MALDI-TOF Mass Spectrometry in a Time of *mu-lambda* Testing

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Marshfield Clinic Health System

TIL …

μ, micro

λ, wave

μλ = microwave

Disclosures

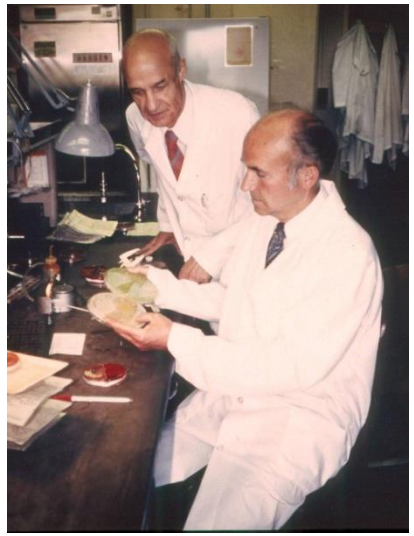
TN: None

JDV: None

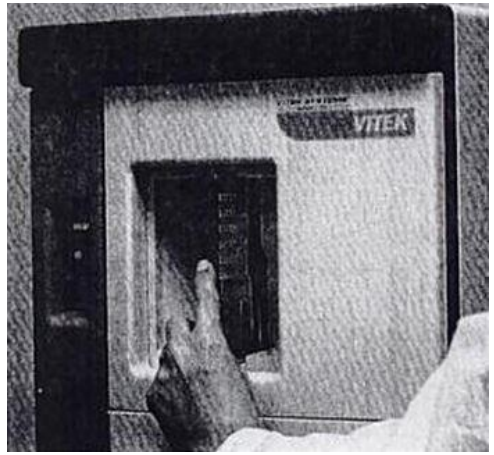
The Paradigms of Medical Bacteriology

Germ Theory
Louis Pasteur
1860

Biochemical ID
Ferdinand Cohn
1875



Standardized AST
Kirby & Bauer
1966



Vitek AMS ID/AST
McDonnell Douglas/NASA
1974

Microbiology Labs: What's Changed, What Hasn't?

San Bernardino County Hospital
(1948)

Texas DSHS Microbiology Lab
(Contemporary)

