

Implementation of the VITEK® MS and Its Use in Microbial Identification

Katalin Kiss, Ph.D., PMP®

Manager, Scientist, Laboratory Testing Services, ATCC

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About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one-third with advanced degrees



Established partner to global researchers and scientists



Certification and accreditation

ISO 9001:2008 certification for quality management system

- Demonstrates commitment to quality products, customer service, and continued improvement



ISO 13485:2003 certification for the design, development, production, testing, and distribution of medical devices

- Applies to synthetic molecular standards, the HIV surveillance kit, and other diagnostic and research kits



ISO Guide 34:2009 accreditation for production

- Applies to Certified Reference Materials (CRMs)



ISO/IEC 17025:2005 accreditation for testing

- Applies to all ATCC cultures, derivatives, and bioproducts tested in our laboratories



ATCC Accessions



- Deposits come from every continent
- Newly identified species, outbreak strains, type strains, etc.
- Grow/expand deposit to a large enough scale to distribute
- Preserve according to the best method for the species
- Some cultures have to be maintained in continuous culture
- Confirm identity noted by the depositor
- Minimum test is purity, viability, and sequence
- Goal is two platforms to confirm the item
- Worldwide distribution
- Ship frozen/live materials

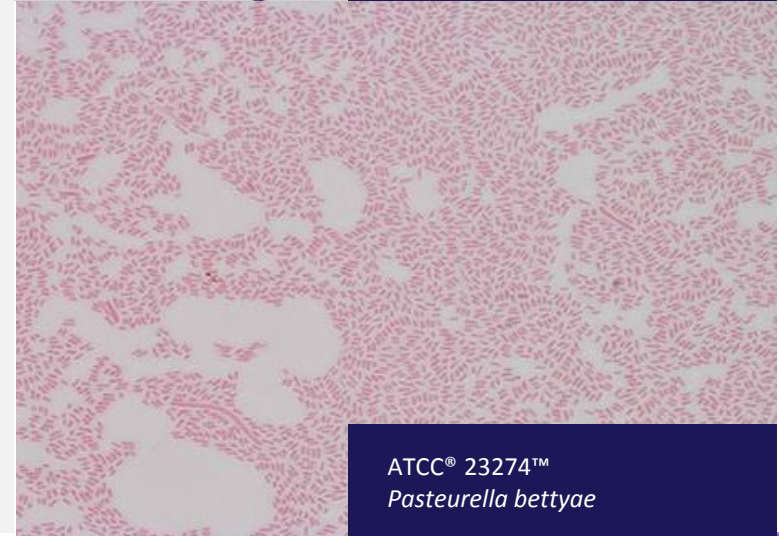
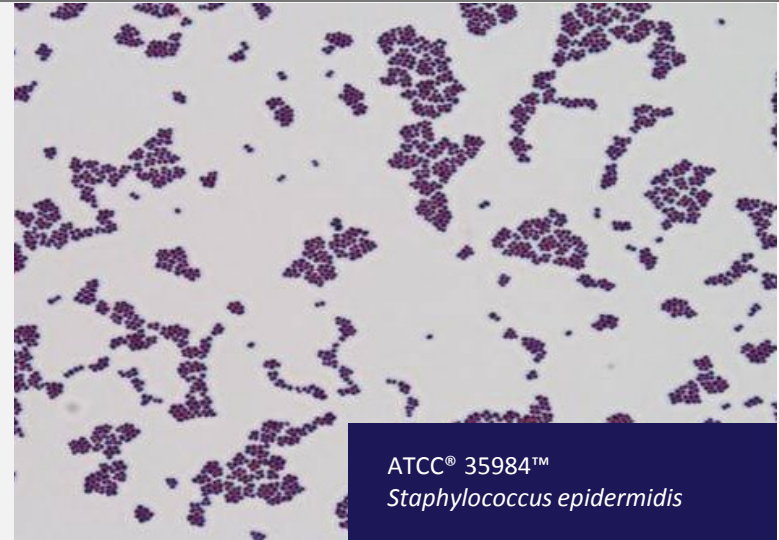
Identification of microbes – After the 19th century

- What does it look like?
 - Macroscopic
 - Microscopic
- How does it grow?
 - Solid media
 - Suspension
 - Biphasic
 - Temperature
 - Atmosphere
 - Carbon source
 - Metabolic by-products
- What does it smell like?
 - Not recommended

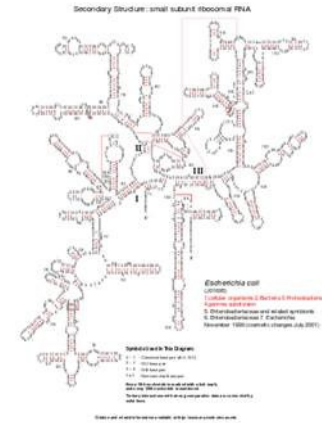
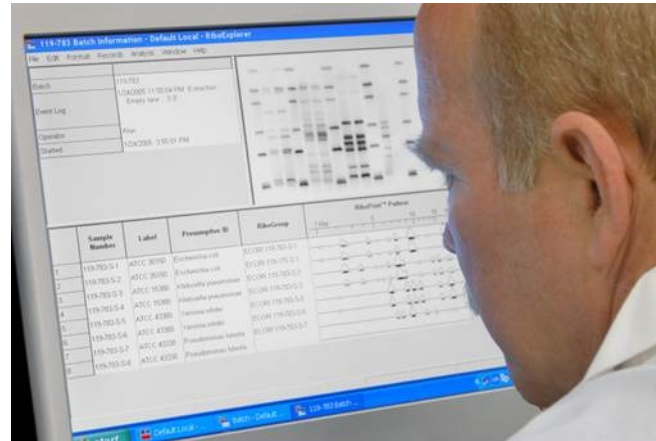


