



MicrobeNet 2.0:

Connecting the Public Health Laboratory to the CDC

New Technologies

John R. McQuiston Ph.D.

Special Bacteriology Reference Laboratory

Bacterial Special Pathogens Branch

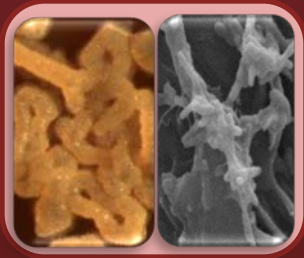
Division of High Consequence Pathogens and Pathology

National Center for Emerging and Zoonotic Infectious Diseases

Centers for Disease Control and Prevention

Overview

- 1) Methods for unknown bacterial identification
- 2) MicrobeNet
- 3) MicrobeNet Modules
 - 1) Phenotypic
 - 2) MALDI-ToF
 - 3) Sequencing
- 4) Getting the word out!
- 5) How to sign up!

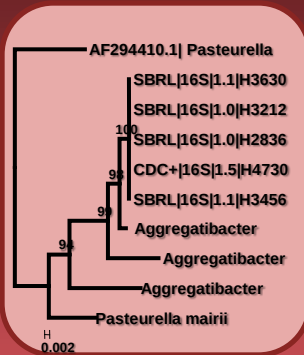


The Special Bacteriology Reference Laboratory (SBRL)

identifying the “unidentifiable”



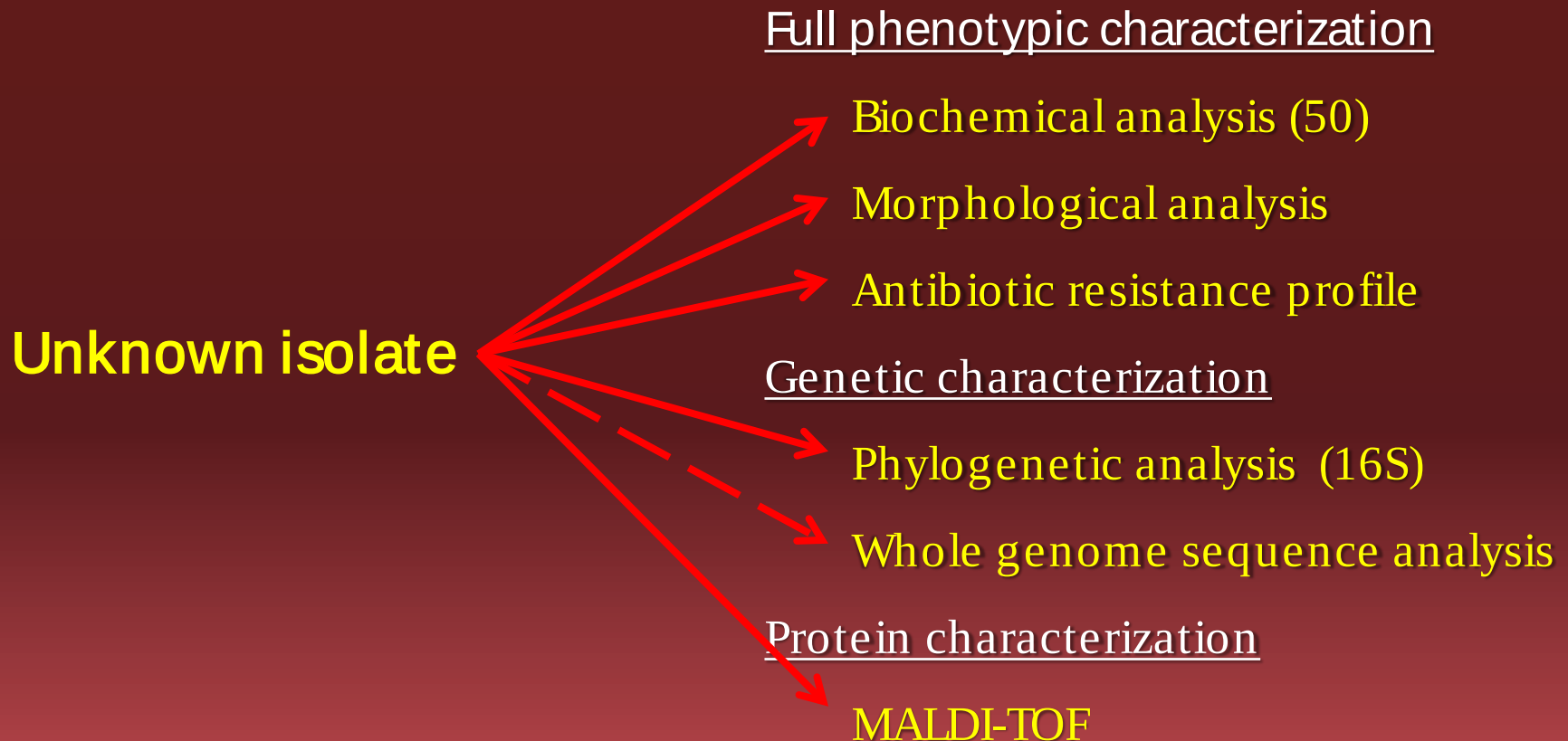
- Performs identification of over 500 rare, unusual and unnamed species
- The extremes of “old and new” technology
 - Traditional microscopy
 - Gram stains and biochemical analysis
 - MALDI-TOF Mass Spec.
 - 16s and *rpoB* sequencing
 - Whole genome sequencing