MALDI-TOF Mass Spectrometry

Matrix-Assisted

Laser

Desorption/Ionization

Time-Of-Flight

Mass Spectrometry

MALDI TOF MS

Two FDA-cleared instruments

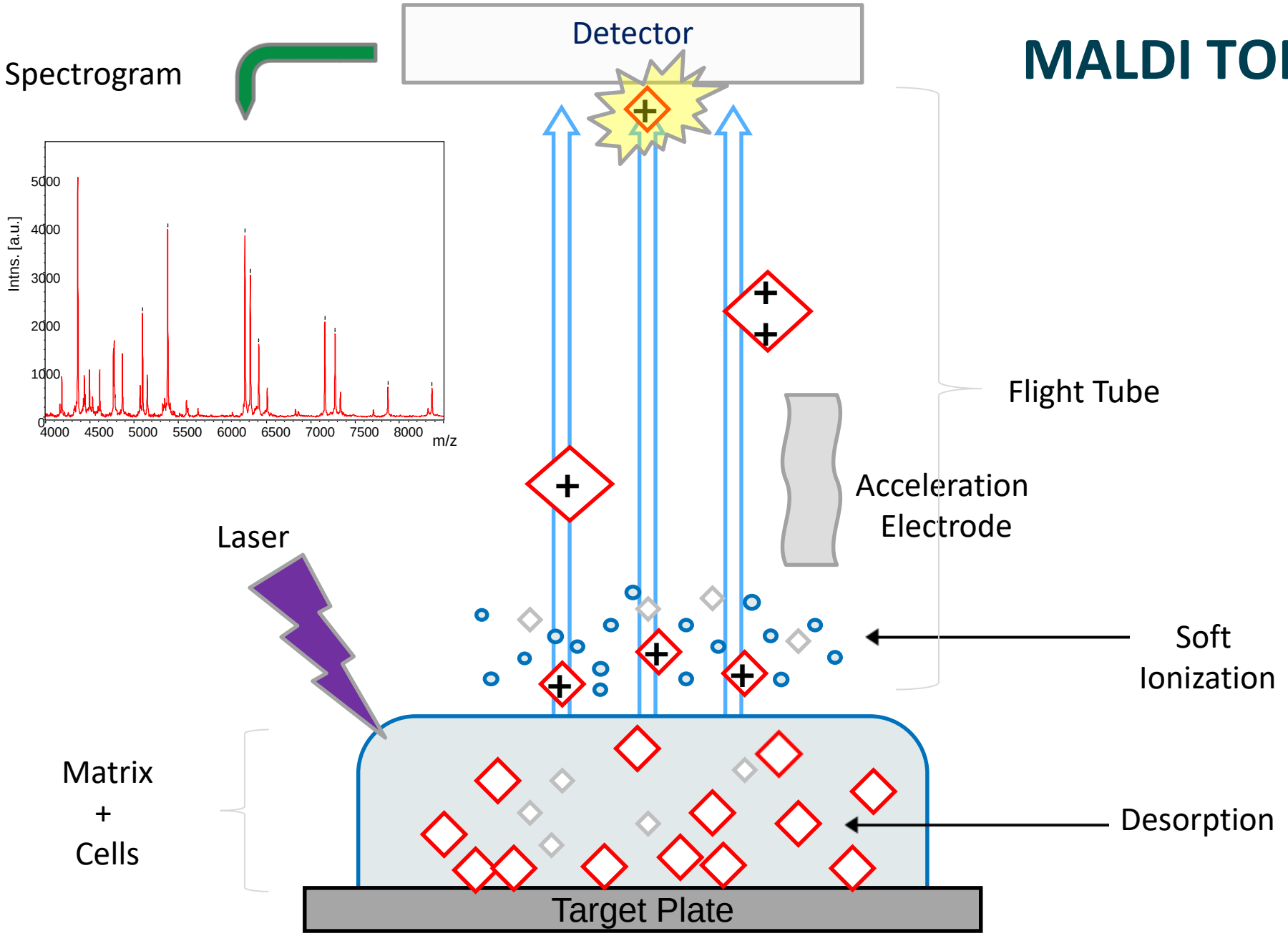
- bioMerieux Vitek® MS
- Bruker MALDI BioTyper® sirius CA

- DB Taxa (as of 3/3/2023)

	FDA- IVD	Non-FDA IVD	Non-clinically validated
BioTyper CA	488	--	≈2,200
Vitek MS [1]	401	915	[2]
Vitek 2 [1]	≈350	--	--

