Modern Biotechnology in the Clinical Microbiology Lab

Faster Methods for Getting Actionable Intelligence to Physicians

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Clinical Laboratory Accreditation

- CLIP = Clinical Laboratory Improvement Program
  - Result of Clinical Laboratory Improvement Amendments (CLIA) of 1988
  - Mechanism by which the Centers for Medicare & Medicaid Services (CMS) regulates laboratory testing
  - All clinical laboratories must be properly certified to receive Medicare/Medicaid payments
- CAP = College of American Pathologists
  - Accrediting body for hospital pathology and laboratory services
  - 2 year accreditation cycle
- Purpose: to ensure quality results, drive implementation of Quality Control/Quality Assurance measures
- Effect: Labs frequently limited to FDA-approved methods and/or labor-intensive laboratory-derived tests

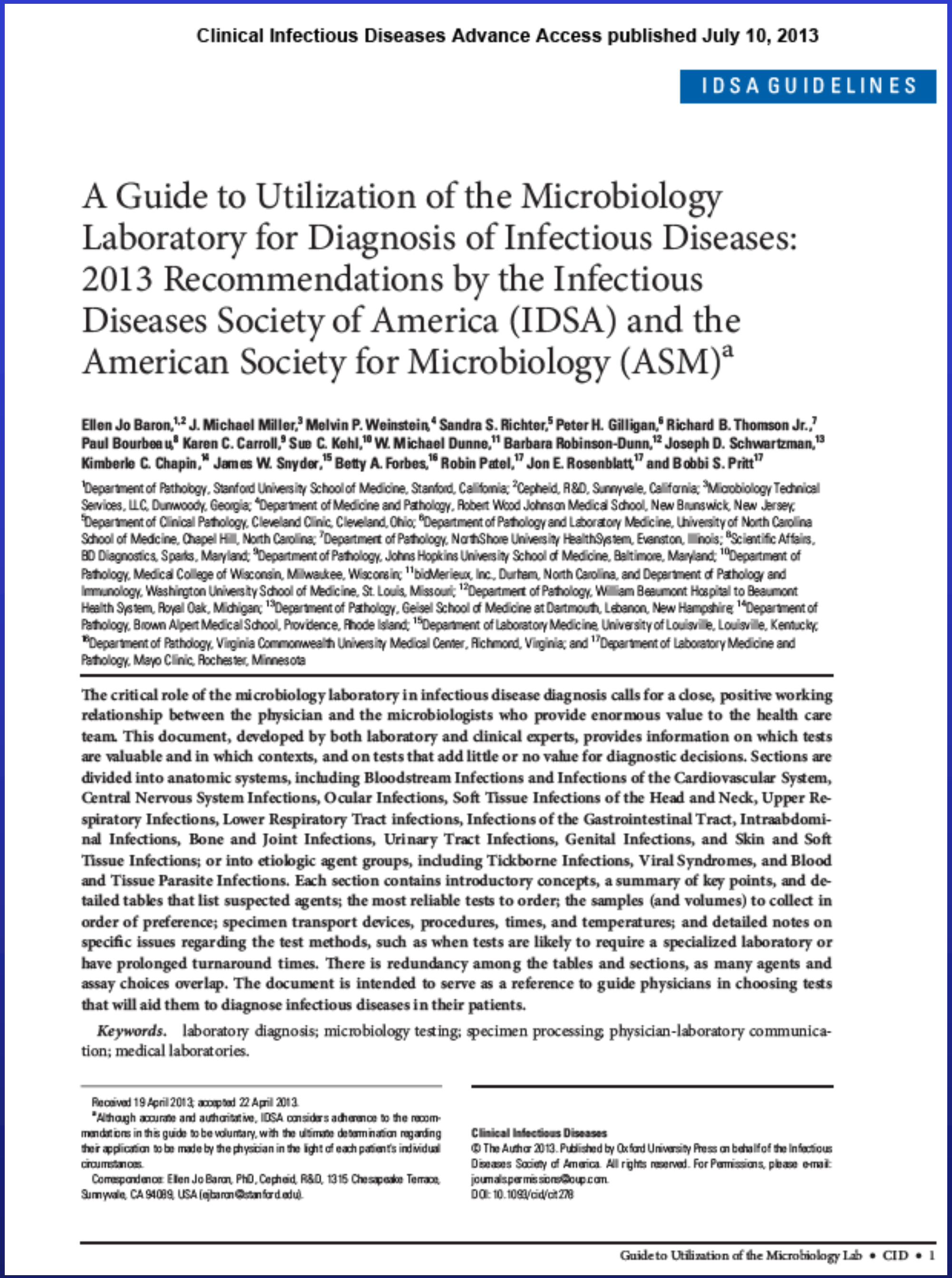
Clinical Scenario #1

50 year old female, recently Army retiree presents 1 year out from a right total knee replacement with worsening knee pain and mild swelling along with some night sweats. She is taken to the OR and the hardware is removed and sent down to the lab for culture…

Bacterial Culture and Identification

Timeline

1 hr →



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IDSA GUIDELINES

A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)[a]

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The critical role of the microbiology laboratory in infectious disease diagnosis calls for a close, positive working relationship between the physician and the microbiologists who provide enormous value to the health care team. This document, developed by both laboratory and clinical experts, provides information on which tests are valuable and in which contexts, and on tests that add little or no value for diagnostic decisions. Sections are divided into anatomic systems, including Bloodstream Infections and Infections of the Cardiovascular System, Central Nervous System Infections, Ocular Infections, Soft Tissue Infections of the Head and Neck, Upper Respiratory Infections, Lower Respiratory Tract infections, Infections of the Gastrointestinal Tract, Intraabdominal Infections, Bone and Joint Infections, Urinary Tract Infections, Genital Infections, and Skin and Soft Tissue Infections; or into etiologic agent groups, including Tickborne Infections, Viral Syndromes, and Blood and Tissue Parasite Infections. Each section contains introductory concepts, a summary of key points, and detailed tables that list suspected agents; the most reliable tests to order; the samples (and volumes) to collect in order of preference; specimen transport devices, procedures, times, and temperatures; and detailed notes on specific issues regarding the test methods, such as when tests are likely to require a specialized laboratory or have prolonged turnaround times. There is redundancy among the tables and sections, as many agents and assay choices overlap. The document is intended to serve as a reference to guide physicians in choosing tests that will aid them to diagnose infectious diseases in their patients.

Keywords. laboratory diagnosis; microbiology testing; specimen processing; physician-laboratory communication; medical laboratories.

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[a]Although accurate and authoritative, IDSA considers adherence to the recommendations in this guide to be voluntary, with the ultimate determination regarding their application to be made by the physician in the light of each patient's individual circumstances.

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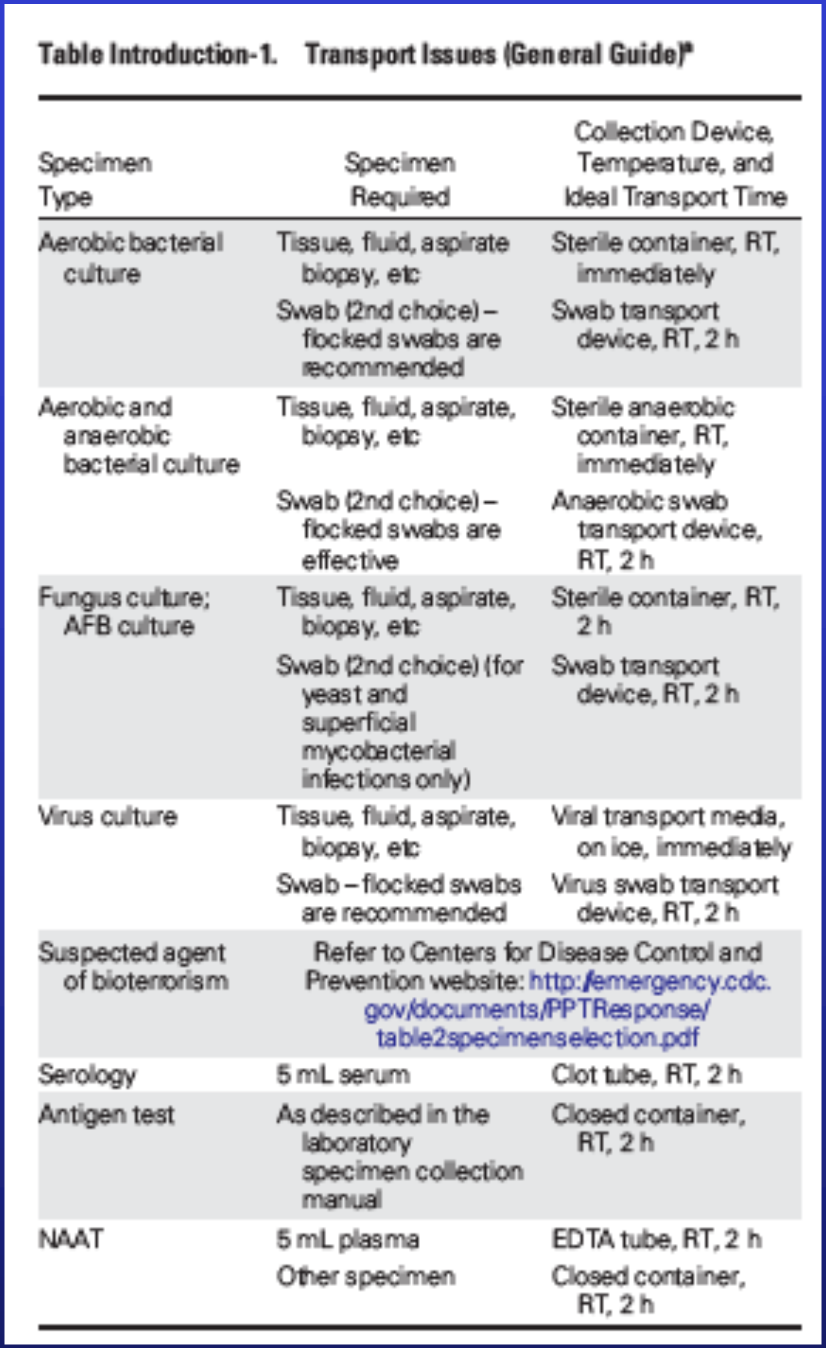


Table Introduction-1. Transport Issues (General Guide)[a]