Identification of microbes – After the 19th Century

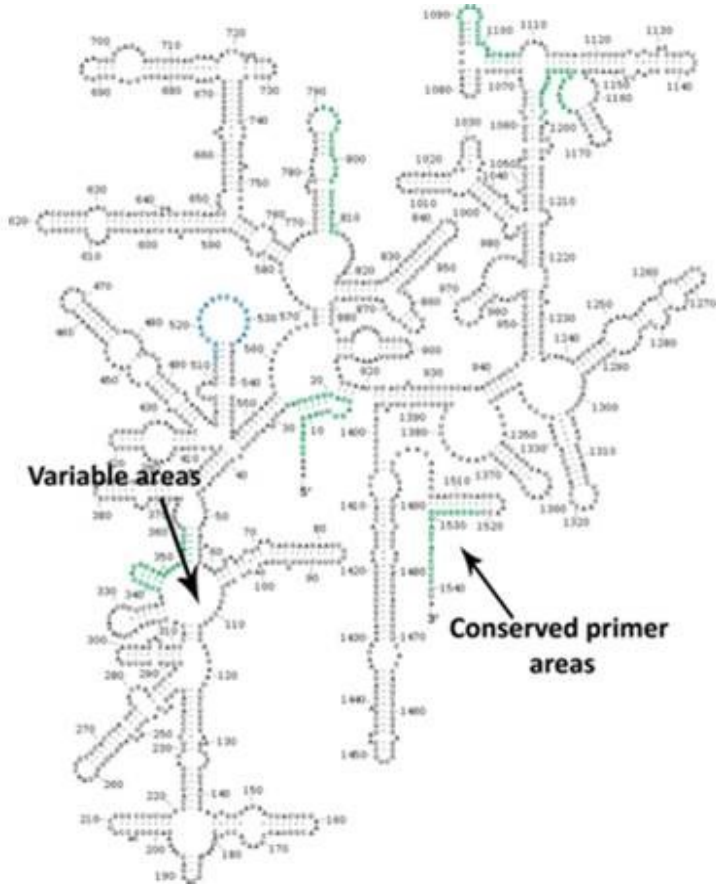
- How does it stain?
- To name a few:
 - Gram stain
 - Acid-fast stain
 - Gimenez
 - Giemsa



Identification of microbes – Genotypic testing



Identification of microbes – Genotypic testing

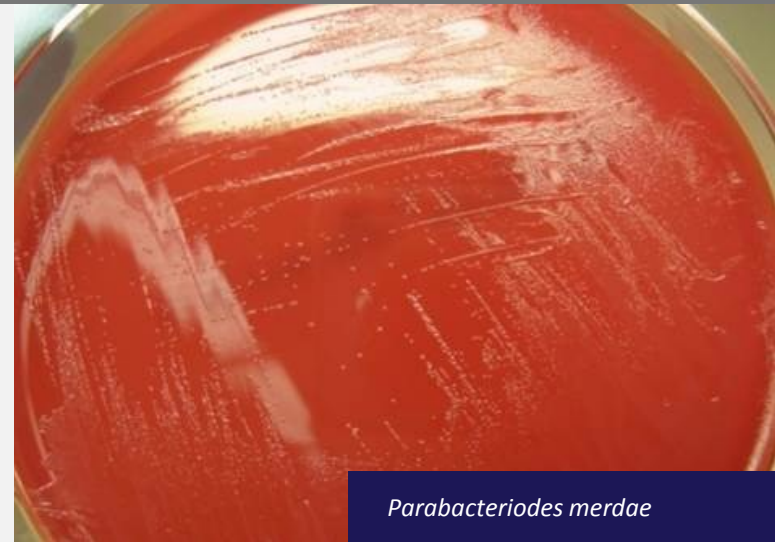


- Confirm identity by genotypic methods to the genus and species level
- Sequencing analysis of the 16S rRNA gene
- Fast and reliable, but limited species and strain resolution
- DNA-DNA hybridization
- Ribotyping

There is no concise definition of a species!

Risks of relying on just phenotypic authentication

- Descriptions can be very subjective
 - You need to know what tests to apply
 - Some genera are biochemically inert
 - Time consuming
-
- Grew anaerobically, 37°C
 - Gram-negative rods
 - Non-motile
 - To differentiate – aerobic growth, catalase, oxidase, motility, variety of carbohydrates, etc.

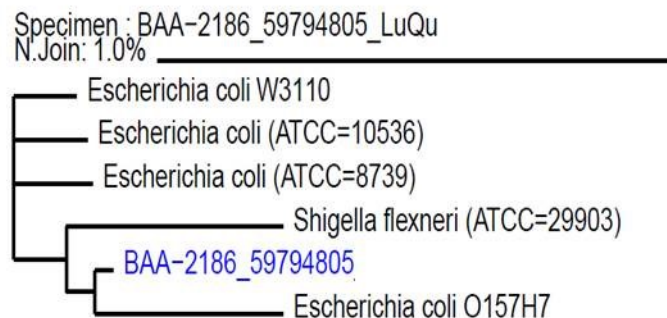


Risks of relying on just genotypic authentication

Not answered by most genotypic testing:

- Which one is it?
- Does it express the trait I need?
- No consensus on definition of a species at the genetic level. Minimum homology can be from 50% to 70%.