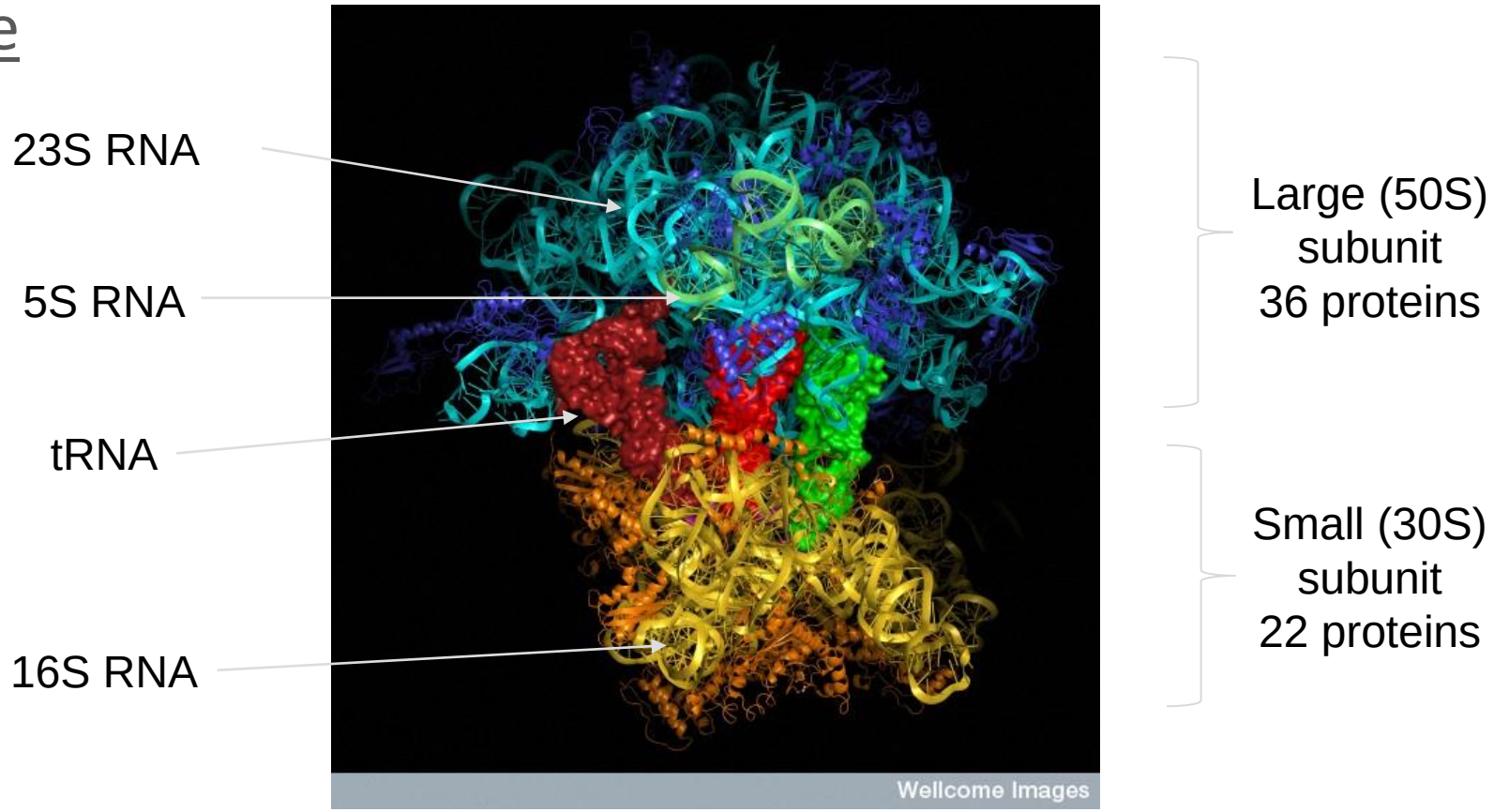
[1] Not mutually inclusive
[2] Extensive RUO DB is available on industrial versions of the VMS only

MALDI TOF MS


Spectrogram
Detector
Intns. [a.u.]
5000
4000
3000
2000
1000
0
4000
4500
5000
5500
6000
6500
7000
7500
8000
m/z
Flight Tube
Acceleration
Electrode
Laser
Soft
Ionization
Matrix
+
Cells
Desorption
Target Plate

The Target

The Ribosome



MALDI TOF MS

Target Plates

Bruker

http://www.bdal.com/uploads/media/MALDI_Biotyper_Consumables_3-2012.pdf

Vitek MS

http://www.biomerieux-industry.com/servlet/srt/bio/industry-microbiology/dynPage?doc=NDY_IND_BPA_PRD_G_PRD_NDY_6

MALDI TOF MS

Bruker MALDI Biotyper sirius CA

(MALDI Biotyper® sirius CA System (US-IVD) | Bruker, accessed 3/2/23)

BioMerieux Vitek MS

Advantages

- Time saving
- Little biomass required
- Cost-per-test is cheaper than biochemical IDs
- High accuracy

Disadvantages

- Can only perform identifications
- Separate instrumentation requires a complex integration system