Specimen Type	Specimen Required	Collection Device, Temperature, and Ideal Transport Time
Aerobic bacterial culture	Tissue, fluid, aspirate biopsy, etc	Sterile container, RT, immediately
	Swab (2nd choice) – flocked swabs are recommended	Swab transport device, RT, 2 h
Aerobic and anaerobic bacterial culture	Tissue, fluid, aspirate, biopsy, etc	Sterile anaerobic container, RT, immediately
	Swab (2nd choice) – flocked swabs are effective	Anaerobic swab transport device, RT, 2 h
Fungus culture; AFB culture	Tissue, fluid, aspirate, biopsy, etc	Sterile container, RT, 2 h
	Swab (2nd choice) (for yeast and superficial mycobacterial infections only)	Swab transport device, RT, 2 h
Virus culture	Tissue, fluid, aspirate, biopsy, etc	Viral transport media, on ice, immediately
	Swab – flocked swabs are recommended	Virus swab transport device, RT, 2 h
Suspected agent of bioterrorism	Refer to Centers for Disease Control and Prevention website: http://emergency.cdc.gov/documents/PPTResponse/table2specimenselection.pdf	
Serology	5 mL serum	Clot tube, RT, 2 h
Antigen test	As described in the laboratory specimen collection manual	Closed container, RT, 2 h
NAAT	5 mL plasma	EDTA tube, RT, 2 h
	Other specimen	Closed container, RT, 2 h

Basic Principles of Specimen Collection

- Healthy, actively growing organisms

(acute, pre-antibiotic)

- Minimize normal flora where you can

(sputum, urine)

- Eliminate normal flora where you must (blood)
- ***Tissue/Fluid is better than Swabs***

(and sometimes essential)

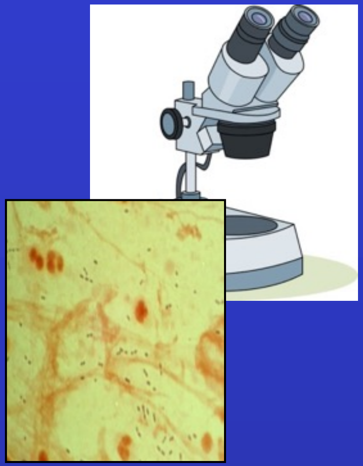
- More Swabs are better than Less Swabs
- Sooner is always better
- Use the right transport media
- Store at the right temperature

Bacterial Culture and Identification

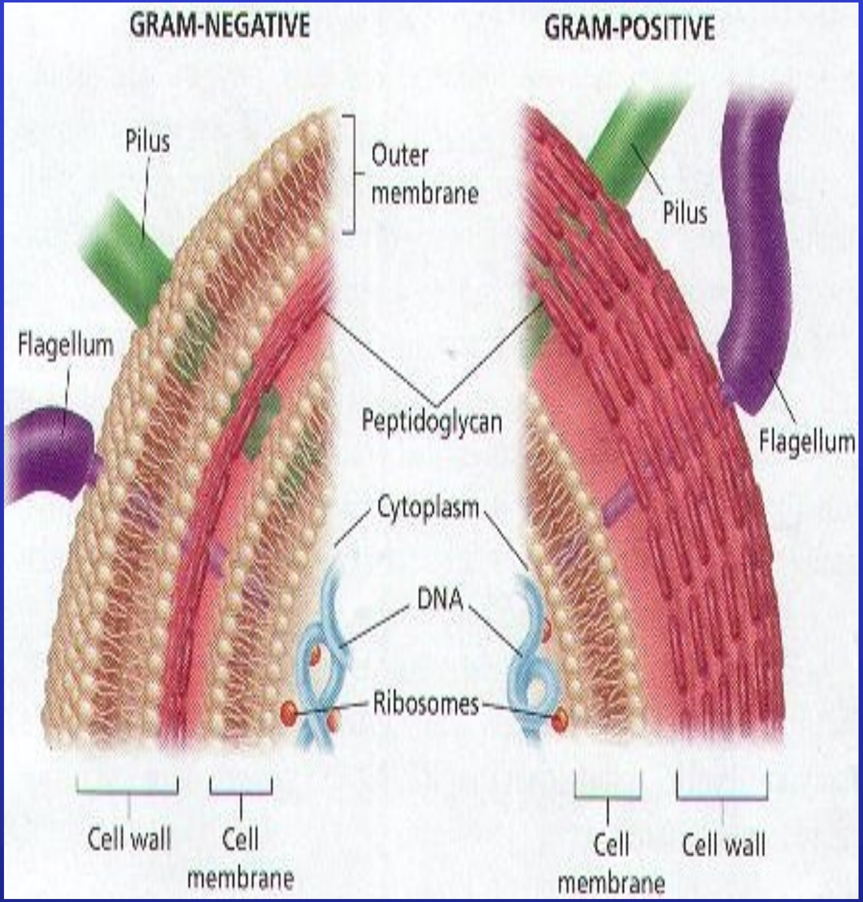
Timeline

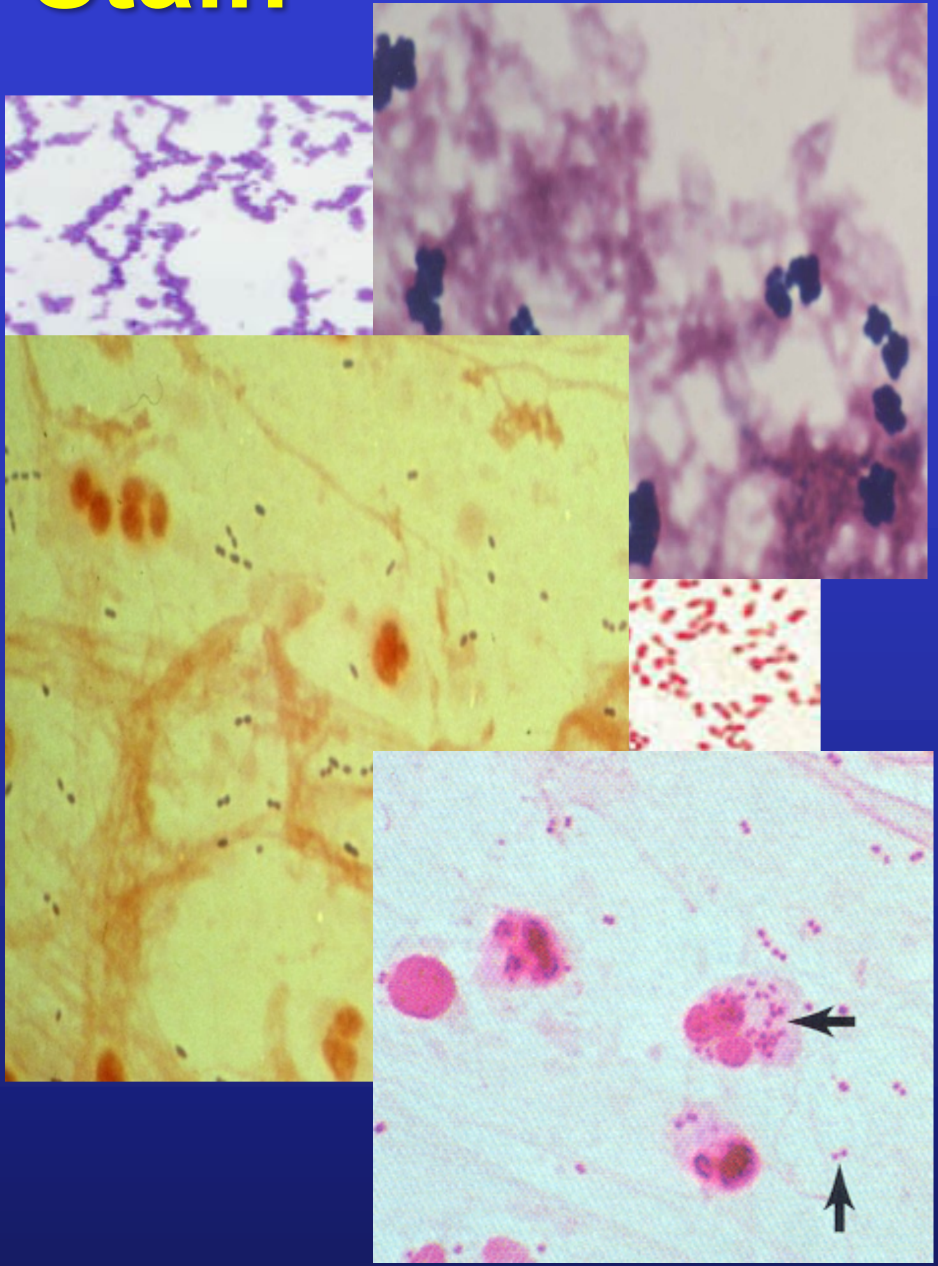
1 hr

1 hr



Gram Stain


GRAM-NEGATIVE
GRAM-POSITIVE
Pilus
Outer membrane
Pilus
Flagellum
Peptidoglycan
Flagellum
Cytoplasm
DNA
Ribosomes
Cell wall
Cell membrane
Cell membrane
Cell wall