Description	Max score	Total score	Query cover	E value	Max ident
Clostridium acetobutylicum strain S512_16S ribosomal RNA gene, partial sequence	2242	2242	100%	0	100%
Bacillus anthracis strain R5-331_16S ribosomal RNA gene, partial sequence	2242	2242	100%	0	100%
Bacillus cereus strain OPP5 3-2_16S ribosomal RNA gene, partial sequence	2242	2242	100%	0	100%



ATCC identification – the most robust system possible



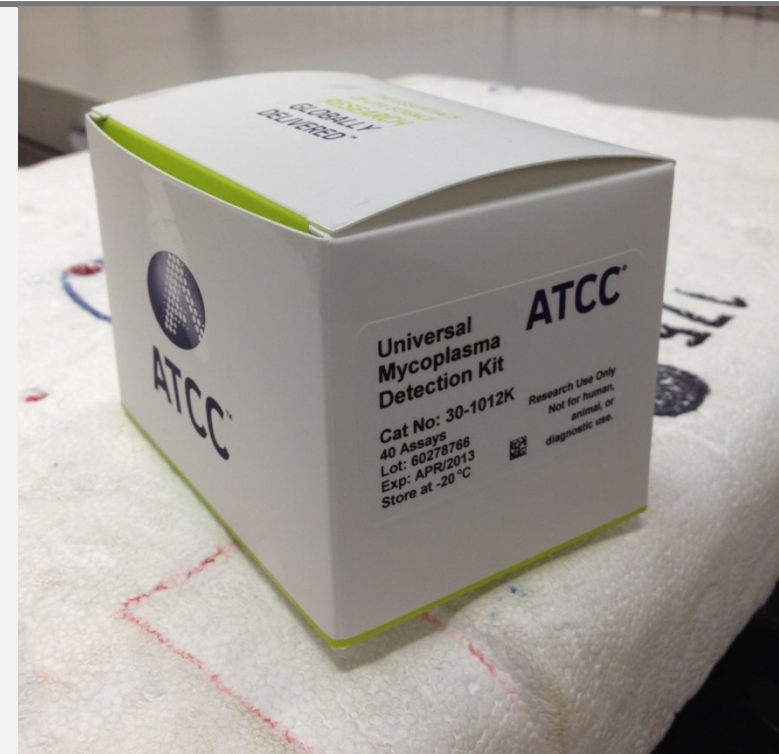
ATCC utilizes both classical and modern techniques

- Phenotypic analysis
- Genotypic analysis
- Functional analysis

No single method of identification is sufficient

ATCC identification – Why do we need the most robust system possible?

- Help our customer make the right choice when selecting a catalog item
- Provide the most consistent product possible
- Perform according to the standard
- Comply with local, state, federal, and international regulation

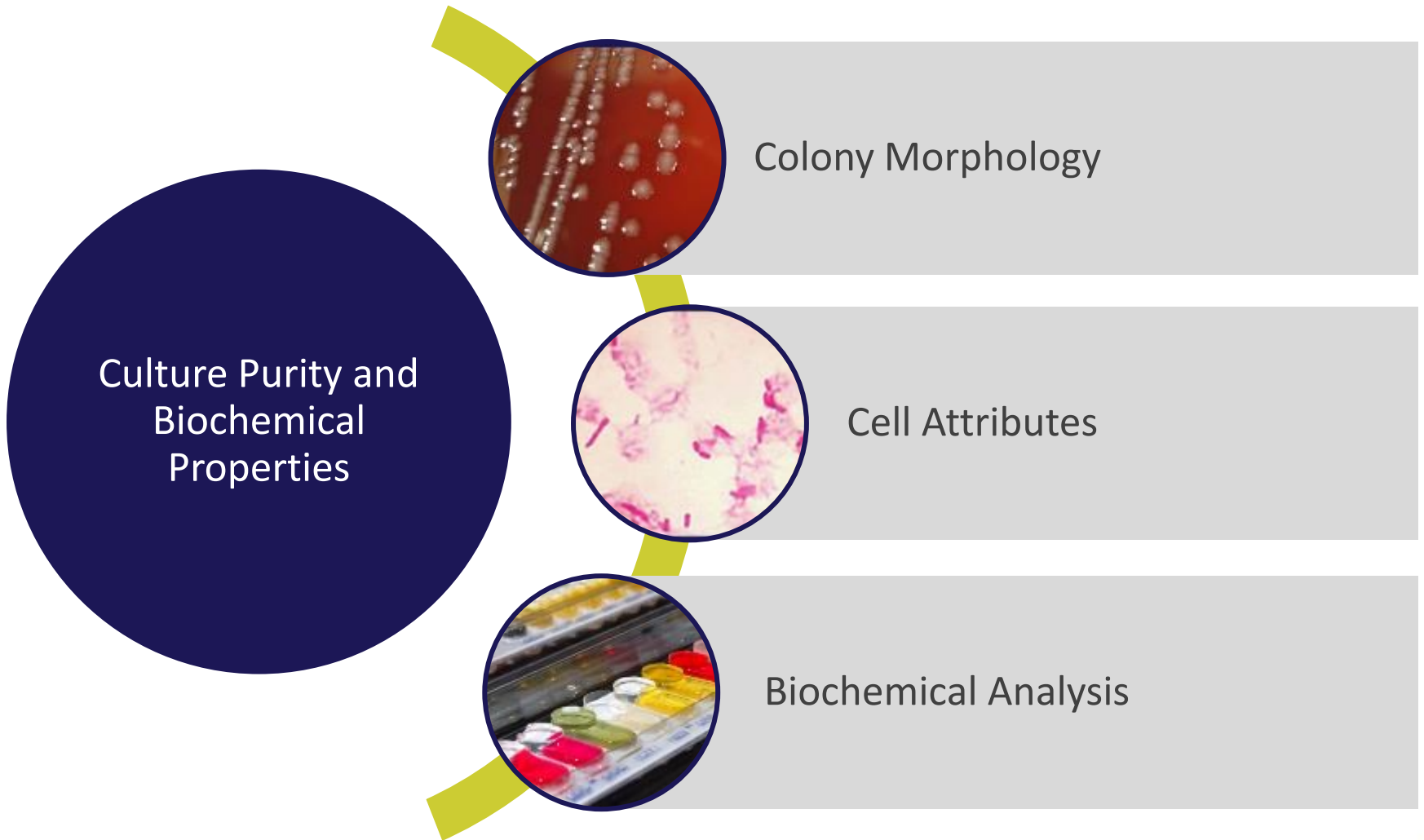


Permits may be required for shipping this product

Distribution requires completion of a Customer Acceptance of Responsibility (CAR) for Commerce Control List Biologicals form.