The Special Bacteriology Reference Laboratory

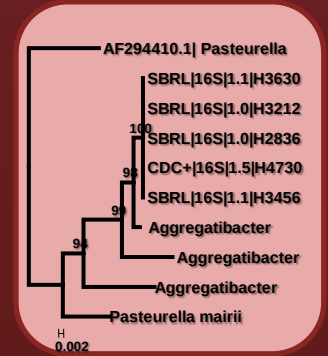
identifying the “unidentifiable”

Reference Diagnostics and New Species Identification

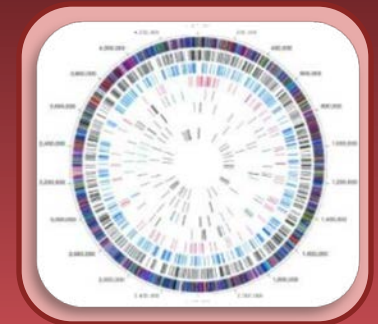
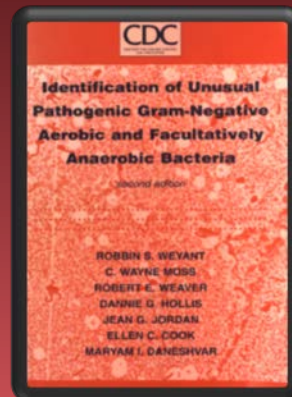
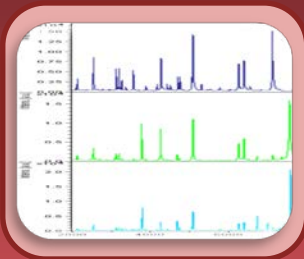




Bacterial Diagnostics



1. Bacterial diagnostic methods are changing rapidly
2. However, many traditional methods are still used worldwide
3. Reliable resources without data overload is a significant problem
4. Finding information in one place for many different pathogens is rare





<https://microbenet.cdc.gov>

MicrobeNet as a support tool for Public Health Laboratories.

- 1.) To have 16s and other gene sequence search capability
- 2.) To have biochemical and phenotype search capability
- 3.) To be heavily curated by SME's from CDC
- 4.) To be limited in redundancy of data for each species
- 5.) To have broad information on many species



Live - Jan. 2013

CDC Home

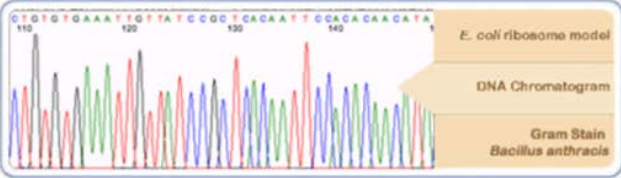


Centers for Disease Control and Prevention
Your Online Source for Credible Health Information

SEARCH

MicrobeNet™

Log In



E. coli ribosome model
DNA Chromatogram
Gram Stain
Bacillus anthracis



Text size: [S](#) [M](#) [L](#) [XL](#)

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Contact Us:

 CDC / MicrobeNet
1600 Clifton Rd
Atlanta, GA 30333

 800-CDC-INFO
(800-232-4636)
TTY: (888) 232-6348
24 Hours/Every Day

 MicrobeNet@cdc.gov

Get Access!

Already have an account? [Click here to log in.](#)

Need an account?

Contact us at MicrobeNet@cdc.gov to request a MicrobeNet account.

What is MicrobeNet?

- A web-based tool for identification of microbial pathogens
- A curated database of genetic and phenotypic properties of microbial pathogens
- A source for information on pathogens, identification algorithms, and testing protocols

What Can I Use MicrobeNet For?

- Identify an unknown organism using 16S rRNA gene comparisons (other targets coming soon)
- View phenotypic portraits of specific organisms or groups of organisms (phenotype based searching coming soon)
- Find information regarding test protocols
- Learn more about a pathogen of interest

[Home](#) [A-Z Index](#) [Site Map](#) [Policies](#) [About CDC.gov](#) [Link to Us](#) [All Languages](#) [Contact CDC](#)

Centers for Disease Control and Prevention 1600 Clifton Rd. Atlanta, GA 30333, USA
800-CDC-INFO (800-232-4636) TTY: (888) 232-6348, 24 Hours/Every Day - cdcinfo@cdc.gov





Trusted sites | Protected M

MicrobeNet 2.0



A CDC Virtual Reference Laboratory

[FEATURES](#)

[SIGN IN](#)

[REQUEST ACCOUNT](#)

Faster and more **accurate** pathogen identification

MicrobeNet helps you to be more efficient at identifying rare and complex pathogens. With powerful tools and information at your fingertips, you get quicker test turnaround and lower costs for your organization.

[LEARN MORE](#)