Limitations

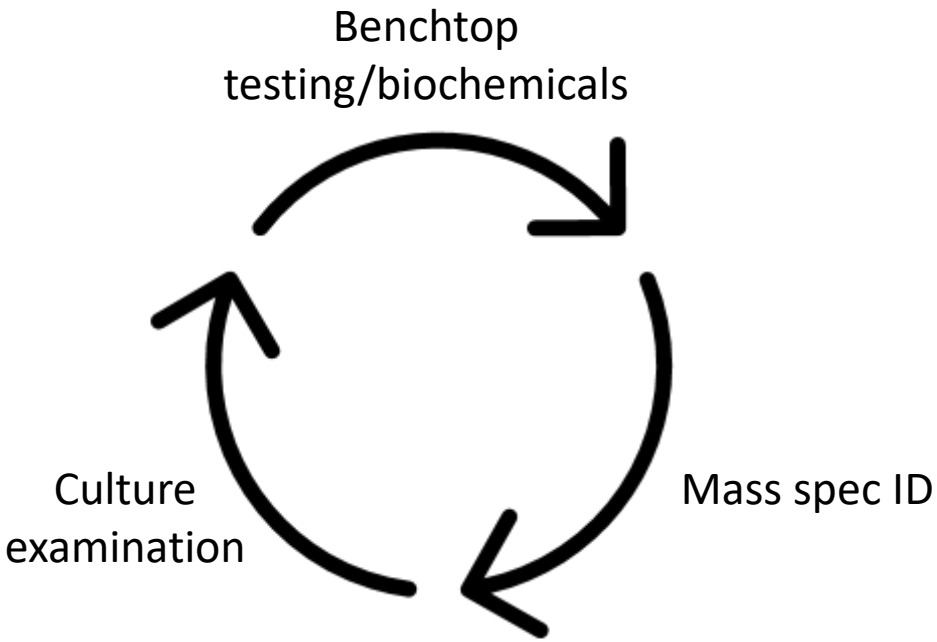
- Growth of colony
- Select organism differentiation
    - Split IDs
    - Organism complexes
- Finite knowledgebase
    - VMS: 1316 organisms (401 FDA cleared)
    - Bruker: 2688+ organisms (488 FDA cleared)

Potential Errors

- Technique dependent
- Specimen inhibition
- Arbitrary identifications
- Generates complacency

Culture examination → Mass spec ID → Culture finalization

VS



How we Attempt to Minimize Complacency

1. Detailed morphology description in the work card
2. Decide if a MALDI is even necessary
    1. Use basic principles: significance, pathogenicity
    2. Sterile sites
    3. ID upon request
3. Treat initial culture examination and spot testing as safety measure
4. Consider the use of rapid ID
5. Preliminary testing becomes 'confirmatory' testing

Case Studies

Marshfield Clinic Health System

Case Study #1: An Aerobic Anaerobe

Background: A new patient's anaerobic blood culture bottle has gone positive

- Sub-cultured aerobically and anaerobically
- Gram stain = Gram Negative bacilli
- BioFire Torch BCID2 Panel = No ID

Backup rapid ID method = MALDI TOF MS

- Spotted from the aerobic subculture growth at 24 hours
- ID = 99.9% Prevotella oris

Does everything make sense so far?

A. Yes

B. No

Immediate issue:

this is an obligate anaerobe!

What about the Anaerobe Subculture?

We incubate our anaerobic cultures for 48 hours before their first look:

- Growing just as well anaerobically as it was aerobically
- Gram stain from anaerobe plates = Gram negative bacilli
- MALDI TOF MS ID (tested twice) = 99.9 % Prevotella oris!

The cultures correlate, what are the next steps?

A. Perform additional biochemicals

B. Nothing, the cultures match

C. Repeat the MALDI on both subcultures

Why?

The Plan

Aerobically:

- Perform an APNA Gram stain confirmation
    - No color change observed
    - This presents new issues...