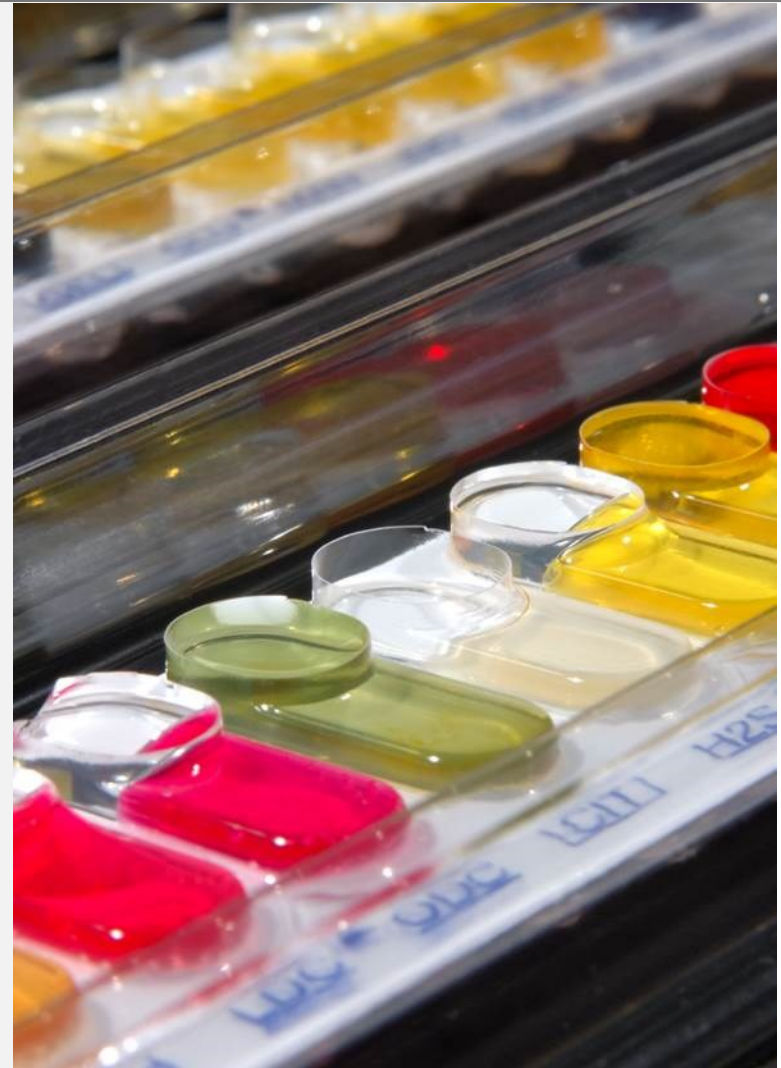
Customers located in Hawaii will need to contact the Hawaii Department of Agriculture to determine if an Import Permit is required.

