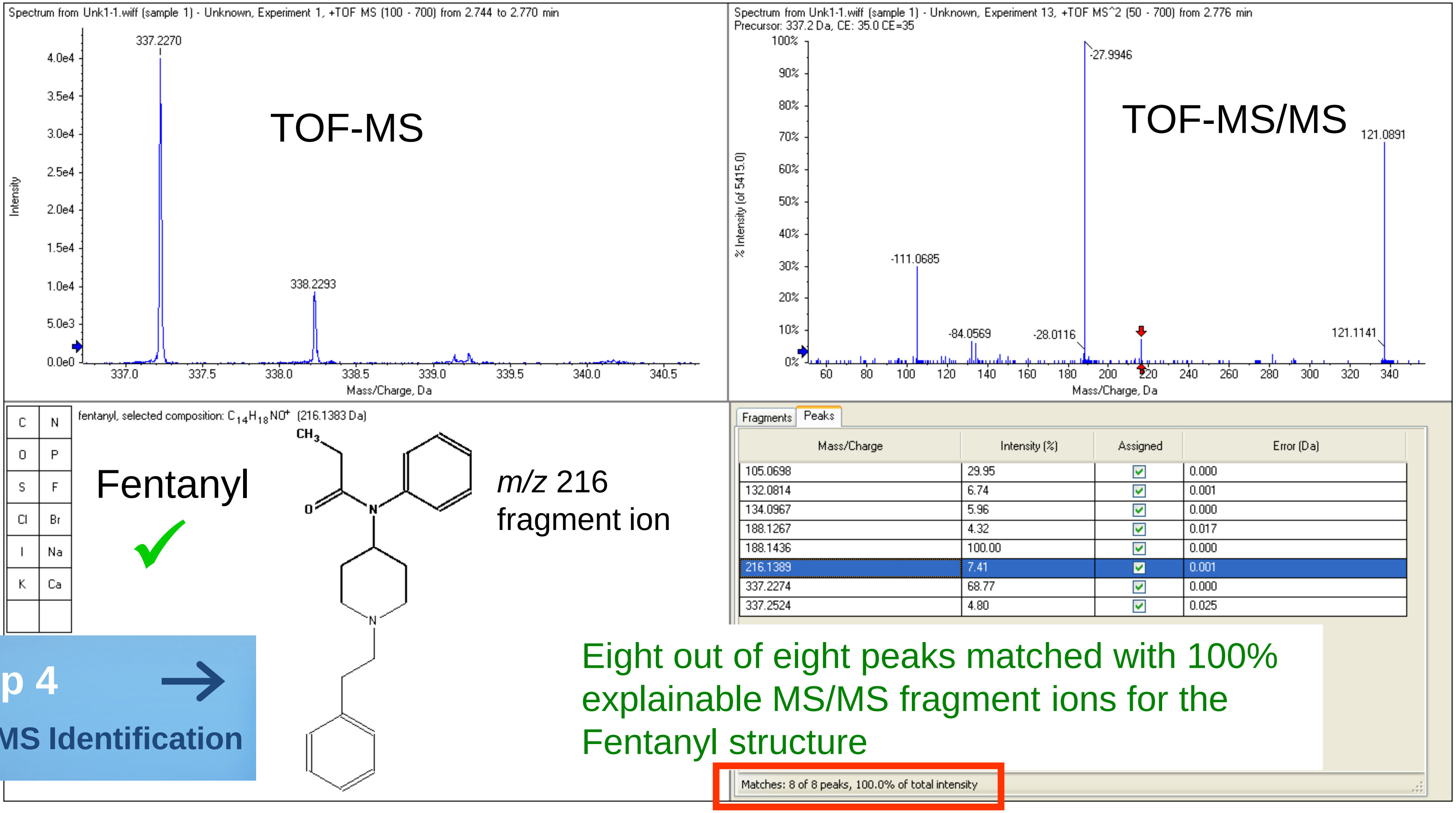
1 ☐ Fentanyl
2 ☐ N-[1-(1-Methyl-2-phenethyl)-4-piperidiny...
3 ☐ 4-(1-Azepanyl)-2,2-diphenylbutanamide
4 ☐ N-(1-Benzyl-4-piperidinyl)-N-phenylbuta...
5 ☐ N-[1-(4-Methylphenyl)-3-(1-piperidinyl)pr...
6 ☐ N-[3-(Cyclohexylamino)-1-phenylpropyl]...
7 ☐ N-(2,2,6,6-Tetramethyl-4-piperidinyl)-4-b...
8 ☐ 1-(2,8-Dimethyl-4-o-tolylamino-3,4-dihyd...
9 ☐ N-Phenyl-N-[1-(3-phenylpropyl)-4-piperi...
10 ☐ N-(3-Methylphenyl)-N-[1-(2-phenylethyl]...
11 ☐ Acetamide, N-phenyl-N-[1-[2-(3-methylp...
12 ☐ Acetamide, N-Phenyl-N-[1-(2-phenylpro...