- Attempt to get an ANC card ID
    - Normally wouldn't have gone this direction
    - Card did not work

Anaerobically:

- Set up potency disks

Disk Type	Expected	Obtained
Bile	S	R
Colistin	V	R
Kanamycin	R	S
Vancomycin	R	S

Resolution

In the end, we sent the isolate to Mayo:

- Identified as *Paenibacillus etheri*

Correlation with our manual testing:

- Gram Positive Rod
- Facultative anaerobe
- Readily decolorizes
- Not in the VMS database or list of organisms available on the ANC card

Case Study #2: A Strange *P. aeruginosa*

Background: A new patient's aerobic blood bottle has gone positive

- Sub-cultured aerobically
- Gram Stain = Gram Negative bacilli
- BioFire Torch BCID2 Panel = No ID

Backup rapid ID method = MALDI TOF MS

- Performed with 3-hour growth
- Came back with no ID
    - Culture was set aside until adequate growth was obtained (between 16 and 24 hours)

Culture Examination

Close macroscopic examination revealed two separate colony morphologies:

- One morphology appropriately identified as *Acinetobacter ursingii*
- The other was small, grey, and wrinkled, with a concentric circle pattern
    - MALDI results from pure isolate = *Pseudomonas aeruginosa*!

What is/are the issue(s) so far?

A. The BioFire did not detect as Pseudomonas aeruginosa

B. The BioFire and VMS contradict each other

C. The morphology is not consistent with Pseudomonas aeruginosa

D. All the above

A GNI card was set up as a potential confirmatory method:

- Came back with a low-discrimination split ID
    - *Pseudomonas aeruginosa*/*Pseudomonas fluorescens*/*Pseudomonas stutzeri*

Too many things were not adding up

- Reported as *Pseudomonas species* and sent out for further identification

In the End…

Identification came back as *Pseudomonas nitroreducens*

Considerations from this experience:

- This organism is not an ID available on the Vitek MS or the GNI card
- The critical nature of this culture is what prompted us to go so far

Case Study #3: A Pure Culture?

Background: A foot tissue culture growing numerous grey, non-hemolytic colonies

My Culture Examination:

- Colony presentation was suggestive of *Enterococcus species*
    - Benchtop tests correlated!

My Reviewer's Examination:

- *Streptococcus agalactiae* with a positive latex test to prove it

Questions I Asked Myself

Did I miss the hemolysis?

Was I fooled by the peachy-pink of a Group-B Strep PYR?

What did the MALDI have to say?

***Enterococcus faecalis/Streptococcus agalactiae* with 50/50 confidence**

What would be your next step?

A. Repeat the MALDI

B. Check instrument limitations

C. Examine plate with stereoscope

D. Repeat the biochemicals

The Verdict

Utilizing our stereo dissecting scope, we examined the culture closer:

- One distinct, non-hemolytic grey colony
- One potentially, ever-so-slightly, lighter grey non-hemolytic colony