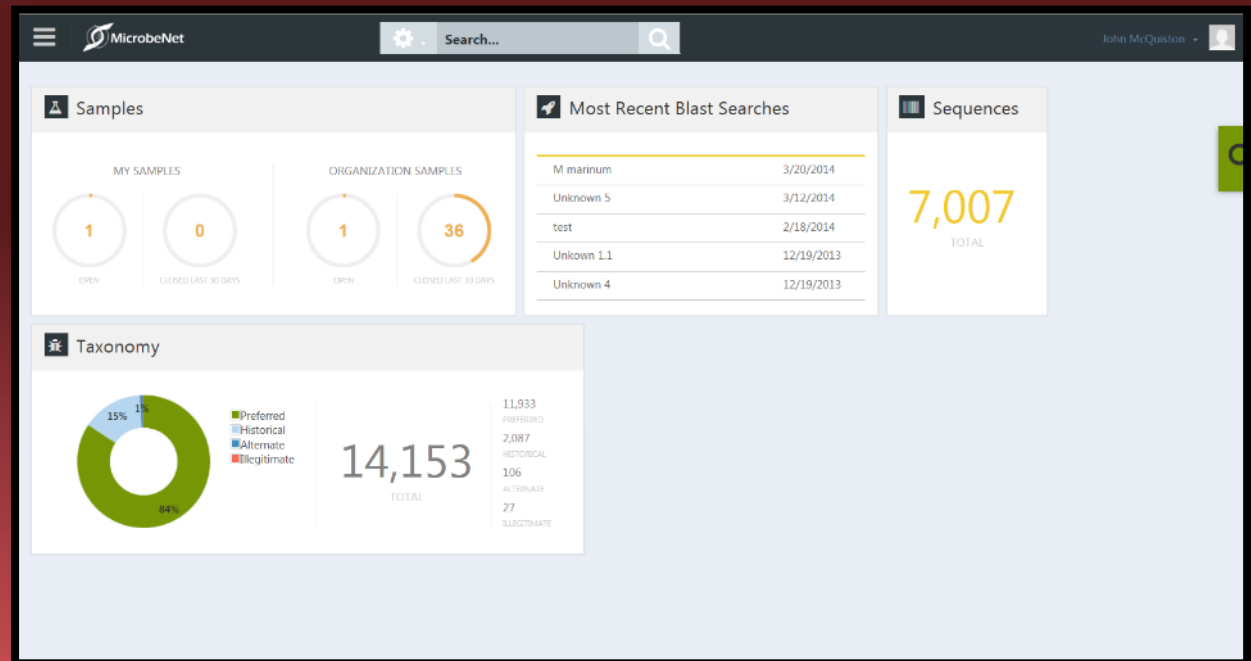
The tools and content are ideal for researchers and students.





Modules

- MicrobeNet 2.0 is a modular system where technology platforms can be added
 - 16S
 - Phenotypic testing
 - MALDI-ToF
 - Genomics



Bacterial Identification by 16S Sequence Analysis

16SrRNA sequences

- Highly conserved within species and divergent between species
- Reasonable length (~1500 bp)
- Ubiquitous in bacteria
- MicrobeNet database is curated by CDC SMEs

MicrobeNet Search...

BLAST Search

Query Name:
Unknown X1234

Results to Display

(Algorithm: BLASTn)

```
GACGAACGCTGGCGGCTGCTTAAACATGCAAGTCGAGCGGTAAGGCCCTTCGGGGTACAGAGCGGC
GAACGGGTGAGTAACACGTGGGTGATCTGCCTCGCACTTCGGGATAAGCCTGGGAAACTGGGTCTAATA
CCGGATATGACCTGCTGTCGCATGGCGGTGGGTGGAAAGATTATCGGTGCGAGATGGGCCCGCGGCCT
ATCAGCTTGTGGTGGGTAATGGCTACCAAGCGGACGACGGGTAGCCGGCTGAGAGGGCGACCGGC
CACACTGGGACTGAGACAGCGCCAGACTCCTACGGGAGGACGAGTGGGGAATATTGCACAAATGGGCG
AAAGCCTGATCGAGCGACCCCGCTGAGGGATGACGSCCTTCGGGTGTAAACCTCTTTCGACAGGGAC
GAAGCGTAAGTGACGTACCTGTAGAAGAAGCACCGGCCAACTACGTGCCAGACCGCGGTAAATACGT
AGGGTGGAGCGTTGTCCGGAATTACTGGGCGTAAAGAGCTTGTAGCGGTTTGTGCGCTGCTCTGTGA
AAACTCACAGCTCAACTGTGAGCTTGCAGGCGATACGGGACAGACTTGAATCTGACAGGGGAGACTGGAA
TTCTGGTGTAGCGGTGAJATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGCGGGTCTCTGGGCA
GTAACTGACCTGAGAAGCGAAGCGTGGGTAGCGAAGCAGGATTAGATACCTTGTAGTCCACGCCGTA
AACGTTGGGCGCTAGGTGTGGTTTCCCTCCACGGGATCCGTGCCGTAGCTAACGCAATTAAGCGCCCG
CTGGGGAGTACGGCGCAAGGCTAAAACCTCAAAGGAATTGACGGGGGCGCCACAAGCGCGGAGCAT
GTGGATTAAATCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATACACCGAAGAACCTGCAGAGATG
TAGGCCCCCTTGTGGTGGGTGATCAGGTGGTGCATGGCTGCTCAGCTCGTGTCTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCTTGTCTGTGTTGCCAGCGGTAATGGCGGGGACTCGCAGGAGAC
TGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCAATGCCCTTATGTCCAGGGCTTCA
CACATGCTACAATGGCCGGTACAGAGGGCTGCGATACCGTGAGGTGGAGCGAATCCCTTAAAGCCGGTC
TCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTTGGAGTCGTAGTAATCGCAGATCAGCAAC
GCTGGGTGAATACGTTCCCGGGCTTGTACACACCCCGCTCACGTGATGAAAGTCGTTAACACCCGA
AGCCGTTGGCTAACCCCTTGTGGGAGGGAGCCGTCGAAGGTGGGATCGGCGATTGGGACG
```