Antibiotic Therapies

Gram Positive vs. Gram Negative

GP

GN

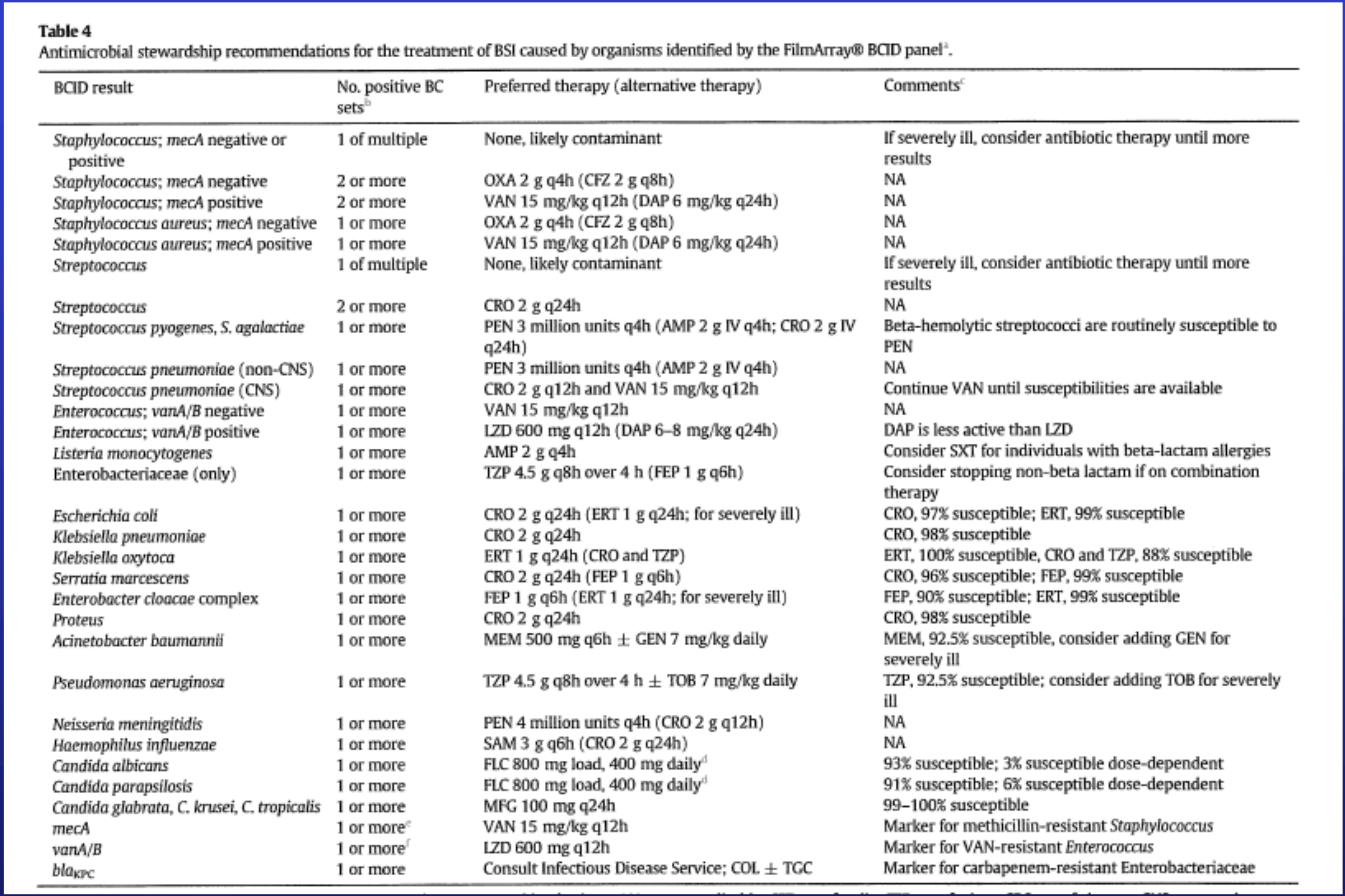


Table 4

Antimicrobial stewardship recommendations for the treatment of BSI caused by organisms identified by the FilmArray® BCID panel[a].

BCID result	No. positive BC sets[b]	Preferred therapy (alternative therapy)	Comments[c]
Staphylococcus; *mecA* negative or positive	1 of multiple	None, likely contaminant	If severely ill, consider antibiotic therapy until more results
Staphylococcus; *mecA* negative	2 or more	OXA 2 g q4h (CFZ 2 g q8h)	NA
Staphylococcus; *mecA* positive	2 or more	VAN 15 mg/kg q12h (DAP 6 mg/kg q24h)	NA
Staphylococcus aureus; *mecA* negative	1 or more	OXA 2 g q4h (CFZ 2 g q8h)	NA
Staphylococcus aureus; *mecA* positive	1 or more	VAN 15 mg/kg q12h (DAP 6 mg/kg q24h)	NA
Streptococcus	1 of multiple	None, likely contaminant	If severely ill, consider antibiotic therapy until more results
Streptococcus	2 or more	CRO 2 g q24h	NA
Streptococcus pyogenes, *S. agalactiae*	1 or more	PEN 3 million units q4h (AMP 2 g IV q4h; CRO 2 g IV q24h)	Beta-hemolytic streptococci are routinely susceptible to PEN
Streptococcus pneumoniae (non-CNS)	1 or more	PEN 3 million units q4h (AMP 2 g IV q4h)	NA
Streptococcus pneumoniae (CNS)	1 or more	CRO 2 g q12h and VAN 15 mg/kg q12h	Continue VAN until susceptibilities are available
Enterococcus; *vanA/B* negative	1 or more	VAN 15 mg/kg q12h	NA
Enterococcus; *vanA/B* positive	1 or more	LZD 600 mg q12h (DAP 6–8 mg/kg q24h)	DAP is less active than LZD
Listeria monocytogenes	1 or more	AMP 2 g q4h	Consider SXT for individuals with beta-lactam allergies
Enterobacteriaceae (only)	1 or more	TZP 4.5 g q8h over 4 h (FEP 1 g q6h)	Consider stopping non-beta lactam if on combination therapy
Escherichia coli	1 or more	CRO 2 g q24h (ERT 1 g q24h; for severely ill)	CRO, 97% susceptible; ERT, 99% susceptible
Klebsiella pneumoniae	1 or more	CRO 2 g q24h	CRO, 98% susceptible
Klebsiella oxytoca	1 or more	ERT 1 g q24h (CRO and TZP)	ERT, 100% susceptible, CRO and TZP, 88% susceptible
Serratia marcescens	1 or more	CRO 2 g q24h (FEP 1 g q6h)	CRO, 96% susceptible; FEP, 99% susceptible
Enterobacter cloacae complex	1 or more	FEP 1 g q6h (ERT 1 g q24h; for severely ill)	FEP, 90% susceptible; ERT, 99% susceptible
Proteus	1 or more	CRO 2 g q24h	CRO, 98% susceptible
Acinetobacter baumannii	1 or more	MEM 500 mg q6h ± GEN 7 mg/kg daily	MEM, 92.5% susceptible, consider adding GEN for severely ill
Pseudomonas aeruginosa	1 or more	TZP 4.5 g q8h over 4 h ± TOB 7 mg/kg daily	TZP, 92.5% susceptible; consider adding TOB for severely ill
Neisseria meningitidis	1 or more	PEN 4 million units q4h (CRO 2 g q12h)	NA
Haemophilus influenzae	1 or more	SAM 3 g q6h (CRO 2 g q24h)	NA
Candida albicans	1 or more	FLC 800 mg load, 400 mg daily[d]	93% susceptible; 3% susceptible dose-dependent
Candida parapsilosis	1 or more	FLC 800 mg load, 400 mg daily[d]	91% susceptible; 6% susceptible dose-dependent
Candida glabrata, *C. krusei*, *C. tropicalis*	1 or more	MFG 100 mg q24h	99–100% susceptible
mecA	1 or more[e]	VAN 15 mg/kg q12h	Marker for methicillin-resistant *Staphylococcus*
vanA/B	1 or more[f]	LZD 600 mg q12h	Marker for VAN-resistant *Enterococcus*
bla_{KPC}	1 or more	Consult Infectious Disease Service; COL ± TGC	Marker for carbapenem-resistant Enterobacteriaceae

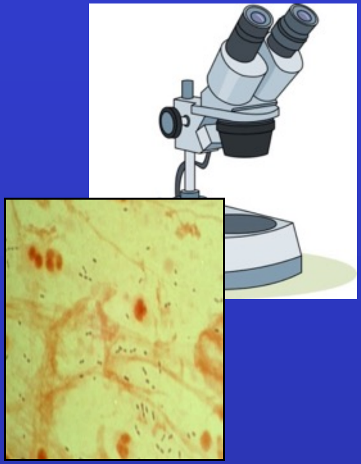
Southern TR et al. Diagnostic Microbiology and Infectious Disease. 2015; 81(2):96-101