We subbed each colony type and repeated our spot testing the next day

Closing Thoughts

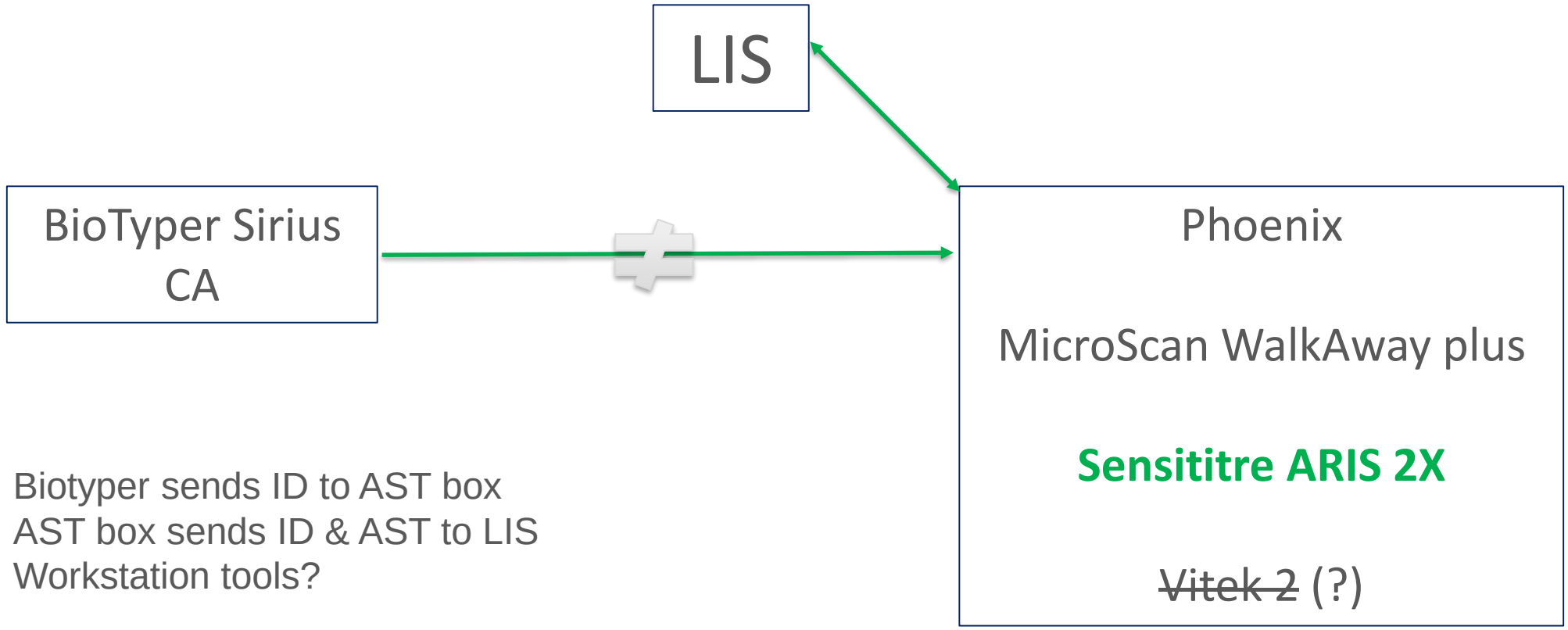
- You can never be too careful in the macroscopic examination of your culture
- In a clinical lab, mass spectrometry is a tool tantamount to conventional microbiology
    - It is not infallible—keep it open to the same level of scrutiny as you would when performing biochemicals
- All-in-all, MALDI TOF MS is a reliable technology
- It is always up to the operating tech to determine the acceptability of an MS ID

Bruker vs Vitek MS

And Why Marshfield Labs Switched

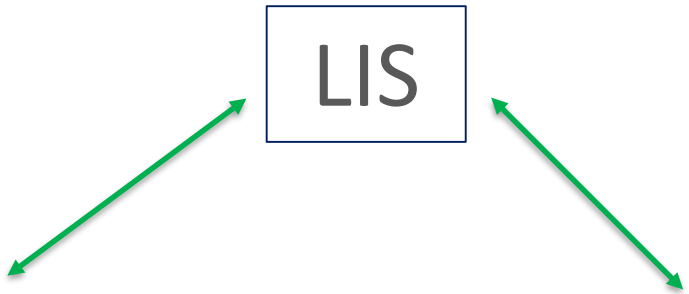
Marshfield Clinic Health System

ID/AST Architecture: Bruker



1. Biotyper sends ID to AST box
2. AST box sends ID & AST to LIS
3. Workstation tools?

ID/AST Architecture: Bruker



BioTyper Sirius CA

1. Biotyper sends ID to LIS
2. LIS sends ID to AST box
3. AST box sends AST to LIS
4. LIS assembles ID & AST report
5. Workstation tools?

BD Phoenix

MicroScan WalkAway plus

Sensititre ARIS 2X

~~Vitek 2~~ (?)