Phenotypic testing

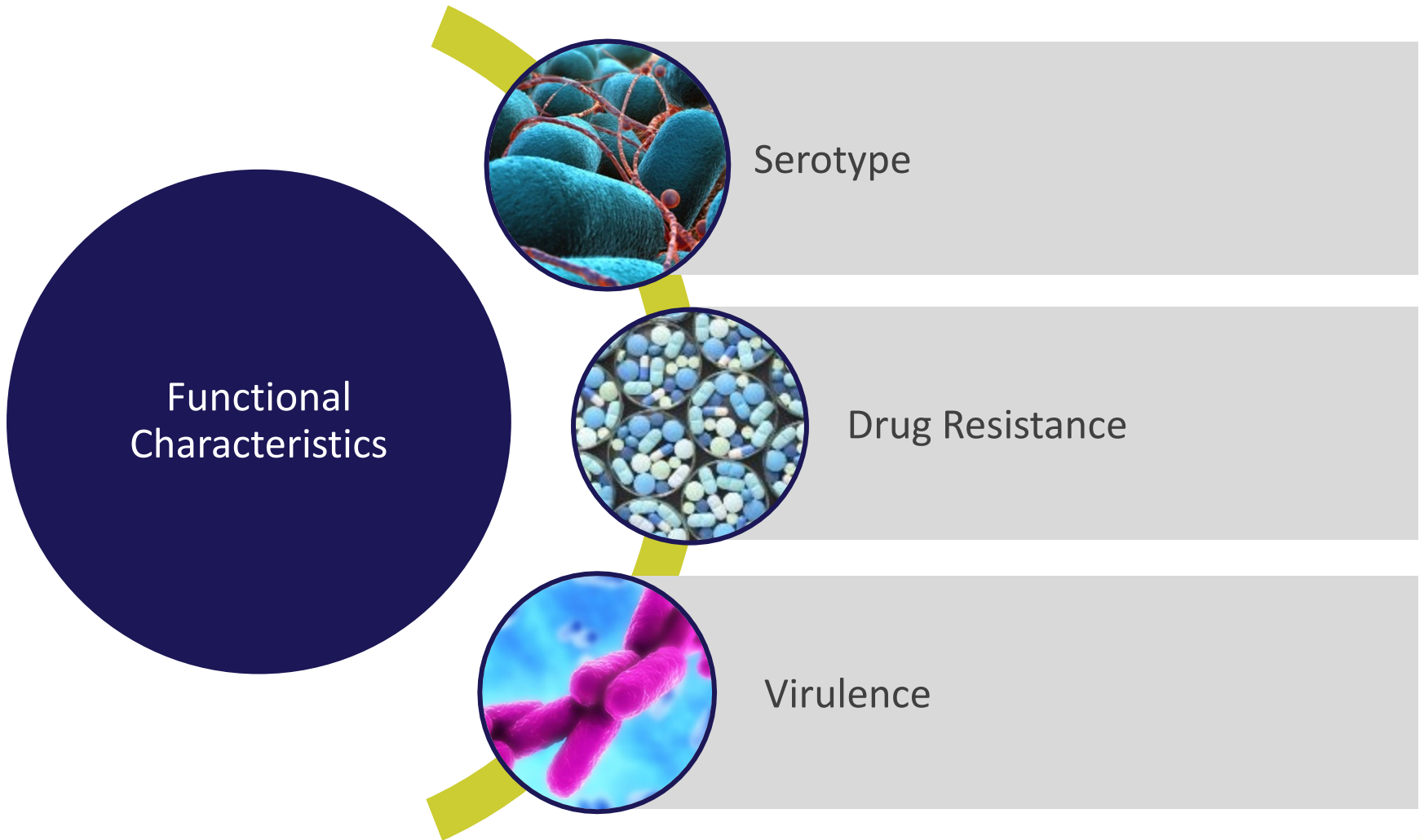


Phenotypic testing platforms at ATCC

- API® ID
- Remel RapID™
- Biolog Microbial ID
- VITEK® 2
- Serotyping
- Hundreds of biochemical tests



Functional testing



Functional testing platforms at ATCC



Photo credit: James Gathany

- AST
- Toxin assay
- IFA
- ELISA
- Cytotoxicity assay

Genotypic testing

Sequencing



Toxinotyping



Ribotyping



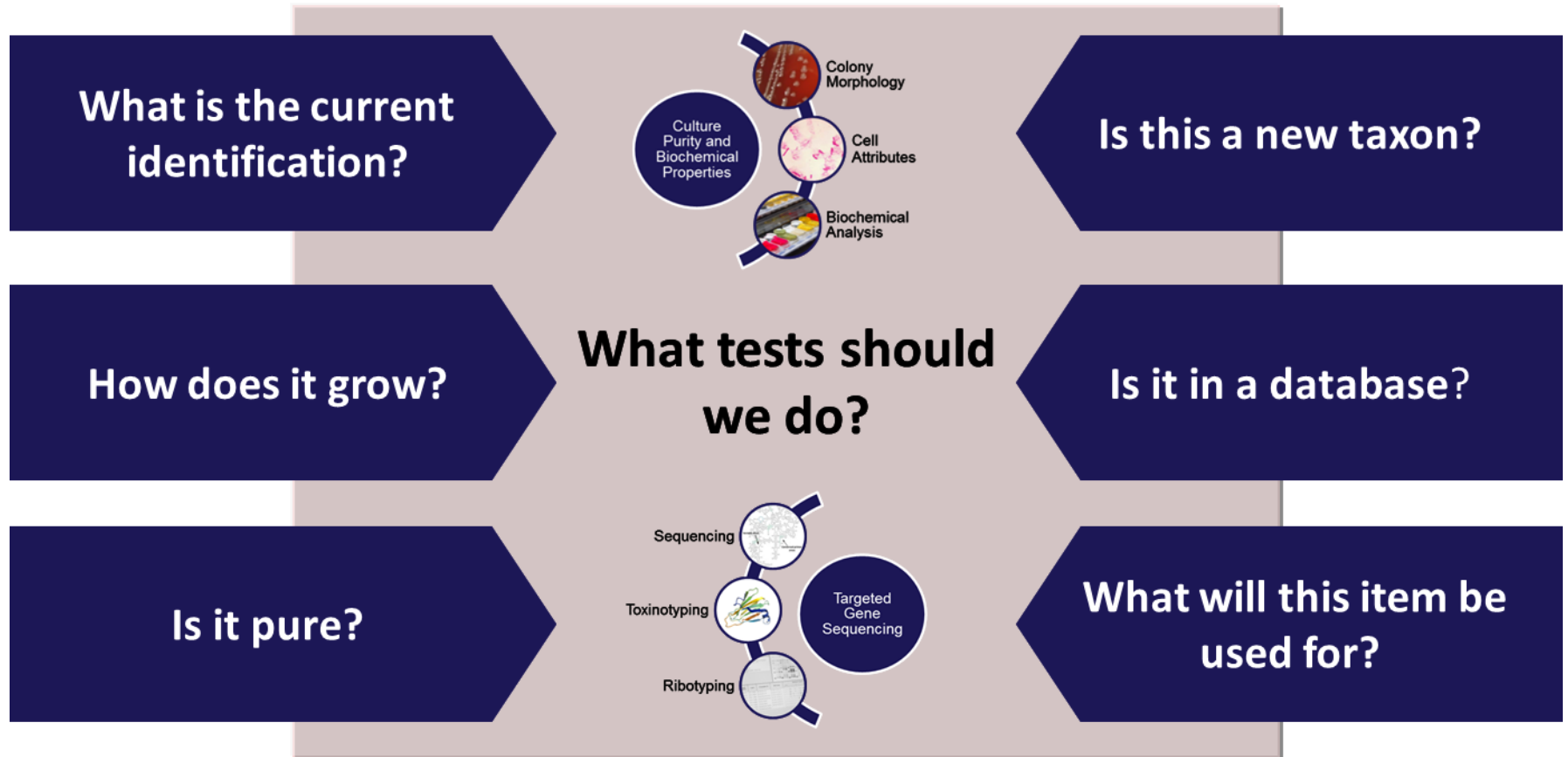
Targeted Gene Sequencing

Genotypic testing platforms at ATCC


- ABI 3500 XL analyzers
 - MicroSeq[®] database
 - Public databases
 - Sequence provided by the depositor
- PCR
- DuPont[™] RiboPrinter[®]
- Illumina[®] MiSeq



Steps to the identification of microbes at ATCC



What is the current identification?



ATCC™ | THE ESSENTIALS OF LIFE SCIENCE RESEARCH
GLOBALLY DELIVERED™

FOR ATCC™ USE ONLY

ACQUISITION ID: _____

ITEM NUMBER: _____

BACTERIOLOGY, MYCOLOGY, & PROTISTOLOGY DEPOSIT DESCRIPTION FORM

INSTRUCTIONS TO DEPOSITOR: Please fill in relevant and available information about each deposit. Additional information, references, or pages may be attached as needed. This information helps us better characterize and preserve your deposit.