Search Reset Upload FASTA File

Bacterial Identification by 16S Sequence Analysis

MicrobeNet

Query Name: Unknown X1234

Select Sequences ☐ Clear Selected Sequences

Analyze Sequences

☒ Include query sequence in analysis

Showing 1 to 25 of 25 entries

	Genus	Species	SubSpecies	Strain	
☐	Nocardia	arthritidis		DSM_44731	25
☐	Nocardia	arsoensis		DSM_44729	25
☐	Nocardia	beijingensis		W7678	25
☐	Nocardia	exalbidia		W9709	24
☐	Nocardia	gamkensis		DSM_44956	24
☐	Nocardia	amamiensis		DSM_45066	24
☐	Nocardia	brevicatena		ATCC_15333	24
☐	Nocardia	xishanensis		W9867	24
☐	Nocardia	takedensis		W9878	24
☐	Nocardia	paucivorans		ATCC_BAA-278	24
☐	Nocardia	elegans		W9693	24
☐	Nocardia	aobensis		W8766	24
☐	Nocardia	vermiculata		W9868	24
☐	Nocardia	lijiangensis		DSM_44933	25



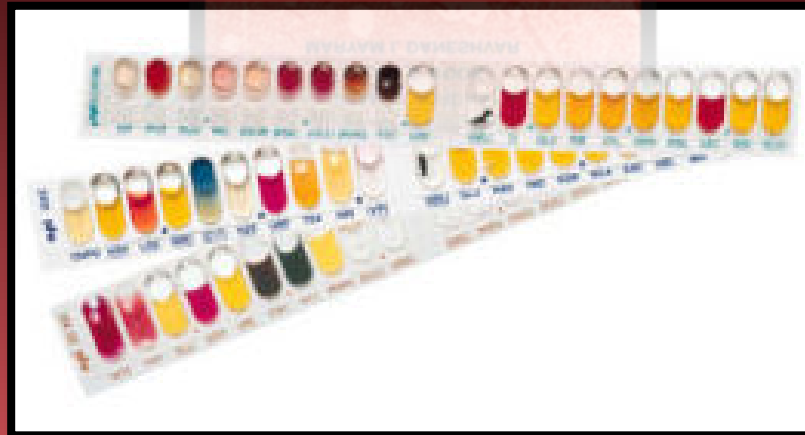
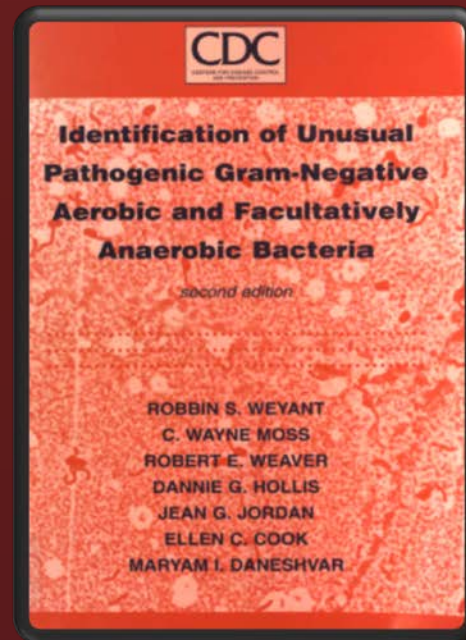
Contact Information:

Species Contact Info:
The Special Bacteriology
Reference Lab (SBR/USP)
5501 Glick Rd
(404) 639-0270

W9693, 20100604, 0.6X COLONY
(2010)

Genus/Species/Strain/Isolation/Environment

Phenotypic Testing



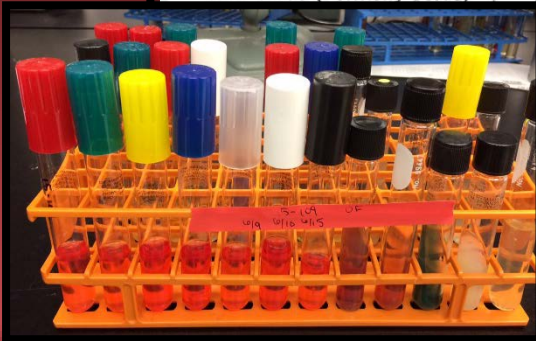
Phenotypic Testing

CULTURE NO. [REDACTED] DATE REC'D. 1/23/15 IDENTIFIED AS: *Chryseobacterium*
 SENDER: WA STATE H.D. *humilis (simultaneous growth)*
 SOURCE: Blood PATIENT: [REDACTED]
 MORPHOLOGY OF ORIGINAL CULT.: GRAM - Rods (Short, thin, faint staining)
 PURE CULT.: H.I. SLANT (24 HRS.): GRAM - Rods (Med to long, thin)
 H.I. BROTH (24 HRS.): FLAGELLA 1/2 none seen
 TYPE OF GROWTH: BLOOD AGAR (H/R) (ER HR. CO₂)
Circular, Entire, low convex 1-2mm Grey
 H.I. SLANT: 1+ A.F.: SPORE:
 GROWTH TEMP.: 5° 25° 1+ 35° 3+ 42° Ng 52° O₂ REQUIREMENTS ODOR