Bacterial Culture and Identification

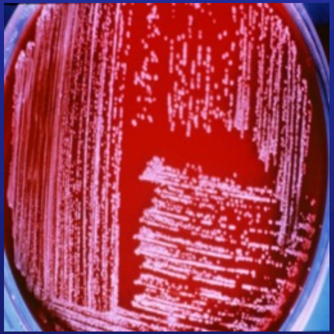
Timeline

1 hr

1 hr

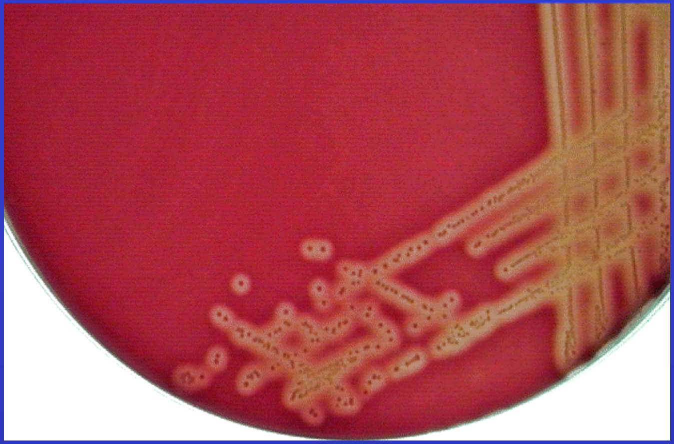


~18 hrs

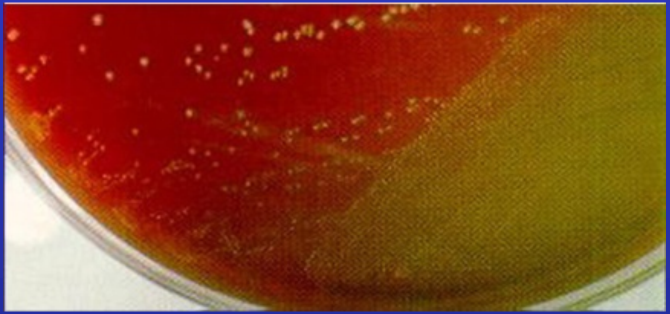


Sheep’s Blood Agar Plates

β-hemolysis

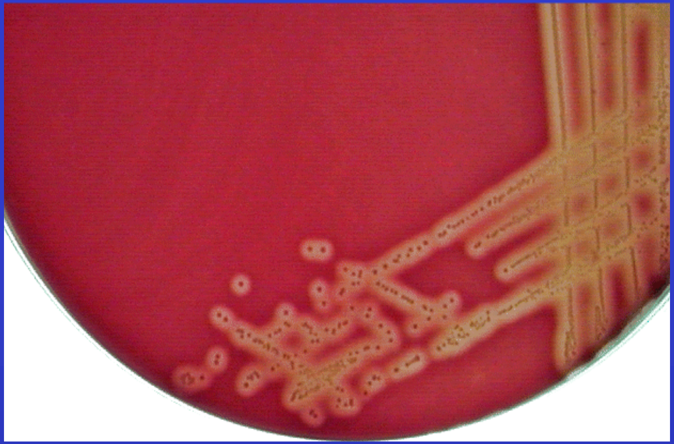


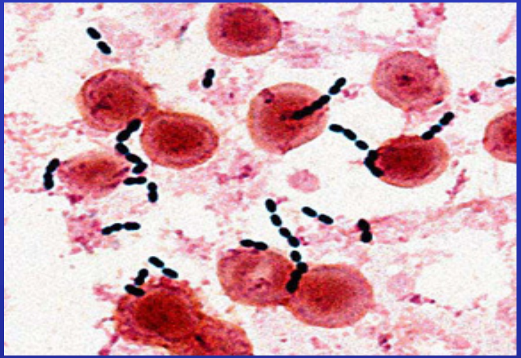
α-hemolysis



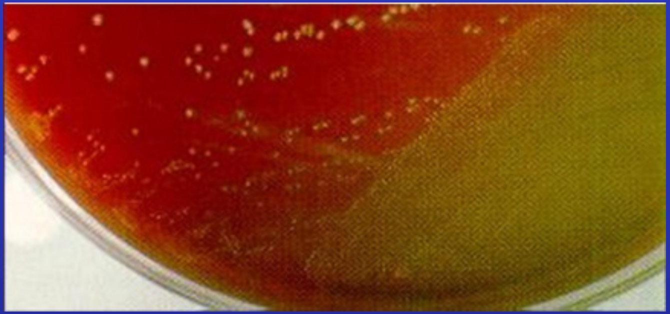
γ-hemolysis

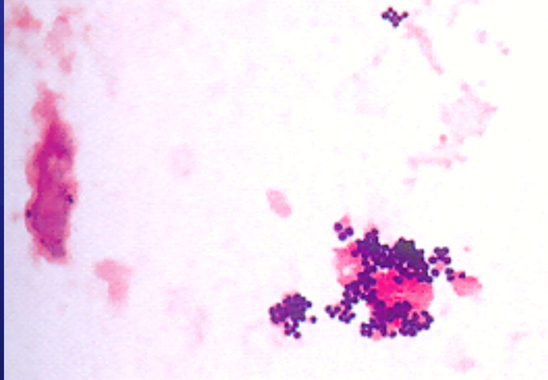
Streptococcus pyogenes





***Enterococcus* spp.**



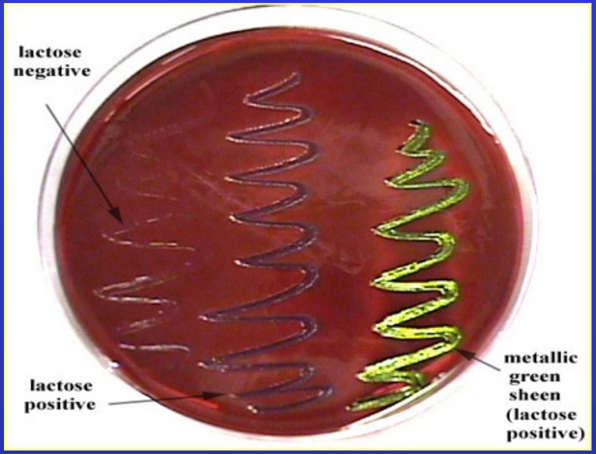


Staphylococcus epidermidis

Selective Differential Media

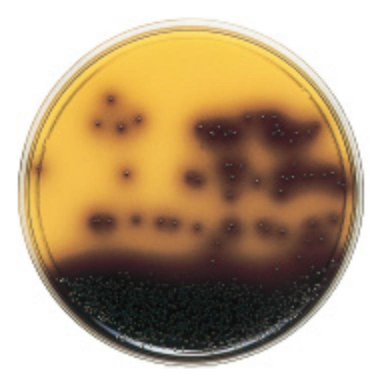
E.coli O157:H7

Clostridium difficile

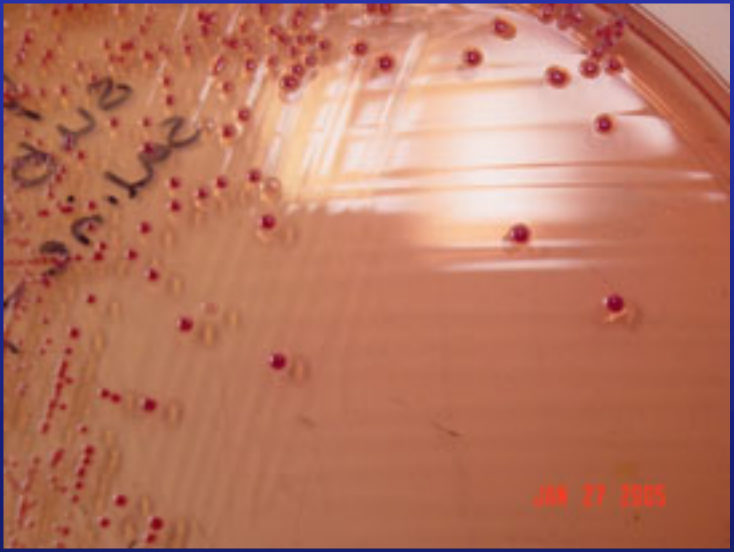


Enterobacteriaceae

Salmonella spp./Shigella spp.



Bacteriodes spp.



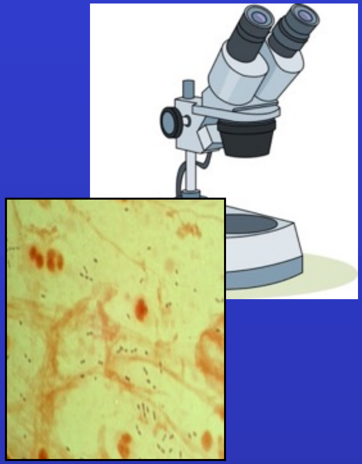
Yersinia spp. (i.e. Plague)

Bacterial Culture and Identification

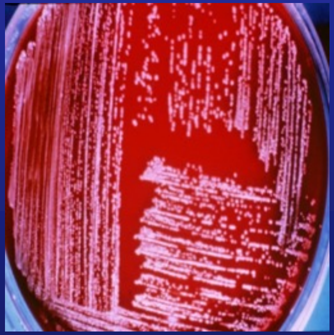
Timeline

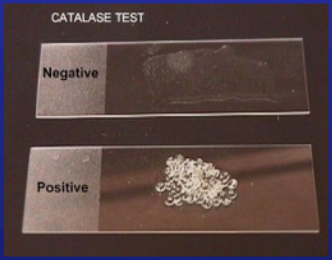
1 hr

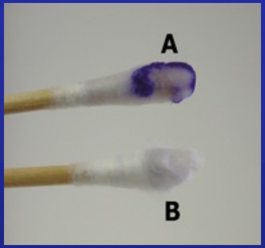
1 hr



~18 hrs







Biochemically-Based Identification Methods

est (*Staph/Strep*)

Indole test *(E.coli)*

Lancefield Group typing (S*trep species*)

Automated Biochemical Systems

- Identification and Susceptibilities
- ID based on biochemical profile
- Antibiotic breakpoints based on CLSI standards
- Some susceptibilities reported out as standard, some withheld and reported out by exception

- Pure culture required
- ~18 hour cycle

Examples:

- Biomerieux Vitek
- Becton Dickinson Pheonix
- Beckman Coulter MicroScan

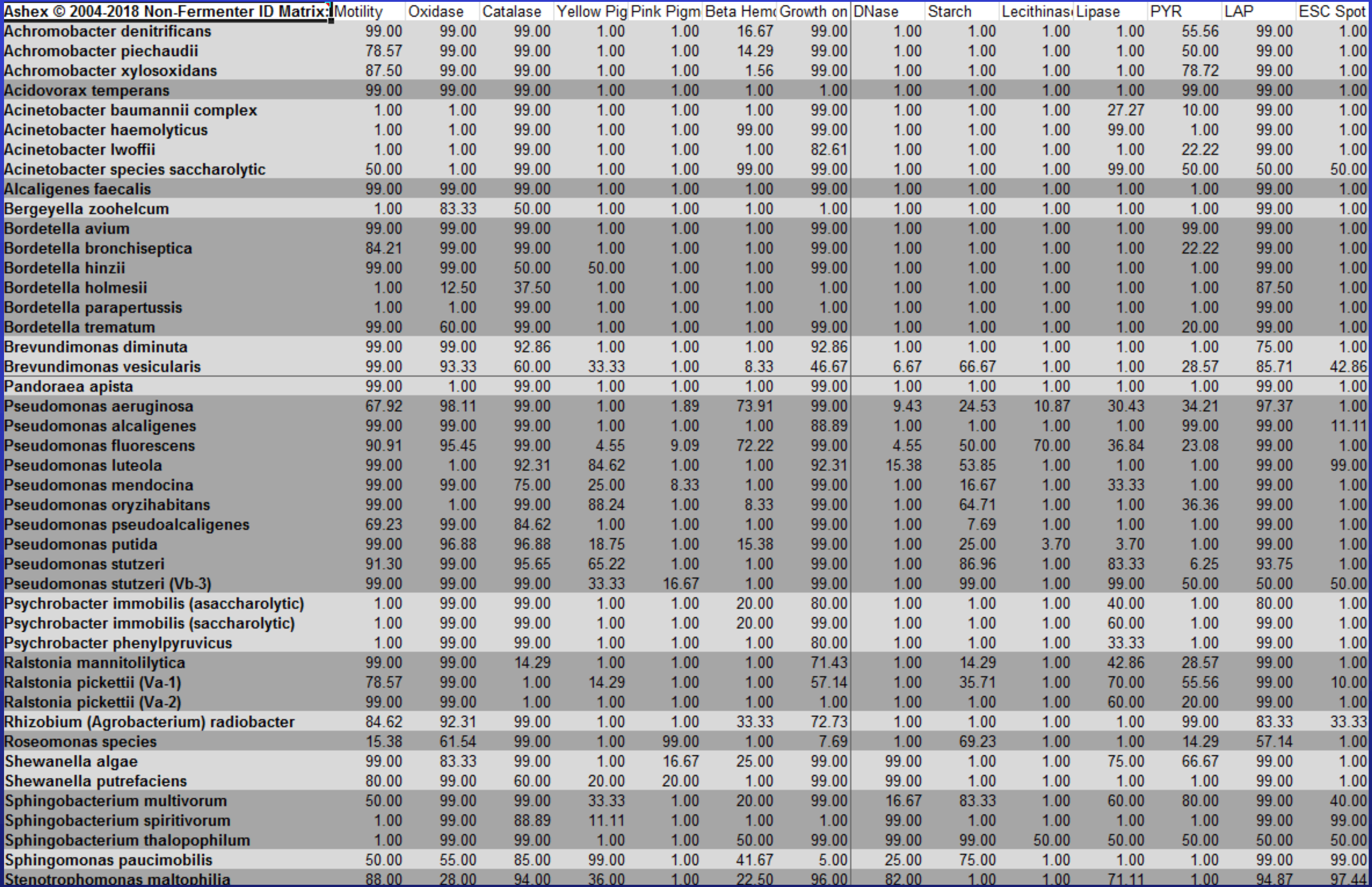
Laboratory Diagnosis

- Biochemical Tests
  - Triple Sugar Iron Agar / Kligler Iron Agar
  - Indole production
  - Methyl Red test
  - Voges-Proskauer test
  - Citrate utilization
  - Nitrate reduction
  - Urease production
  - Oxidase activity
  - Carbohydrate fermentation (Adonitol, Arabinose, Inositol, Sucrose)
  - Decarboxylation of Lysine, Ornithine, and Arginine (amino acids)
  - Phenylalanine Deaminase production
  - o-Nitrophenyl-β-D-galctopyranoside (ONPG)
    - Tests for β-galactosidase; helpful in identifying late lactose fermenters
  - Hydrogen Sulfide production
  - Motility