Type of Deposit (please select one of the following): Bacteriology Mycology Protistology

1. BACKGROUND INFORMATION

a. The "MATERIAL" subject to this Deposit Form is:

Taxonomic Classification:

Family:

Genus:

Species:

Subspecies/Strain:

Biological Agent/Common Name / Name of Other State:

b. Is this material a type strain of the species? Yes No

c. Method of identification (e.g. molecular methods):

d. Date of identification:

e. Geographic source of material (country, state, locale, GPS coordinates or latitude/longitude):

f. Source of isolate:

Was this material isolated from:

American Type Culture Collection (ATCC)®
P.O. Box 1549
Manassas, VA 20110 USA
www.atcc.org

(800) 638-6597 or 703-365-2700
Fax: 703-365-2750
Doc ID: 26714

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Effective Date: 05/14/2014
Revision: 2

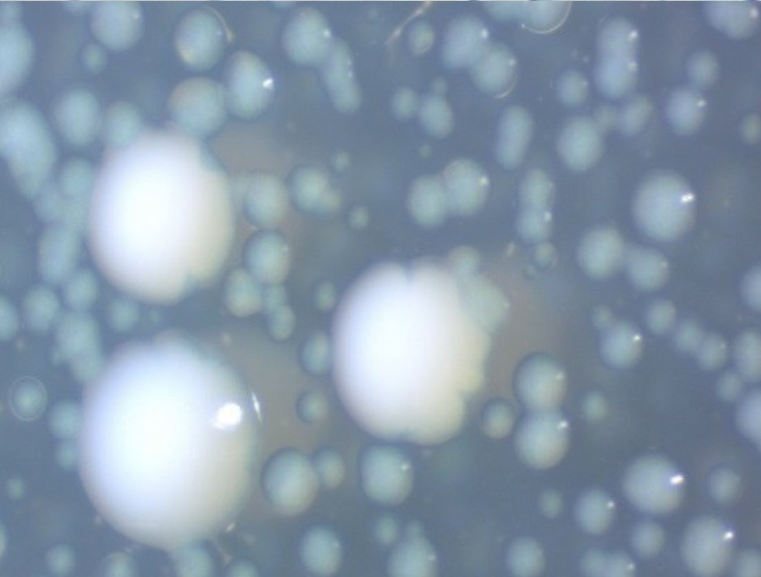
Steps to identification- How does it grow?

How do we grow it?

- Media
 - Atmosphere
 - Temperature
 - Incubation time
-
- Access to thousands of media formulations
 - >15 temperatures available at all times
 - Anaerobic chambers
 - Cannula system for custom gas mixtures
 - Lighted incubators



Steps to identification – Is it pure?



- Contaminants are not tolerated
- We avoid selective media for growth
- We spot check when the material is passaged
- We check all anaerobic cultures for aerobic contaminants

Steps to identification – Is it a new taxon? Is it described in a database or literature?

- Check depositor information
- Peer reviewed literature
- Text books, such as Bergey's Manual of Systematic Bacteriology
- Online databases, NCBI, RDP, etc.
- Not every catalog item has been described in depth
- Minimal test is purity, viability, and sequencing
- If we have access, we can confirm 16S rRNA sequence

Steps to identification – What will this be used for?



How does VITEK® MS compare to current methods?

Complexity

Method	Complexity
API® ID	Moderate
Biolog Microbial ID	Moderate
VITEK® 2	Moderate
Hundreds of biochemical tests	Moderate-Advanced
ABI 3500 XL analyzers	Advanced
DuPont™ RiboPrinter®	Moderate
PCR	Moderate-Advanced
VITEK® MS	Easy

How does VITEK® MS compare to current methods?

Turnaround time

Method	Turnaround time
API® ID	24-48 hours
Biolog Microbial ID	8 hours
VITEK® 2	4-6 hours
Hundreds of biochemical tests	24 hours or longer
ABI 3500 XL analyzers	1 day
DuPont™ RiboPrinter®	1 day
PCR	4 hours
VITEK® MS	Minutes

How does VITEK® MS compare to current methods?

Sample preparation

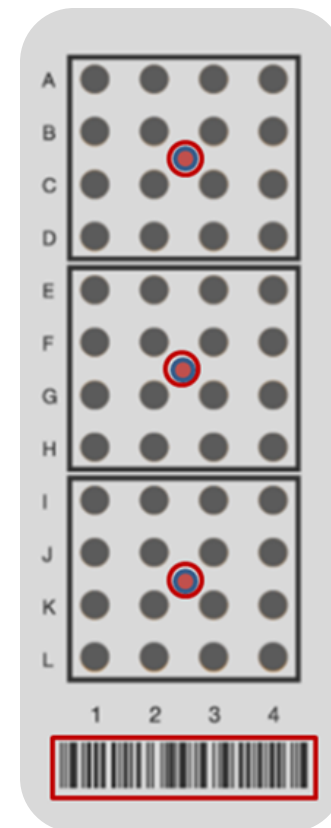


Method	Sample Preparation
API® ID	Fresh, pure
Biolog Microbial ID	Fresh, pure
VITEK® 2	Fresh, pure
Hundreds of biochemical tests	Fresh, pure
ABI 3500 XL analyzers	Pure
DuPont™ RiboPrinter®	Pure
PCR	Pure
VITEK® MS	Fresh, Pure

How does VITEK[®] MS compare to current methods?

Sample preparation

- Basic protocol for bacteria:
 - Grow bacteria on a solid surface for isolation
 - Transfer a spot from the colony to the slide
 - Add matrix
 - Allow to dry
 - Read slide
- For fungi, an additional lysis reagent is needed
- Sample prep take less than five minutes



How does VITEK® MS compare to current methods?