BASE	24	48	1 WK	BASE	24	48	1 WK	BASE	24	48	1 WK	24	NC
GLUCOSE	AA	AA	A	CATALASE	+			TSI SI / BUTT	NG/A	B/B		LITMUS	48 NC
D-XYLOSE	bb	bb	B	OXIDASE K/ROUT.	+			H ₂ S PAPER BUTT	wt/-	wt/-		MILK	
MANNITOL	nc	nc	B	MAC CONKEYS	Ng Ng	Ng	Ng	MR /VP ROUT.				1 WK	alk
LACTOSE	nc	nc	B	S.S.	Ng Ng	Ng	Ng	MR /VP SPEC.				PIGMENT: GROWTH	
SUCROSE	QA	QA	A	CITRATE	-	-	-	MOTILITY WP DIFCO	-	+	-	WATER SOLUBLE:	
MALTOSE	AA	AA	A	CETRIMIDE	Ng Ng	Ng	Ng	GELATIN				FLO.	- - -
FRUCTOSE				UREA SERUM PLAIN	-	-	-	ESCULIN HVD.	+	+	+	TECH.	- - -
CONTROL	bb	bb	B	NITRATE INF. ROUT.	wt/wt			% 0	3+4+	4+	4+	HIT.	- - -
10% SLANT LACTOSE				INDOLE INF. ROUT.	+			NaCL 6	Ng Ng	Ng	Ng		- - -

CDC 52.55 (Formerly 56.16)

LABORATORY DATA CARD



Identified Organism: ☐ Mark Sample as Complete/Incomplete

☒ **Get Identification Results**

	25°C (L)	35°C (M)	42°C (M)		H ₂ S Butt (+)	H ₂ S Paper (-)	
					TSI Butt (+)	TSI Slant (+)	
	NaCl 0%	NaCl 6%	Gel	Pig		O (-)	Cat (-)
Suc(OF)	Suc(OF) (A)	Malt(OF) (A)	MacC	SS	Cit	Cet	Mot

MicrobeNet Phenotypic Testing

Actinobacillus

Type Species: *Actinobacillus tignieresii*

Genus Overview

- a
- c
- d
- e
- h
- i
- l
- m
- p
- r
- s
- scotiae
- seminis
- succinogenes
- suis**
- u

Actinobacillus suis

Taxonomy / Strain

Strains in MicrobeNet:
KC560

Overview

Gram-negative coccobacillary to rod form, nonmotile; ferments glucose, xylose, lactose, sucrose, maltose, D-mannose, and sometimes mannitol; oxidase-, urease-, and esculin-positive; indole-negative, usually produces a clear zone of hemolysis; usually grows on MacConkey agar; reduces nitrate, usually produces catalase. Van Dorssen and Jaartsveld (van Dorssen, Jaartsveld, 1962), Zimmerman (Zimmerman 1964), and Kilian and Frederiksen (Kilian, Frederiksen et al. 1981) have variously described *Actinobacillus suis*. *Bergey's Manual of Systematic Bacteriology* describes *A. suis* as mannitol-negative. Strains we have encountered and considered to be *A. suis* may represent a heterogeneous group and should be studied further.

References

Species References

- van Dorssen, C.A. and F.H.J. Jaartsveld, 1962.
van Dorssen, C.A. and F.H.J. Jaartsveld. 1962. *Actinobacillus suis* (novo species) een bij het varken voorkomende bacterie. Tijdschr. Diergeneesk. 87:450-458.
- Zimmerman, T. 1964.
Zimmerman, T. 1964. Untersuchungen über die Actinobacilliose des Schweines I. Mitteilung: Isolierung und charakterisierung der erreger. Dtsch. Tierärztl. Wochenschr. 71:457-461.
- Kilian, M. and W. Frederiksen, 1981.
Kilian, M. and W. Frederiksen. 1981. Identification tables for the *Haemophilus*-*Pasteurella*-*Actinobacillus* group. In M. Kilian, W. Frederiksen, and E.L. Biberstein (Eds.) *Haemophilus, Pasteurella, and Actinobacillus*. Academic Press, New York, pp. 286-297.
- Bisgaard, M., et al. 1984.
Bisgaard, M., K. Diechulte, Y.-T. Ying, W. Frederiksen and W. Mannheim. 1984. Prevalence of organisms described as *Actinobacillus suis* or haemolytic *Actinobacillus equi* in the

Contact Information:

Species Contact Info:
The Special Bacteriology
Reference Lab (SBRL/BSPB)
SBRL@cdc.gov
(404) 639-0270

Number of Taxon Matches to Display: 5

Number of Recommended Tests to Display: 5

Matched Taxon Results

Showing 1 to 5 of 5 entries

Genus	Species	SubSpecies	Score	Atypical Test	Chosen Answer	Prob
Actinobacillus	suis		73.19135	Nitrite Reduction	reduction, C, +C, +W	0
				H2S TSI Butt	positive, tr, s+, +w, 1, 1+, 2, 2+, 3, 3+, 4, 4+	1.0000
				H2S TSI Paper	negative	1.0000
				TSI Test: Butt	positive, tr, s+, +w, 1, 1+, 2, 2+, 3, 3+, 4, 4+	0
				TSI Test: Slant	positive, tr, s+, +w, 1, 1+, 2, 2+, 3, 3+, 4, 4+	0
				D-Xylose Oxidation Fermentation	no indicator change, N, -	0
				D-Mannitol Oxidation Fermentation	no indicator change, N, -	0
				MacConkey Agar	-, NG, -/	6.0000
				Motility	+, 7+	0
Provisional	0-2		15.84634			
Pannonibacter	phragmitetus		6.397359			
Capnocytophaga	cynodegmi		1.316513			
Psychrobacter	phenylpyruvicus		0.6233055			

Recommended Next Tests

Showing 1 to 5 of 5 entries

Test Name	Separated Taxon Pairs
Maltose Oxidation Fermentation	3660
D-Glucose Oxidation Fermentation	3648
Simmons' Citrate Agar	2670
Lactose Oxidation Fermentation	2240
Sucrose Oxidation Fermentation	1824