Make up the 13-tube biochemical ID panel

Biochemical Testing

	LF?	Motility	Indole	Methyl Red	Voges Proskauer	H_2S	Citrate	Urease
K. pneumoniae	Yes	-	-	-	+	-	+	+
K. oxytoca	Yes	-	+	-	+	-	+	+
Y. pestis	No	-	-	+	-	-	-	-
Y. enterocolitica	No	+ (25 C)	V(50%)	+	-	-	-	+
E. coli	Yes	+	+	+	-	-	-	-
Shigella	No	-	-/+	+	-	-	-	-
Salmonella	No	+	-	+	-	+	+	-
S. Typhi	No	+	-	+	-	+ (wk)	-	-
P. mirabilis	No	+ (sw.)	-	+	-	+	+/-	++
P. vulgaris	No	+ (sw.)	+	+	-	+	-	++



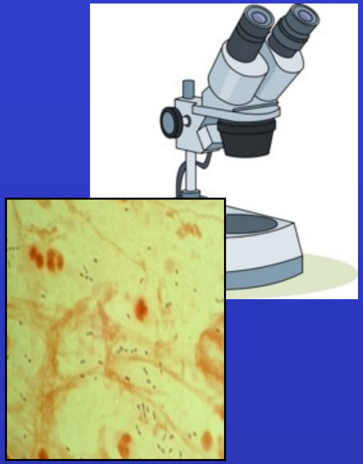
Ashex © 2004-2018 Non-Fermenter ID Matrix:	Motility	Oxidase	Catalase	Yellow Pig	Pink Pigm	Beta Hem	Growth on	DNase	Starch	Lecithinas	Lipase	PYR	LAP	ESC Spot
Achromobacter denitrificans	99.00	99.00	99.00	1.00	1.00	16.67	99.00	1.00	1.00	1.00	1.00	55.56	99.00	1.00
Achromobacter piechaudii	78.57	99.00	99.00	1.00	1.00	14.29	99.00	1.00	1.00	1.00	1.00	50.00	99.00	1.00
Achromobacter xylosoxidans	87.50	99.00	99.00	1.00	1.00	1.56	99.00	1.00	1.00	1.00	1.00	78.72	99.00	1.00
Acidovorax temperans	99.00	99.00	99.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	99.00	99.00	1.00
Acinetobacter baumannii complex	1.00	1.00	99.00	1.00	1.00	1.00	99.00	1.00	1.00	1.00	27.27	10.00	99.00	1.00
Acinetobacter haemolyticus	1.00	1.00	99.00	1.00	1.00	99.00	99.00	1.00	1.00	1.00	99.00	1.00	99.00	1.00
Acinetobacter lwoffii	1.00	1.00	99.00	1.00	1.00	1.00	82.61	1.00	1.00	1.00	1.00	22.22	99.00	1.00
Acinetobacter species saccharolytic	50.00	1.00	99.00	1.00	1.00	99.00	99.00	1.00	1.00	1.00	99.00	50.00	50.00	50.00
Alcaligenes faecalis	99.00	99.00	99.00	1.00	1.00	1.00	99.00	1.00	1.00	1.00	1.00	1.00	99.00	1.00
Bergeyella zoohelcum	1.00	83.33	50.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	99.00	1.00
Bordetella avium	99.00	99.00	99.00	1.00	1.00	1.00	99.00	1.00	1.00	1.00	1.00	99.00	99.00	1.00
Bordetella bronchiseptica	84.21	99.00	99.00	1.00	1.00	1.00	99.00	1.00	1.00	1.00	1.00	22.22	99.00	1.00
Bordetella hinzii	99.00	99.00	50.00	50.00	1.00	1.00	99.00	1.00	1.00	1.00	1.00	1.00	99.00	1.00
Bordetella holmesii	1.00	12.50	37.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	87.50	1.00
Bordetella parapertussis	1.00	1.00	99.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	99.00	1.00
Bordetella trematum	99.00	60.00	99.00	1.00	1.00	1.00	99.00	1.00	1.00	1.00	1.00	20.00	99.00	1.00
Brevundimonas diminuta	99.00	99.00	92.86	1.00	1.00	1.00	92.86	1.00	1.00	1.00	1.00	1.00	75.00	1.00
Brevundimonas vesicularis	99.00	93.33	60.00	33.33	1.00	8.33	46.67	6.67	66.67	1.00	1.00	28.57	85.71	42.86
Pandoraea apista	99.00	1.00	99.00	1.00	1.00	1.00	99.00	1.00	1.00	1.00	1.00	1.00	99.00	1.00
Pseudomonas aeruginosa	67.92	98.11	99.00	1.00	1.89	73.91	99.00	9.43	24.53	10.87	30.43	34.21	97.37	1.00
Pseudomonas alcaligenes	99.00	99.00	99.00	1.00	1.00	1.00	88.89	1.00	1.00	1.00	1.00	99.00	99.00	11.11
Pseudomonas fluorescens	90.91	95.45	99.00	4.55	9.09	72.22	99.00	4.55	50.00	70.00	36.84	23.08	99.00	1.00
Pseudomonas luteola	99.00	1.00	92.31	84.62	1.00	1.00	92.31	15.38	53.85	1.00	1.00	1.00	99.00	99.00
Pseudomonas mendocina	99.00	99.00	75.00	25.00	8.33	1.00	99.00	1.00	16.67	1.00	33.33	1.00	99.00	1.00
Pseudomonas oryzihabitans	99.00	1.00	99.00	88.24	1.00	8.33	99.00	1.00	64.71	1.00	1.00	36.36	99.00	1.00
Pseudomonas pseudoalcaligenes	69.23	99.00	84.62	1.00	1.00	1.00	99.00	1.00	7.69	1.00	1.00	1.00	99.00	1.00
Pseudomonas putida	99.00	96.88	96.88	18.75	1.00	15.38	99.00	1.00	25.00	3.70	3.70	1.00	99.00	1.00
Pseudomonas stutzeri	91.30	99.00	95.65	65.22	1.00	1.00	99.00	1.00	86.96	1.00	83.33	6.25	93.75	1.00
Pseudomonas stutzeri (Vb-3)	99.00	99.00	99.00	33.33	16.67	1.00	99.00	1.00	99.00	1.00	99.00	50.00	50.00	50.00
Psychrobacter immobilis (asaccharolytic)	1.00	99.00	99.00	1.00	1.00	20.00	80.00	1.00	1.00	1.00	40.00	1.00	80.00	1.00
Psychrobacter immobilis (saccharolytic)	1.00	99.00	99.00	1.00	1.00	20.00	99.00	1.00	1.00	1.00	60.00	1.00	99.00	1.00
Psychrobacter phenylpyruvicus	1.00	99.00	99.00	1.00	1.00	1.00	80.00	1.00	1.00	1.00	33.33	1.00	99.00	1.00
Ralstonia mannitolilytica	99.00	99.00	14.29	1.00	1.00	1.00	71.43	1.00	14.29	1.00	42.86	28.57	99.00	1.00
Ralstonia pickettii (Va-1)	78.57	99.00	1.00	14.29	1.00	1.00	57.14	1.00	35.71	1.00	70.00	55.56	99.00	10.00
Ralstonia pickettii (Va-2)	99.00	99.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	60.00	20.00	99.00	1.00
Rhizobium (Agrobacterium) radiobacter	84.62	92.31	99.00	1.00	1.00	33.33	72.73	1.00	1.00	1.00	1.00	99.00	83.33	33.33
Roseomonas species	15.38	61.54	99.00	1.00	99.00	1.00	7.69	1.00	69.23	1.00	1.00	14.29	57.14	1.00
Shewanella algae	99.00	83.33	99.00	1.00	16.67	25.00	99.00	99.00	1.00	1.00	75.00	66.67	99.00	1.00
Shewanella putrefaciens	80.00	99.00	60.00	20.00	20.00	1.00	99.00	99.00	1.00	1.00	1.00	1.00	99.00	1.00
Sphingobacterium multivorum	50.00	99.00	99.00	33.33	1.00	20.00	99.00	16.67	83.33	1.00	60.00	80.00	99.00	40.00
Sphingobacterium spiritivorum	1.00	99.00	88.89	11.11	1.00	1.00	1.00	99.00	1.00	1.00	1.00	1.00	99.00	99.00
Sphingobacterium thalophilum	1.00	99.00	99.00	1.00	1.00	50.00	99.00	99.00	99.00	50.00	50.00	50.00	50.00	50.00
Sphingomonas paucimobilis	50.00	55.00	85.00	99.00	1.00	41.67	5.00	25.00	75.00	1.00	1.00	1.00	99.00	99.00
Stenotrophomonas maltophilia	88.00	28.00	94.00	36.00	1.00	22.50	96.00	82.00	1.00	1.00	71.11	1.00	94.87	97.44