Interpretation of data

Method	Data Output	Ease of Interpretation
API® ID	Genus, species, score	Easy
Biolog Microbial ID	Genus, species, score	Easy
VITEK® 2	Genus, species, score	Easy
Hundreds of biochemical tests	+ or -	Moderate
ABI 3500 XL analyzers	FASTA File	Complex
DuPont™ RiboPrinter®	Genus, species, score	Easy
PCR	+ or - / size amplicon	Easy-Moderate
VITEK® MS	Genus, species, score	Easy

How does VITEK® MS compare to current methods?

Interpretation of data – Biochemical results

Assay	<i>L. grayi</i>	<i>L. innocua</i>	<i>L. ivanovii</i>	<i>L. monocytogenes</i>
Mannitol	+	-	-	-
L-Rhamnose	-	d	-	+
Soluble starch	+	-	-	-
D-xylose	-	-	+	-
B-hemolysis	-	-	+	+

How does VITEK® MS compare to current methods?

Interpretation of data – VITEK® 2 card

Identification Information	Card:	GN	Lot Number:	241287420	Expires:	Oct 27, 2014 13:00 EDT											
	Completed:	Jun 27, 2014 15:48 EDT	Status:	Final	Analysis Time:	6.00 hours											
Selected Organism	96% Probability		Klebsiella pneumoniae ssp rhinoscleromatis														
	Bionumber: 6205710542560200		Confidence:		Excellent identification												
SRF Organism																	
Analysis Organisms and Tests to Separate:																	
Analysis Messages:																	
Contraindicating Typical Biopattern(s)																	
Klebsiella pneumoniae ssp rhinoscleromatis CMT(11),																	
Biochemical Details																	
2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

How does VITEK® MS compare to current methods?

Interpretation of data – MicroSeq® database search

Hit List Table

Specimen	Library	Library Entry Name	% Match	Consensus Length	Library Entry Length	Total Mismatches
6908_622520 91_SaLi	AB_BacterialF ullGeneLib_2. 0	Klebsiella pneumoniae rhinoscleroma tis (ATCC=13884)	100.0	812	1488	0
6908_622520 91_SaLi	AB_BacterialF ullGeneLib_2. 0	Klebsiella pneumoniae ozaenae (ATCC=11296)	99.61	812	1488	4
6908_622520 91_SaLi	AB_BacterialF ullGeneLib_2. 0	Klebsiella pneumoniae pneumoniae (ATCC=10031)	99.48	812	1457	6
6908_622520 91_SaLi	AB_BacterialF ullGeneLib_2. 0	Klebsiella pneumoniae pneumoniae (ATCC=13883)	99.48	812	1488	4
6908_622520 91_SaLi	AB_BacterialF ullGeneLib_2. 0	Enterobacter cancerogenus (ATCC=33241)	99.11	812	1488	9

How does VITEK[®] MS compare to current methods?

Interpretation of data – API[®] strip

apiweb[™] - Identification result

ATCC - Manassas

API LISTERIA V1.2

1 2 4 1 2 4 1 2 4 1

DIM ESC αMAN DARL XYL RHA MDG RIB G1P TAG BHEM

↑ 6 ↑ 5 ↑ 1 ↑ 0 ↑

REFERENCE DATE
7/9/14

COMMENT
19113_62671205

GOOD IDENTIFICATION

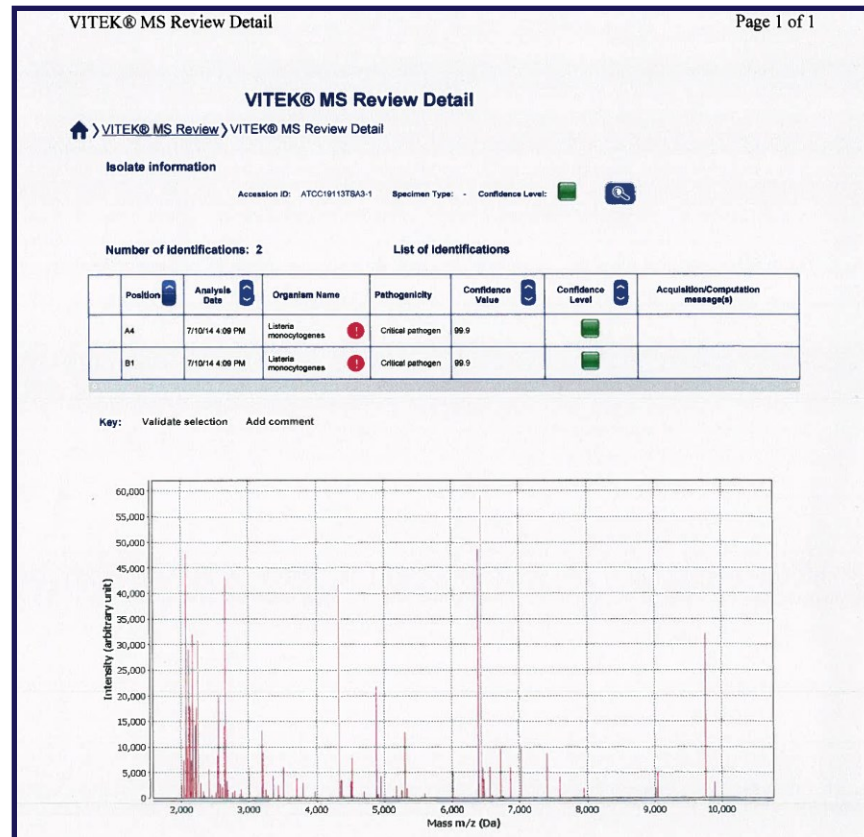
Strip API LISTERIA V1.2
Profile 6 5 1 0
Note

Significant taxa	% ID	T	Tests against
Listeria monocytogenes	98.6	1.0	

Next taxon	% ID	T	Tests against
Listeria innocua	1.3	0.58	DIM 99%

How does VITEK® MS compare to current methods?

Interpretation of data – VITEK® MS



How does VITEK® MS compare to current methods?