Phenotypic Testing SOP's

▼ Antimicrobial Susceptibilities

▼ Acid production from Gordon Sugars

▼ Growth Characteristics

▼ Other Characteristics

▲ Hydrolysis of

Adenine

Casein

Hypoxanthine

Tyrosine

Xanthine

Esculin

Urea

Esculin SBRL-11.pdf

Find

1 / 5

60.2%

Sign

CDC

CDC Infectious Diseases Laboratories
Bacterial Special Pathogens Branch

Esculin Medium Used to Determine Ability of Organism to Hydrolyze Esculin

Doc. No. SBRL-11 Rev. No. 01 Effective Date: 02/6/2014 Page 1 of 5

1.0 Purpose/Principle

This medium is used to determine whether an organism will hydrolyze the glycoside esculin. Esculin hydrolysis is a useful aid in the identification of bacteria species.

If an organism is able to hydrolyze esculin, the esculin molecules are split into dextrose and esculetin. The esculetin reacts with the ferric ions contained within the medium, turning it from its original amber color to a dark brown to black. (See Appendix A). An organism that darkens the medium to any extent is considered esculin positive. An organism that grows but the medium remains the normal amber color is considered negative.

2.0 Scope

This procedure applies to laboratory personnel responsible for performing microbiology testing in the Special Bacteriology Reference Laboratory.

3.0 Related Documents:

Title	Document Control Number
Characterization and Identification of Unusual Bacterial Isolates	SBRL-PC.C.001

4.0 Responsibility

Position	Responsibility
Microbiologist	Perform testing and review results for reporting

5.0 Definitions

Term	Definition
Hydrolysis	The breaking down of a chemical compound into two or more simpler compounds by reacting with water. Esculin → Glucose + Esculetin
Wood's lamp	Lamp which emits long wave UV-A ultraviolet light

6.0 Equipment

6.1 Incubator

6.2 Wood's (UV) lamp

7.0 Reagents and Media

7.1 Esculin agar slants, (HIA base)

7.1.1 Storage: Store tubes at 2-8°C. Avoid freezing and overheating.

7.1.2 Stability: Up to the stated expiration date. Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

Controlled Document - DO NOT DUPLICATE

MALDI-ToF!

Matrix-**A**ssisted **L**aser **D**esorption/**I**onization **T**ime of **F**light



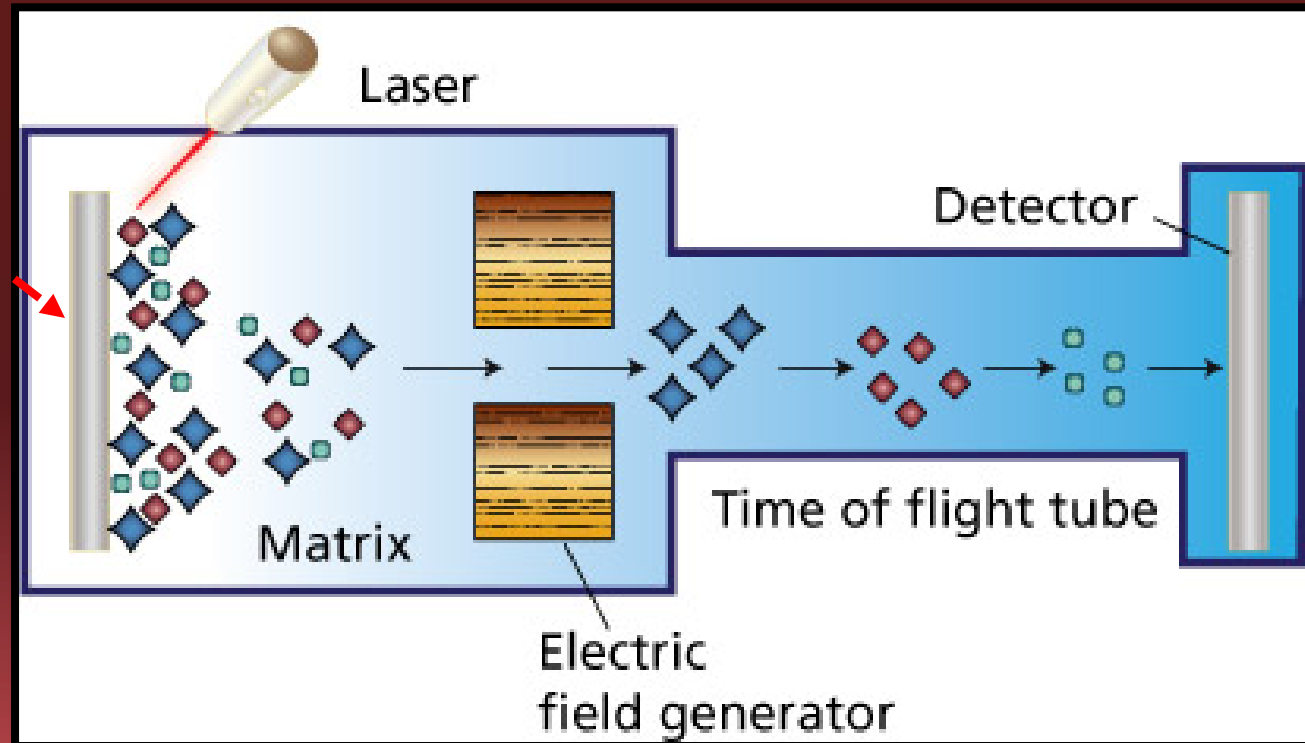
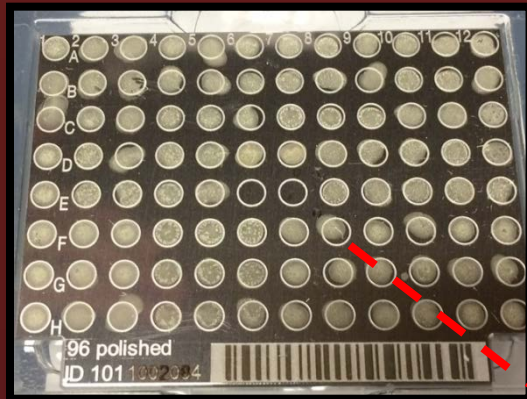
Bruker BioTyper