Bacterial Culture and Identification

Timeline

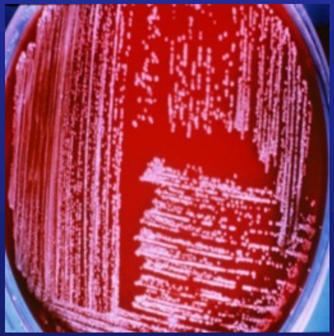
1 hr

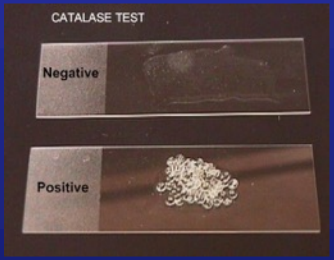
1 hr

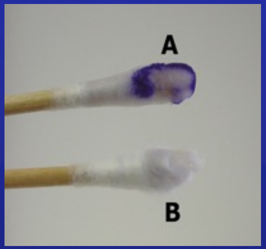


Total time to ID: ~48 hours
Total time to antibiotic profile: ~48 hours

~18 hrs







~24 hrs

Antibiotic Therapies

Knowing the species matters

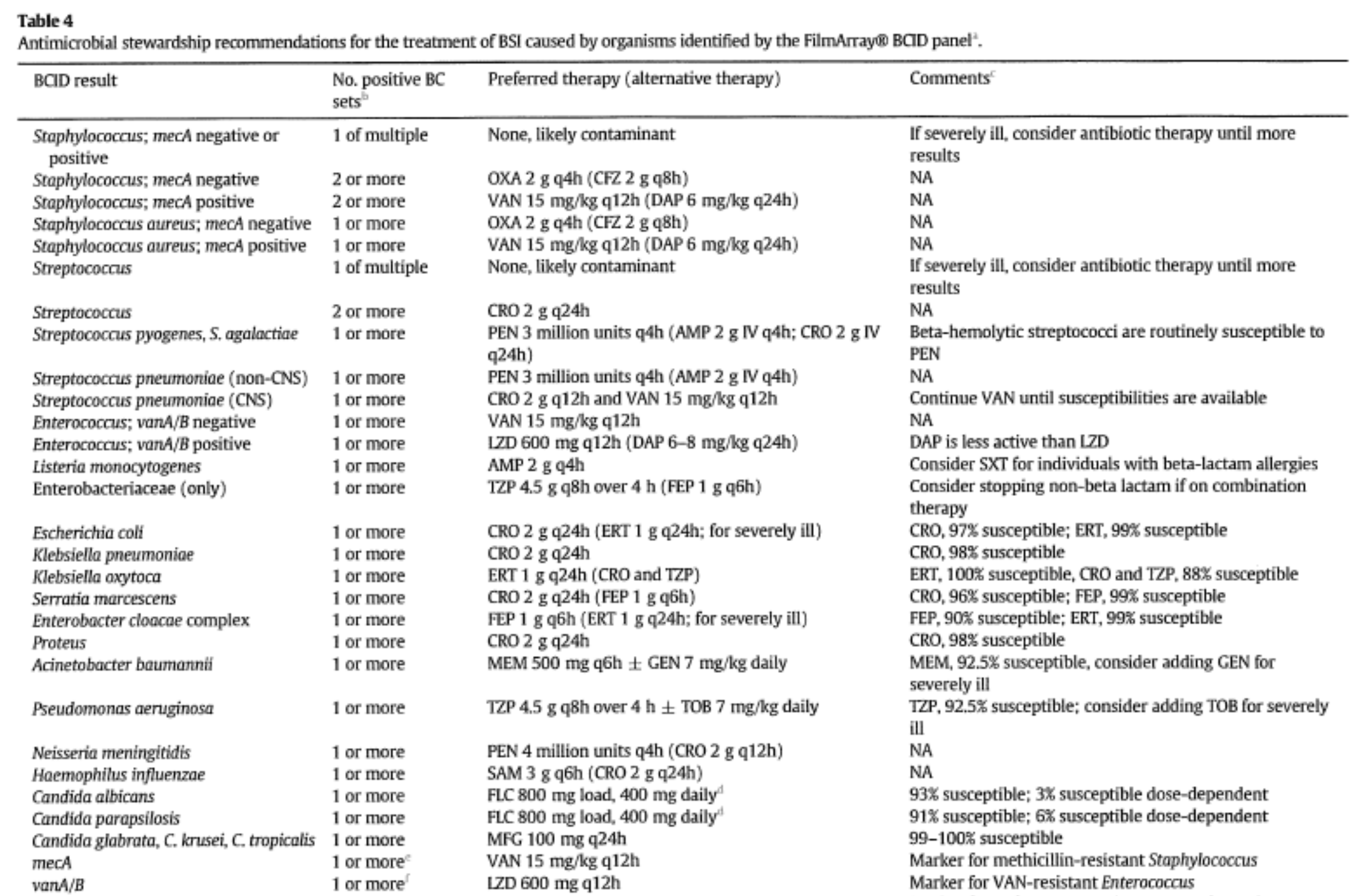


Table 4

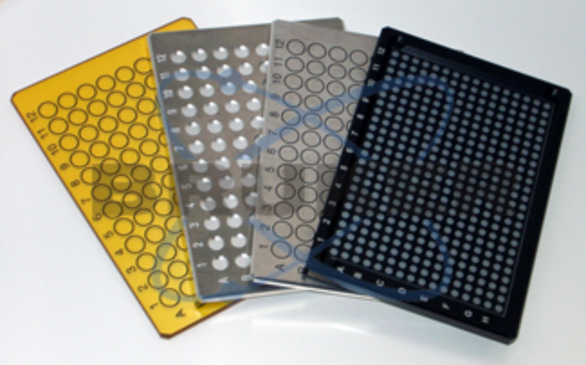
Antimicrobial stewardship recommendations for the treatment of BSI caused by organisms identified by the FilmArray® BCID panel[a].

BCID result	No. positive BC sets[b]	Preferred therapy (alternative therapy)	Comments[c]
Staphylococcus; *mecA* negative or positive	1 of multiple	None, likely contaminant	If severely ill, consider antibiotic therapy until more results
Staphylococcus; *mecA* negative	2 or more	OXA 2 g q4h (CFZ 2 g q8h)	NA
Staphylococcus; *mecA* positive	2 or more	VAN 15 mg/kg q12h (DAP 6 mg/kg q24h)	NA
Staphylococcus aureus; *mecA* negative	1 or more	OXA 2 g q4h (CFZ 2 g q8h)	NA
Staphylococcus aureus; *mecA* positive	1 or more	VAN 15 mg/kg q12h (DAP 6 mg/kg q24h)	NA
Streptococcus	1 of multiple	None, likely contaminant	If severely ill, consider antibiotic therapy until more results
Streptococcus	2 or more	CRO 2 g q24h	NA
Streptococcus pyogenes, *S. agalactiae*	1 or more	PEN 3 million units q4h (AMP 2 g IV q4h; CRO 2 g IV q24h)	Beta-hemolytic streptococci are routinely susceptible to PEN
Streptococcus pneumoniae (non-CNS)	1 or more	PEN 3 million units q4h (AMP 2 g IV q4h)	NA
Streptococcus pneumoniae (CNS)	1 or more	CRO 2 g q12h and VAN 15 mg/kg q12h	Continue VAN until susceptibilities are available
Enterococcus; *vanA/B* negative	1 or more	VAN 15 mg/kg q12h	NA
Enterococcus; *vanA/B* positive	1 or more	LZD 600 mg q12h (DAP 6–8 mg/kg q24h)	DAP is less active than LZD
Listeria monocytogenes	1 or more	AMP 2 g q4h	Consider SXT for individuals with beta-lactam allergies
Enterobacteriaceae (only)	1 or more	TZP 4.5 g q8h over 4 h (FEP 1 g q6h)	Consider stopping non-beta lactam if on combination therapy
Escherichia coli	1 or more	CRO 2 g q24h (ERT 1 g q24h; for severely ill)	CRO, 97% susceptible; ERT, 99% susceptible
Klebsiella pneumoniae	1 or more	CRO 2 g q24h	CRO, 98% susceptible
Klebsiella oxytoca	1 or more	ERT 1 g q24h (CRO and TZP)	ERT, 100% susceptible, CRO and TZP, 88% susceptible
Serratia marcescens	1 or more	CRO 2 g q24h (FEP 1 g q6h)	CRO, 96% susceptible; FEP, 99% susceptible
Enterobacter cloacae complex	1 or more	FEP 1 g q6h (ERT 1 g q24h; for severely ill)	FEP, 90% susceptible; ERT, 99% susceptible
Proteus	1 or more	CRO 2 g q24h	CRO, 98% susceptible
Acinetobacter baumannii	1 or more	MEM 500 mg q6h ± GEN 7 mg/kg daily	MEM, 92.5% susceptible, consider adding GEN for severely ill
Pseudomonas aeruginosa	1 or more	TZP 4.5 g q8h over 4 h ± TOB 7 mg/kg daily	TZP, 92.5% susceptible; consider adding TOB for severely ill
Neisseria meningitidis	1 or more	PEN 4 million units q4h (CRO 2 g q12h)	NA
Haemophilus influenzae	1 or more	SAM 3 g q6h (CRO 2 g q24h)	NA
Candida albicans	1 or more	FLC 800 mg load, 400 mg daily[d]	93% susceptible; 3% susceptible dose-dependent
Candida parapsilosis	1 or more	FLC 800 mg load, 400 mg daily[d]	91% susceptible; 6% susceptible dose-dependent
Candida glabrata, *C. krusei*, *C. tropicalis*	1 or more	MFG 100 mg q24h	99–100% susceptible
mecA	1 or more[e]	VAN 15 mg/kg q12h	Marker for methicillin-resistant *Staphylococcus*
vanA/B	1 or more[f]	LZD 600 mg q12h	Marker for VAN-resistant *Enterococcus*
bla$_{KPC}$	1 or more	Consult Infectious Disease Service; COL ± TGC	Marker for carbapenem-resistant Enterobacteriaceae

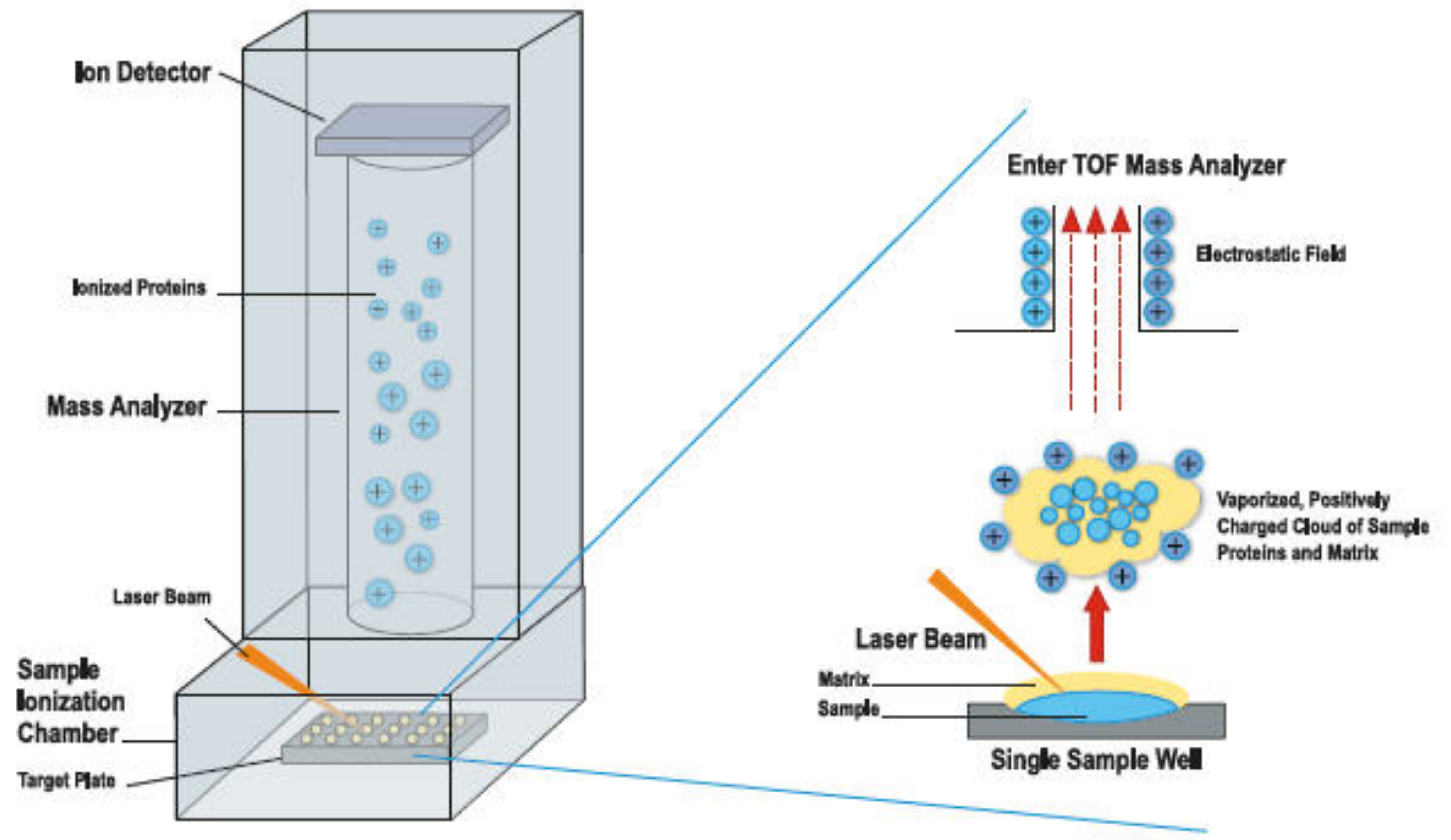
Southern TR et al. Diagnostic Microbiology and Infectious Disease. 2015; 81(2):96-101