Fentanyl
Composition: C22H28N2O, Mass:336.2196

Fentanyl

Unknown Screening and Identification

Fragmentation Prediction Tool in PeakView® Software



Spectrum from Unk1-1.wiff (sample 1) - Unknown, Experiment 1, +TOF MS (100 - 700) from 2.744 to 2.770 min

TOF-MS

Spectrum from Unk1-1.wiff (sample 1) - Unknown, Experiment 13, +TOF MS^2 (50 - 700) from 2.776 min
Precursor: 337.2 Da, CE: 35.0 CE=35

TOF-MS/MS

fentanyl, selected composition: $C_{14}H_{18}NO^+$ (216.1383 Da)

Fentanyl

✓

m/z 216
fragment ion

Mass/Charge	Intensity (%)	Assigned	Error (Da)
105.0698	29.95	☑	0.000
132.0814	6.74	☑	0.001
134.0967	5.96	☑	0.000
188.1267	4.32	☑	0.017
188.1436	100.00	☑	0.000
216.1389	7.41	☑	0.001
337.2274	68.77	☑	0.000
337.2524	4.80	☑	0.025

Eight out of eight peaks matched with 100% explainable MS/MS fragment ions for the Fentanyl structure

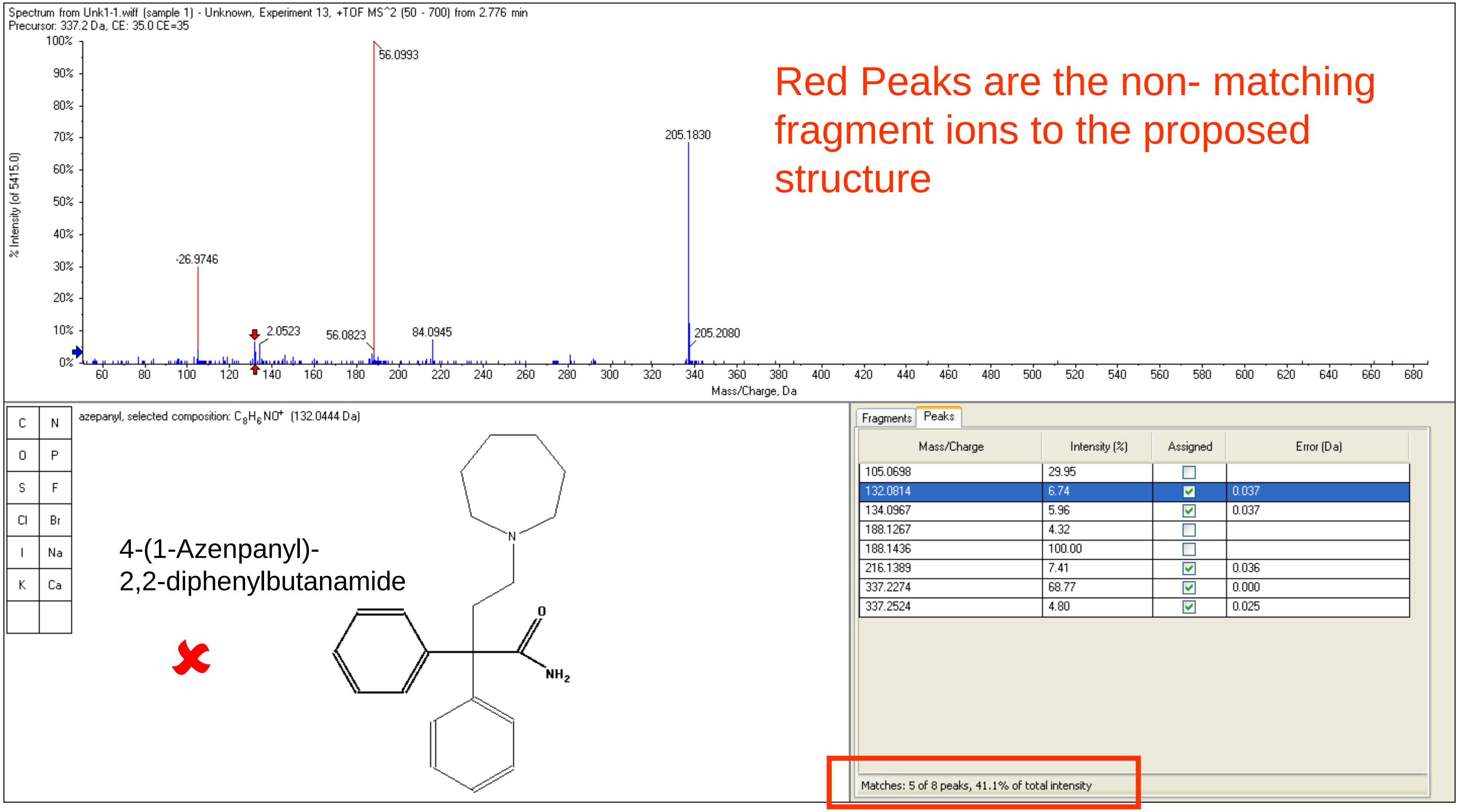
Matches: 8 of 8 peaks, 100.0% of total intensity