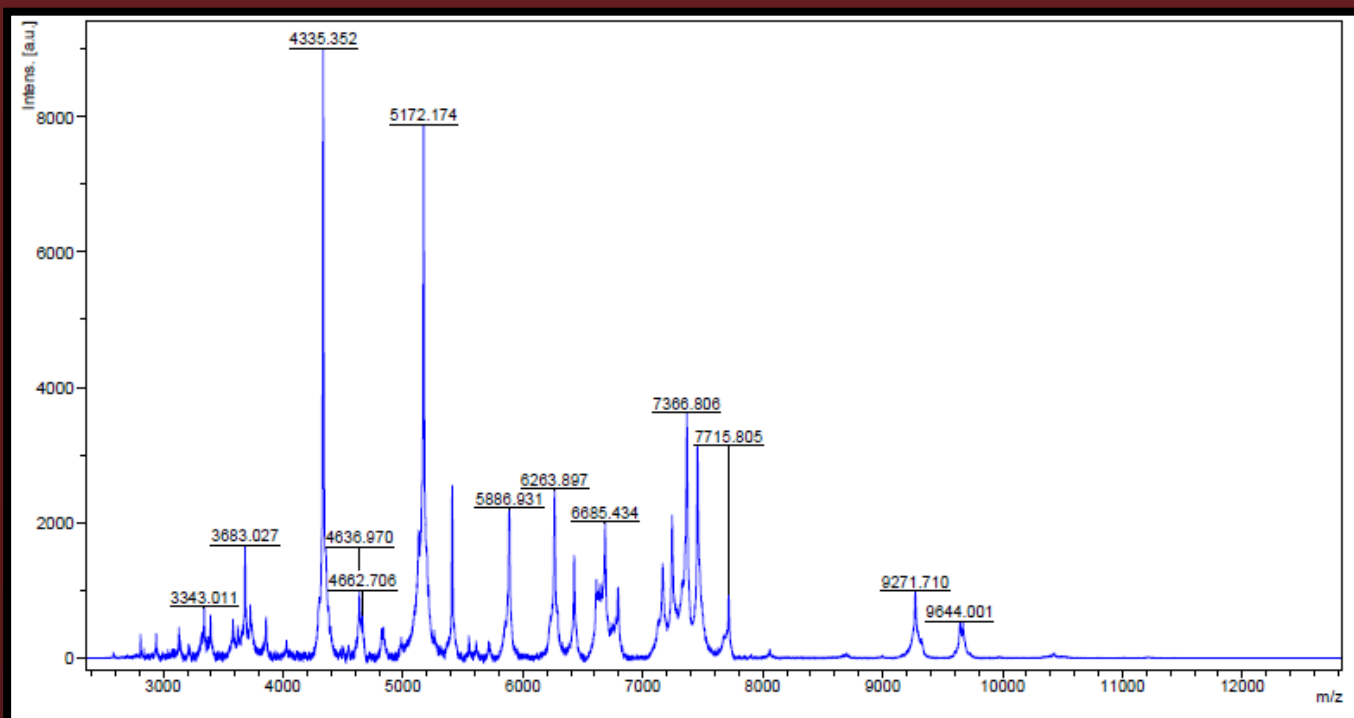
bioMerieux Vitek MS

MALDI-ToF!

Matrix-Assisted Laser Desorption/Ionization Time of Flight



Protein Spectrum Match



<u>E4</u> (-)(C)	H6382	not reliable identification	1.425	not reliable identification	1.346
<u>E5</u> (-)(C)	H6382	not reliable identification	1.357	not reliable identification	1.319
<u>E6</u> (+++)(A)	H6383	Flavobacterium lindanitolerans	2.552	not reliable identification	1.398
<u>E7</u> (+++)(A)	H6383	Flavobacterium lindanitolerans	2.589	not reliable identification	1.41

MALDI-ToF!

Advantages:

10 min preparation - Extremely fast for a lab test

\$0.25 per sample – Extremely inexpensive

70 - 95% match – Extremely reproducible *

*dependent on database

Disadvantages:

Cost of equipment - \$200K - \$300K starting

Building your own database.

MALDI-ToF in MicrobeNet

Users will be able to search from their instrument to our database at CDC

The screenshot displays the MicrobeNet web interface. At the top, there is a navigation bar with the MicrobeNet logo, a search bar, and a user profile for John McQuiston. The main content area shows a mass spectrum plot with peaks labeled A1 through A7. Below the plot, there is a table of search results. The table has columns for ID, NAME, POSITION, CHIP, MBT STAR-BL, DETECTED SPECIES, SCORE, COMMENT, DESCRIPTION, CONFIDENCE, EXPORT STATE, and SPECIES TO EXPORT. The results show seven entries, with the first six having high confidence and the seventh having an unknown confidence level.