Matrix Assisted Laser Desorption Ionization-Time Of Flight (MALDI-TOF)

- Protein-based identification
- Accuracy comparable to Nucleic Acid Sequencing
  - >98% accuracy compared to 16S RNA sequencing
- Much faster than traditional methods
  - ~200 IDs an hour from a pure colony (requires initial culturing)
- Cost effective (with large initial capital investment)
  - Less than a dollar per test
- Two commercially-available, FDA-approved platforms
  - Bruker MS Biotyper
  - Biomerieux Vitek MS

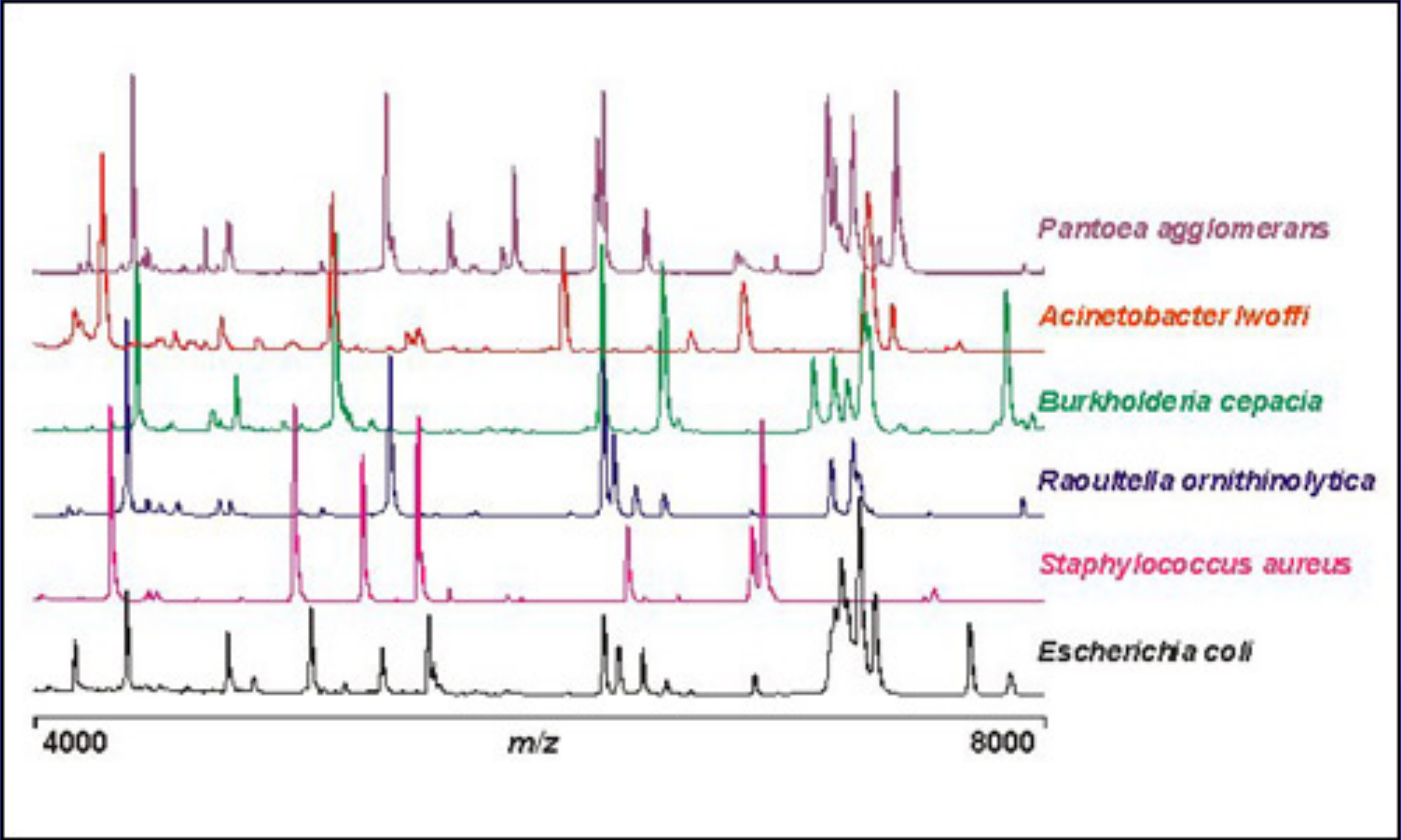


MALDI-TOF



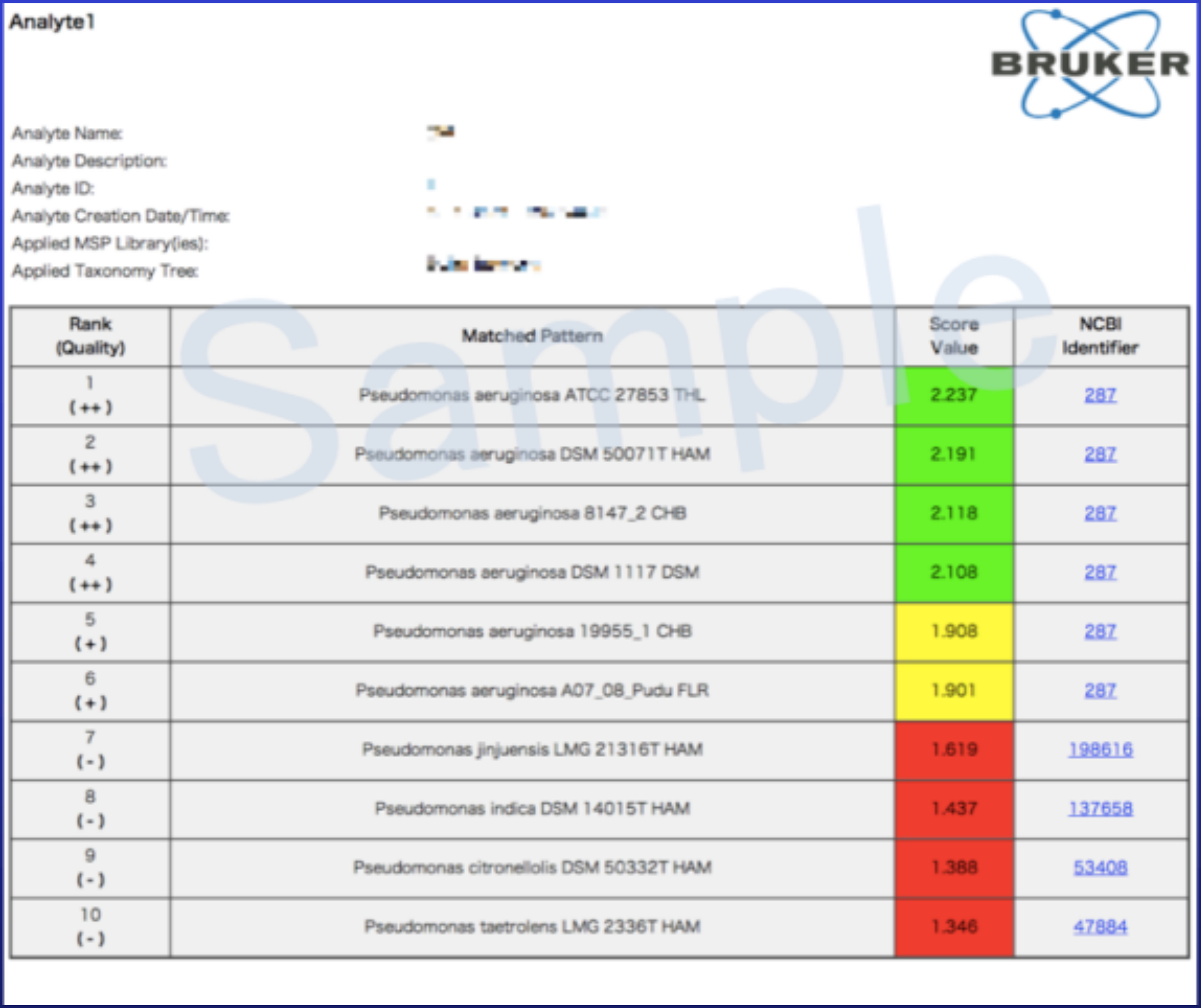
- Measures highly abundant proteins found in all organisms (ex. 16S Ribosomal proteins)
- Creates a spectra fingerprint to compare against a database of known organisms

MALDI-TOF



- Measures highly abundant proteins found in all organisms (ex. 16S Ribosomal proteins)
- Creates a spectra fingerprint to compare against a database of known organisms

MALDI-TOF



Analyte1

BRUKER

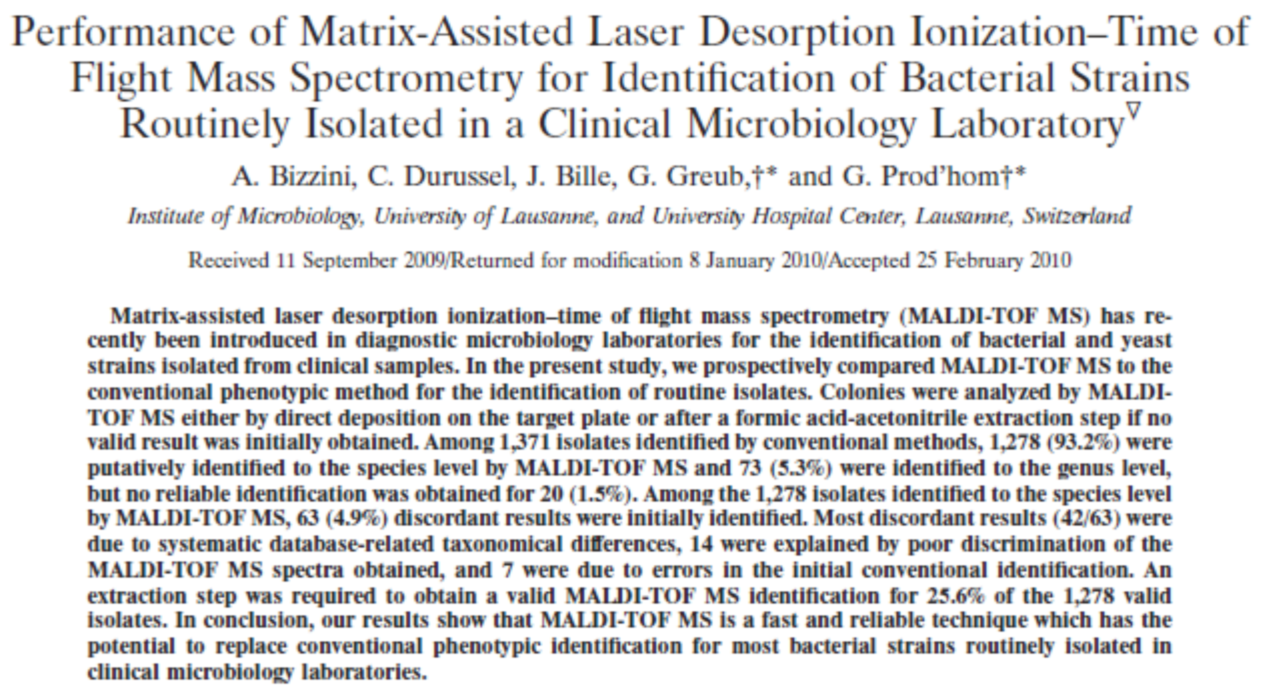
Analyte Name: [redacted]
Analyte Description:
Analyte ID: [redacted]
Analyte Creation Date/Time: [redacted]
Applied MSP Library(ies):
Applied Taxonomy Tree: [redacted]