Database

Method	Data Output	Size of database
API® ID	Genus, species, score	>300
Biolog Microbial ID	Genus, species, score	>1500
VITEK® 2	Genus, species, score	>400 species
Hundreds of biochemical tests	+ or -	?
ABI 3500 XL analyzers	FASTA File	?
DuPont™ RiboPrinter®	Genus, species, score	>1400
PCR	+ or - / size amplicon	NA
VITEK® MS	Genus, species, score	>2000

How does VITEK® MS compare to current methods?

Expandability

Method	Size of database	Can we expand the database?
API® ID	>300	No
Biolog Microbial ID	>1500	No
VITEK® 2	>400 species	No
Hundreds of biochemical tests	?	No
ABI 3500 XL analyzers	?	Yes
DuPont™ RiboPrinter®	>1400	Yes
PCR	NA	NA
VITEK® MS	>2000	Yes

Summary – Methods

Method	Complexity	Turnaround Time	Sample Preparation	Ease of Interpretation	Size of the Database
API® ID	Moderate	24-48 hours	Fresh, pure	Easy	>300
Biolog Microbial ID	Moderate	8 hours	Fresh, pure	Easy	>1500
VITEK® 2	Moderate	4-6 hours	Fresh, pure	Easy	>400 species
Hundreds of biochemical tests	Moderate-Advanced	24 hours or longer	Fresh, pure	Moderate	?
ABI 3500 XL analyzers	Advanced	1 day	Pure	Complex	?
DuPont™ RiboPrinter®	Moderate	1 day	Pure	Easy	>1400
PCR	Moderate-Advanced	4 hours	Pure	Easy-Moderate	NA
VITEK® MS	Easy	Minutes	Fresh, Pure	Easy	>2000

How does VITEK® MS compare to current methods?

Installation:

- Required only one modification to change an electrical socket
- Reset climate control to 68°F to ensure proper functioning of machine
- Very few moving parts of the machine, can be remotely monitored and manipulated if needed

Training:

We have over ten people trained on the use of the machine. We used a 'train the trainer approach' where one of our team members went to the bioMérieux training and is responsible for training our staff in sample preparation and machine usage.



Photo credit: BioMérieux

Implementation into the ATCC Process



Did we receive the right thing?

- Morphology/Microscopic observation
- Sequence analysis
- VITEK® MS

Is it pure?

- Morphology/Microscopic observation
- VITEK® MS
- Sequence analysis
- API® ID
- Remel RapID™
- Biolog Microbial ID
- VITEK® 2
- Serotyping
- Hundreds of biochemical tests

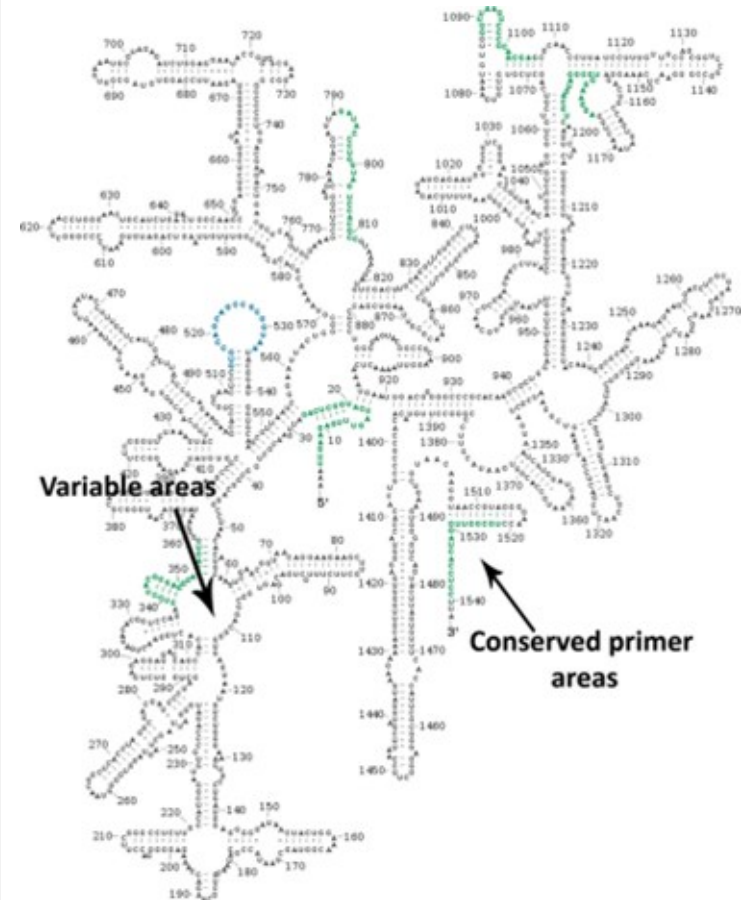
Authenticate the material

- Morphology/Microscopic observation
- Sequence analysis
- VITEK® MS
- API® ID
- Remel RapID™
- Biolog Microbial ID
- VITEK® 2
- Serotyping
- Hundreds of biochemical tests
- PCR
- DuPont™ RiboPrinter®

Challenges to implementation?

- Developing protocols for broth grown cultures
- Developing protocols for filamentous fungi
- There will always be slash calls

There is no concise definition of a species!



Conclusion

- VITEK[®] MS is a user-friendly platform
- Training is straightforward with some practice required
- Data is easy to interpret
- It has one of the largest, curated databases available
- Sample preparation is comparable to other methods currently being used
- Turnaround time is faster than other methods currently being used
- Installation was simple

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Thank you for joining today!

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- **October 29, 2015**
10:00 AM, 3:00 PM EST
Barry R. Bochner, Ph.D., *CEO & CSO*, Biolog, Inc.
High Resolution Phenomic Analysis of Microbial and Mammalian Cells
- **November 12, 2015**
10:00 AM, 3:00 PM EST
Bill Hirt, Ph.D., *Director of Accreditation*, ANAB
How Does ISO 17025 Accreditation Build International Confidence?



Please email additional questions to:
tech@atcc.org