ID	NAME	POSITION	CHIP	MBT STAR-BL	DETECTED SPECIES	SCORE	COMMENT	DESCRIPTION	CONFIDENCE	EXPORT STATE	SPECIES TO EXPORT
A1	A1	A1	0	☐	Escherichia coli	2.69	Closely related to Shigella and not definitely distinguishable at the moment		High	Exported	<Best Match>
A2	A2	A2	0	☐	Acidiphilium acidophilum	2.26			High	Exported	<Best Match>
A3	A3	A3	0	☐	Cupriavidus necator	2.73			High	Exported	<Best Match>
A4	A4	A4	0	☐	Anthrobacter sulfureus	2.73			High	Exported	<Best Match>
A5	A5	A5	0	☐	Microbacterium maritimum	2.70			Low	Not Exported	<Best Match>
A6	A6	A6	0	☐	Proteus mirabilis	2.70			Low	Not Exported	<Best Match>
A7	A7	A7	0	☐	No organism identification possible	1.31			Unknown	Not Exported	<Best Match>

- SBRL is in a research collaboration agreement with Bruker Daltonics
- Since 6/2014 SBRL has added 855 new strains to the CDC database
- This database will be accessible from anywhere in the world through MicrobeNet
- All data is confirmed by 16S prior to being added to the database

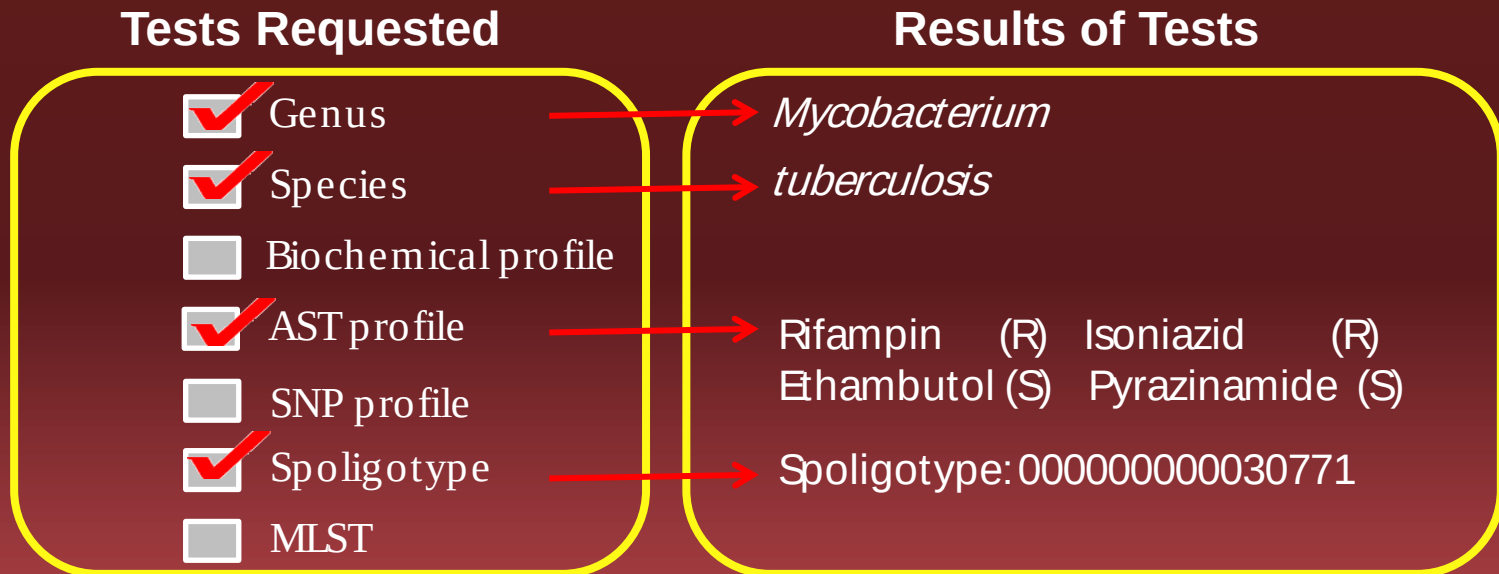
Genomics and AMD in MicrobeNet

- Genomics capabilities module planned for 2016-2017
- Foundational Genome database will need to be built
- AMD support needed for over 1,000 high quality genomes

MicrobeNet and Genomics

Reference Laboratory of the Future

- Public Health Laboratory A
 - Performs whole genome sequencing
 - Selects tests they want to perform through MicrobeNet™



AST, Antimicrobial susceptibility testing
SNP, Single nucleotide polymorphism (mutation)
MLST, Multi-locus sequence typing

Training!

Five training workshops have been held since MicrobeNet went live.

- Organized by NTLN and APHL
- Held at CDC training facilities
- 56 State Health Dept. Scientists trained
- Webinar

Who is using MicrobeNet?

- 100+ users from 37 state health departments, Universities
- International users and Federal agencies.

How You Can Sign Up!

To request a User ID and Password:

Go to <https://microbenet.cdc.gov>

Or email: MicrobeNet@cdc.gov

You will be asked to change your password on your first visit



Acknowledgements

Centers for Disease Control

MicrobeNet IT Development Team

SBRL

Ming Fung
Anne Whitney
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James Graziano
Brent Lasker

SRA

Keith Silberman
Pierre Raymond
Greg Vence
Nivedith Mittipalli
Vishal Nayak

Life Technologies

Chad Alexander
Joan McGuire
Jing Zhai
Sylvia Chang

Bruker Daltonics

Gongyi Shi

Thank You

Questions?