ID/AST Architecture: Bruker

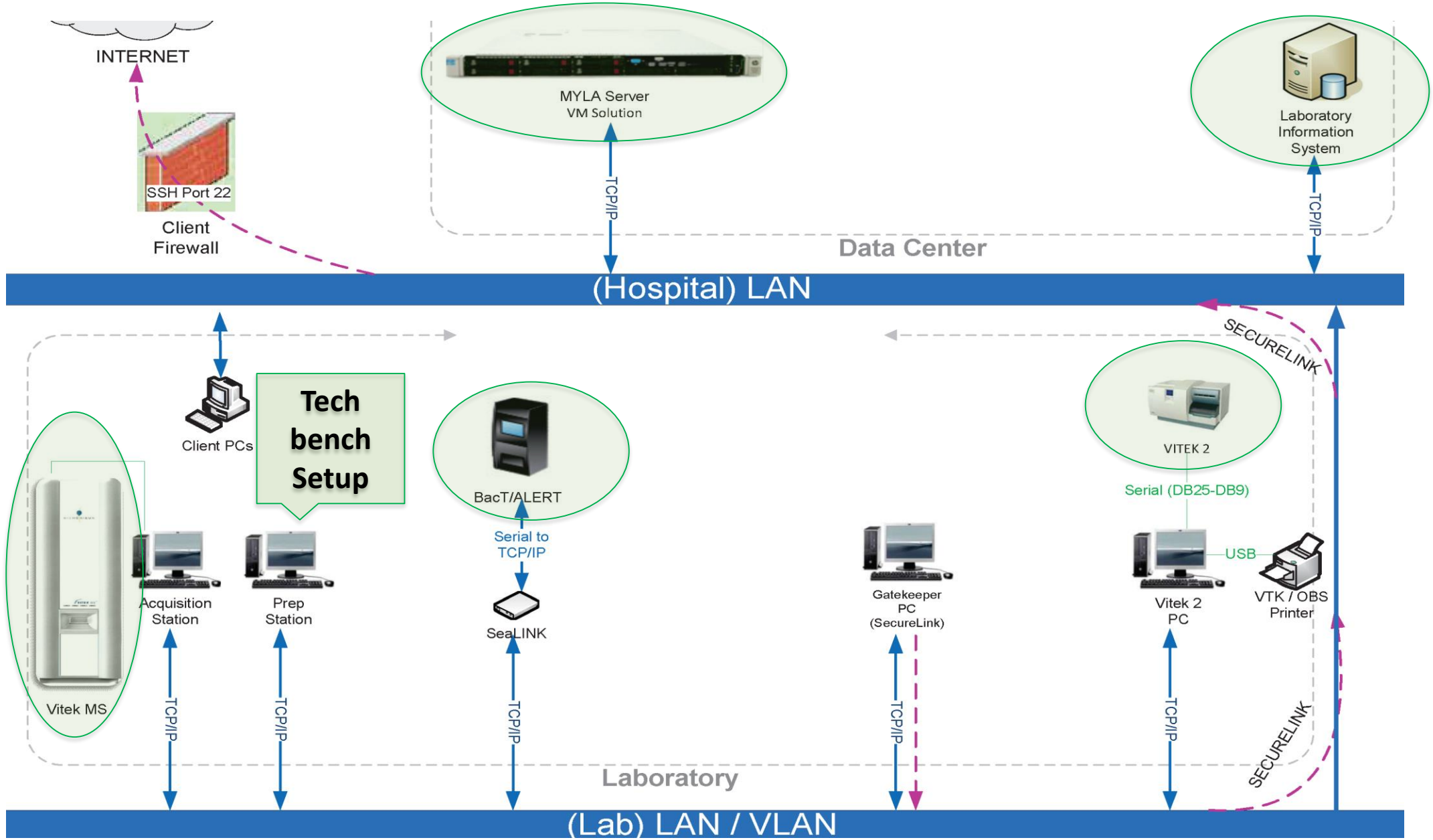
Pros

- Allows wider selection of AST boxes

Cons

- Requires separate P2P connection between boxes
- Depends on AST vendor to maintain the interface

ID/AST Architecture: bioMerieux



ID/AST Architecture: bioMerieux

Pros

- Consistent data integration
- BacT/Alert functionality
- Statistical reports through Myla
- Single vendor solution
- Browser-based user interface at bench tech & management levels
- IT = support

Cons

- IT =more layers
- Single vendor solution = less choice