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	Pseudomonas aeruginosa ATCC 27853 THL	2.237	287
2 (++)	Pseudomonas aeruginosa DSM 50071T HAM	2.191	287
3 (++)	Pseudomonas aeruginosa 8147_2 CHB	2.118	287
4 (++)	Pseudomonas aeruginosa DSM 1117 DSM	2.108	287
5 (+)	Pseudomonas aeruginosa 19955_1 CHB	1.908	287
6 (+)	Pseudomonas aeruginosa A07_08_Pudu FLR	1.901	287
7 (-)	Pseudomonas jinjuensis LMG 21316T HAM	1.619	198616
8 (-)	Pseudomonas indica DSM 14015T HAM	1.437	137658
9 (-)	Pseudomonas citronellolis DSM 50332T HAM	1.388	53408
10 (-)	Pseudomonas taetrolens LMG 2336T HAM	1.346	47884

Version 1.0 Approved Organisms

Acinetobacter baumanii	*Micrococcus luteus*
Aeromonas hydrophilia	*Moraxella catarrhalis*
Aggregatibacter aphrophilus	*Neisseria gonorrhoeae*
Arcanobacteria pyogenes	*Neisseria lactamica*
Bacteroides fragilis	*Peptostreptococcus asaccharolyticus*
Bacteroides uniformis	*Prevotella melaninogenica*
Burkholderia cepacia	*Propionibacterium acnes*
Campylobacter jejuni	*Proteus mirabilis*
Clostridium perfringens	*Pseudomonas aeruginosa*
Clostridium sordelli	*Serratia marcescens*
Corynebacterium diptheriae	*Staphylococcus aureus*
Corynebacterium pseudodiptheriticum	*Staphylococcus epidermidis*
Eikenella corrodens	*Staphylococcus lugdunensis*
Enterobacter aerogenes	*Staphylococcus saprophyticus*
Enterobacter cloacae	*Stenotrophomonas maltophilia*
Enterococcus faecalis	*Streptococcus agalactiae*
Enterococcus faecium	*Streptococcus constellatus*
Enterococcus gallinarum	*Streptococcus dysgalactiae*
Escherichia coli	*Streptococcus equi*
Fusobacterium necrophorum	*Streptococcus pneumoniae*
Haemophilus influenzae	*Streptococcus pyogenes*
Klebsiella oxytoca	*Streptococcus sanguinis*
Klebsiella pneumoniae	

Version 2.0 had hundreds. Version 3.0 at FDA now…



Performance of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of Bacterial Strains Routinely Isolated in a Clinical Microbiology Laboratory$^\nabla$

A. Bizzini, C. Durussel, J. Bille, G. Greub,†* and G. Prod'hom†*

Institute of Microbiology, University of Lausanne, and University Hospital Center, Lausanne, Switzerland

Received 11 September 2009/Returned for modification 8 January 2010/Accepted 25 February 2010

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has recently been introduced in diagnostic microbiology laboratories for the identification of bacterial and yeast strains isolated from clinical samples. In the present study, we prospectively compared MALDI-TOF MS to the conventional phenotypic method for the identification of routine isolates. Colonies were analyzed by MALDI-TOF MS either by direct deposition on the target plate or after a formic acid-acetonitrile extraction step if no valid result was initially obtained. Among 1,371 isolates identified by conventional methods, 1,278 (93.2%) were putatively identified to the species level by MALDI-TOF MS and 73 (5.3%) were identified to the genus level, but no reliable identification was obtained for 20 (1.5%). Among the 1,278 isolates identified to the species level by MALDI-TOF MS, 63 (4.9%) discordant results were initially identified. Most discordant results (42/63) were due to systematic database-related taxonomical differences, 14 were explained by poor discrimination of the MALDI-TOF MS spectra obtained, and 7 were due to errors in the initial conventional identification. An extraction step was required to obtain a valid MALDI-TOF MS identification for 25.6% of the 1,278 valid isolates. In conclusion, our results show that MALDI-TOF MS is a fast and reliable technique which has the potential to replace conventional phenotypic identification for most bacterial strains routinely isolated in clinical microbiology laboratories.

- Overall, ~93% success rate (2010 – databases continually improving)
- Generally:
  - Gram negative identification easier, more successful than Gram positives
  - More common organisms more successful than less common organisms
  - Certain bacteria (Corynebacterium, Actinomycetes), Mycobacteria, Molds, Yeasts lagging behind others, but catching up
  - Lack of identification (poor database coverage) much, much more likely than mis-identification
  - Exceptions in the case of several closely related species
    - NLF *E. coli* and *Shigella*
    - *Streptococcus pneumoniae* and *Streptococcus mitis*
    - Certain *Select Agents*

Evaluation of Matrix-Assisted Laser Desorpti
Flight Mass Spectrometry for Identification o
Nocardia species, and Other Aerobic Actinon

S. P. Buckwalter, S. L. Olson, B. J. Connelly, B. C. Lucas, A. A. Rodning, R. C. Walchak,
Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rocheste

The value of matrix-assisted laser desorption ionization—time of flight mass spec
tion of bacteria and yeasts is well documented in the literature. Its utility for the i
spp. has also been
162 *Mycobacteriu*
tes using both the
performance of a
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Following library
16S rRNA gene se
actinomycetes iso
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rapidly identify sl

Matrix-Assisted Desorption Ionization Time of Flight Mass Spectrometry for the Use with

Positive Blood Cultures: Methodology, Performance, and Optimization

Matthew L. Faron[1], Blake W. Buchan[1,2] and Nathan A. Ledeboer [1,2]

[1]Medical College of Wisconsin, Milwaukee, WI

ositive

Microbial Drug Resistance

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A First Insight into *Escherichia coli* ST131 High-Risk Clone Among Extended-Spectrum Beta-Lactamase-Producing Urine Isolates in Istanbul with the Use of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass-Spectrometry and Real-Time PCR

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Online Ahead of Print: May 3, 2017

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Boaz Bisho
Neuberger

PII:

DOI:

Reference:

To appear in: *Clinica*

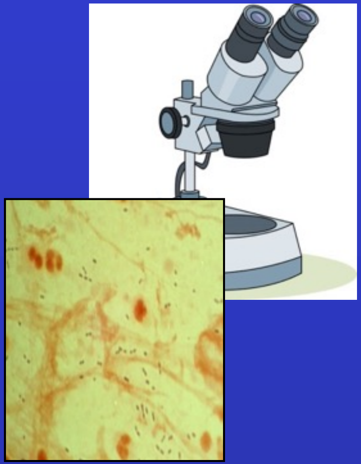
Bin Huang[1,2,¶], Lei Zhang[4,¶], Weizheng Zhang[5,6], Kang Liao[1], Shihong Zhang[1], Zhiquan Zhang[7], Xingyan Ma[1], Jialong Chen[8], Xiuhong Zhang[9], Pinghua Qu[5,6], Shangwei Wu[4], Cha Chen[5,6,*], and Yi-Wei Tang[2,3,*]

Bacterial Culture and Identification

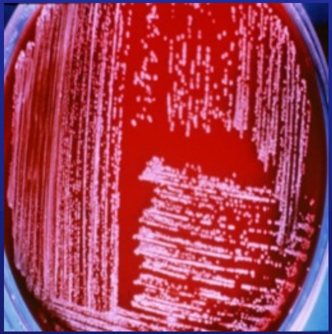
Timeline

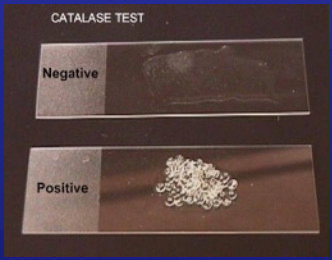
1 hr

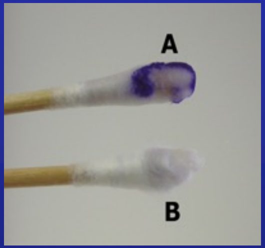
1 hr



~18 hrs







~24 hrs

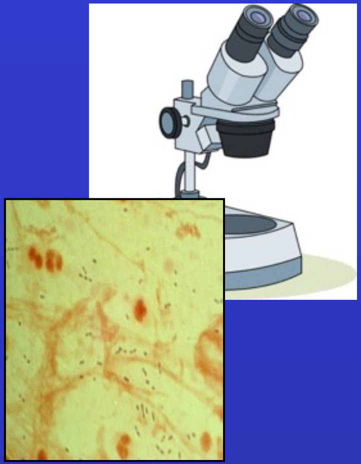
Total time to ID: ~48 hours
Total time to antibiotic profile: ~48 hours

Bacterial Culture and Identification

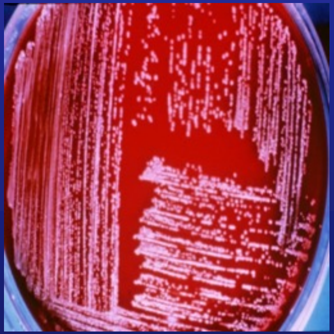
Timeline

1 hr

1 hr



~18 hrs



10 min

~24 hrs

Total time to ID: ~24 hours

Clinical Scenario #1

50 year old recently female retiree presents 1 year out from a right total knee replacement with worsening knee pain and mild swelling along with some night sweats. She is taken to the OR and the hardware is removed and sent down to the lab for culture… After 24 hours she has light growth of a short Gram negative rod growing only on the chocolate agar plate. MALDI identifies it as Haemophilus influenzae. She is treated with ceftriaxone for 6 weeks and her repeat cultures are negative and new hardware is placed and patient doing well to date.

What accreditation does your hospital use?