Step 4 →

MS/MS Identification

Unknown Screening and Identification

Fragmentation Prediction Tool in PeakView® Software



Spectrum from Unk1-1.wiff (sample 1) - Unknown, Experiment 13, +TOF MS^2 (50 - 700) from 2.776 min
Precursor: 337.2 Da, CE: 35.0 CE=35

Red Peaks are the non- matching fragment ions to the proposed structure

azepanyl, selected composition: $C_8H_6NO^+$ (132.0444 Da)

4-(1-Azenpanyl)-2,2-diphenylbutanamide

Mass/Charge	Intensity (%)	Assigned	Error (Da)
105.0698	29.95	☐	
132.0814	6.74	☑	0.037
134.0967	5.96	☑	0.037
188.1267	4.32	☐	
188.1436	100.00	☐	
216.1389	7.41	☑	0.036
337.2274	68.77	☑	0.000
337.2524	4.80	☑	0.025

Matches: 5 of 8 peaks, 41.1% of total intensity

Five out of eight peaks matched explainable MS/MS fragment ions for the 4-(1-Azenpanyl)-2,2-diphenylbutanamide structure

So what do you get from Mass Spec?

- Testosterone – Quality
- Vitamin D – Long term savings
- Drugs of Abuse – Rapid validated result
- Steroids – Panels for better Dxs
- Microbiology – Better, Faster and Cheaper

Mass Spectrometry Companies

- AB SCIEX
- Agilent
- Bruker
- Ionics
- Shimadzu (bioMerieux)
- SimulTOF
- ThermoFisher
- Waters

THE ASSOCIATION FOR

MASS SPECTROMETRY

APPLICATIONS TO THE CLINICAL LAB

MSACL 2014
6th Annual Conference & Exhibits
Sheraton San Diego Hotel & Marina
San Diego, CA
March 1-5, 2014
www.msacl.org

David Herold, MD, PhD, DABCC
University of California, San Diego
Phone: (858) 552-8585 x7758
E-mail: dherold@ucsd.edu

50 year rule – George Ebers, MD Oxford, UK

1. Neural Tube Defect – 1958 through 1991
   - UK still not using folate

2. Small Pox – Variolation vs Vaccination
   a. Africa, India, Turkey
   b. Milk maids – Cow Pox and Jenner 1794
   c. 1840 Vaccination accepted as only method

3. Scurvy – James Lind 1747 – 1794 - 1804

   6 teatment groups with 2 pts each for 6 days
   1. Liter of cider
   2. 25 drops of H_2SO_4
   3. 30 mL of vinegar
   4. 0.5 L seawater
   5. 2 oranges & 1 lemon
   6. 30 mL barley water

4. Cigarettes and Cancer link – first suggested in 1930s

5. Sugar and diabetes?