Foundational Training for the Entry-level Clinical Microbiologist Part Three: CLSI M35-A2 Document for Same-day Identification of Bacteria and Yeast (II)

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The presenter states no conflict of interest and has no financial relationship to disclose relevant to the content of this presentation.

OBJECTIVES

1. Apply basic bench-level skills, along with spot and rapid identification reagents, to the abbreviated identification of commonly-encountered patient isolates;

2. Recognize the utility of the CLSI M35-A2 guideline as an adjunct to routine operations in the clinical microbiology laboratory; and,

3. Review essential strategies in the triage of potential biothreat bacterial agents.

Life Without MALDI

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WE'LL DO BETTER NEXT TIME

- Foundational training

  Hemolysis
  Media
  Odor
  Significance
  Colony morphology
  Growth distribution
  Cellular morphology

- M35-A2 Round One

  Important reviews

- M35-A2 Round Two

  Yeast
  HACEKs
  Anaerobes
  Biothreats

(Re)Introduction to CLSI M35-A2

IMPETUS

- We've been doing this for years (odor, spot testing, *et cetera*); CLSI sought to standardize the practice
- Cost savings, patient benefits

Study	Turnaround Time *P*	Length of Stay *P* [4]	Mortality Index *P* [4]	Average Cost *P* [4]	Notes
Doern *et al.*[1]	< 0.0005	NS[5]	< 0.02	0.01	Rapid MicroScan product
Barenfanger *et al.* [2]	0.001[4]	0.006	0.45	0.04	VITEK product
Kerremans *et al.* [3]	< 0.0001	ND[6]	0.21	ND	VITEK products

[1]J Clin Microbiol 32:1757-1762; 1994
[2]J Clin Microbiol 37:1415-1418; 1999
[3]J Antimicrob Chemother 61:428-435; 2008

WARNINGS

- "Isolates to be tested should match the criteria required for proper identification."

- Isolates conforming to M35-A2 identify organism with >95% accuracy; report without qualification
- Sniffing/wafting can be dangerous

  Once mold colony is ruled out, opening of plates from non-invasive sources (i.e., urine, sputum) is common & relatively safe

CLSI M35-A2; 2008

OVERALL UTILITY I

	n	Ampicillin/Sulbactam	Piperacillin/Tazobactam	Imipenem	Meropenem	Aztreonam	Cefazolin	Ceftriaxone	Cefepime	Gentamicin	Amikacin	Ciprofloxacin	Levofloxacin	Trimethoprim/ Sulfamethoxazole
Escherichia coli	1595	58	96		100	96	89	95	96	93	100	80	80	79
Klebsiella pneumoniae	323	85	96		99	93	90	94	97	98	99	96	96	93
Klebsiella oxytoca	71	49	80		100	97	49	83	92	96	100	85	86	92
Enterobacter cloacae	89		87		97	90		88	98	98	100	94	94	86
Enterobacter aerogenes	38		71		100	84		87	97	100	100	89	89	97
Citrobacter freundii	30		93		100	93		93	100	97	100	96	97	90
Proteus mirabilis	155	84	99		100	100	87	100	100	95	100	81	85	81
Serratia marcescens	34				100	100		100	100	100	100	91	91	100
														Tobra
Pseudomonas aeruginosa	186		89	82	86	67			83	91		87	80	98
Organisms with extended-spectrum beta-lactamases (ESBL): 98														
Haemophilus influenzae beta-lactamase rate: 35.1%*														

OVERALL UTILITY II

	n	Penicillin	Methicillin	Erythromycin	Clindamycin	Vancomycin	Levofloxacin	Nitrofurantoin	Trimethoprim/sulfa	Tetracycline
methicillin-resistant *S. aureus* (45% MRSA rate)	255	0	0	10	69	100	41		100	91
methicillin-susceptible *S. aureus*	314	11	100	69	86	100	89		99	97
coagulase-negative *Staphylococcus*	221	13	48	28	50	100	65		66	91
Enterococcus species*	247-321	71				89		92		
viridans group *Streptococcus***	166	67		56	88	100				
*Streptococcus agalactiae***	167	100		39	54	100	98			
Beta-hemolytic streptococci other than group B**	40	100		83	85	100	100			
*Streptococcus pneumoniae***	81	PCN:	70.3% susceptible			Levofloxacin:		100.0% susceptible		
			18.5% intermediate					0.0% intermediate		
			11.1% resistant					0.0% resistant		

STOCK UP

Bile solubility reagent
Rapid spot CAMP
H_2O_2 (aerobes)
H_2O_2 (anaerobes)
H_2O_2 (*N. gonorrhoeae*)
Germ tube
Rapid hippurate hydrolysis
Spot indole

Indoxyl acetate
MUG test
Spot oxidase
δ-aminolevulinic acid
Rapid trehalose
Urea/phenylalanine deaminase
PYR, LAP
Latex agglutination (*S. aureus*)

SPOT REAGENTS

Reagent	Notes on Protocol	Read Time	Important Notes
Catalase	Smear then drop; different formulations	< 20 seconds	non-platinum loops; no blood agar
Indole	Different formulations	< 20 seconds	not on medium with dyes, not on Mueller-Hinton; indole diffuses
Oxidase	Slow-growers with no growth on MacConkey	< 10 seconds	no wires; no differential media

M35-A2 Rapid Identification

Candida albicans

- Presumptive identification

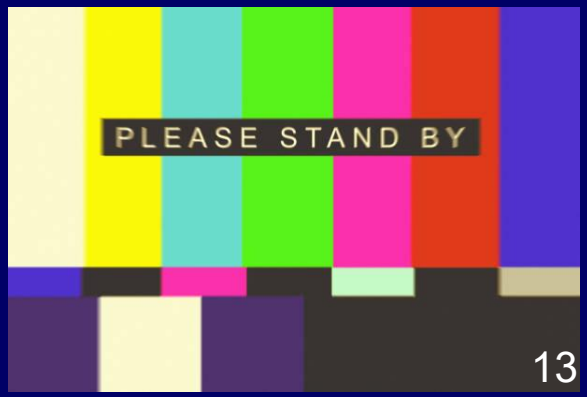
  Budding yeast

- Additional tests for definitive identification

  “Feet” in less than 48 hours OR
  Germ tube-positive

- Notes

  Not easily separated from
  C. dubliniensis



Candida glabrata

- Presumptive identification

  Small yeast in smear with no hyphae
  Better growth on chocolate agar than blood agar

- Additional tests for definitive identification

  Rapid trehalose-positive at 42ºC OR

Evaluation of Growth Characteristics on Blood Agar and Eosin Methylene Blue Agar for the Identification of *Candida (Torulopsis) glabrata*

Martha J. Bale, Carol Yang, and
Michael A. Pfaller

Diagn Microbiol Infect Dis 28:65-67; 1997

TABLE 1 Growth Characteristics on Primary Culture Plates

	C. glabrata	Other Species
EMB growth > BAP[a]	92	0
EMB = or < BAP	10	267
Total	102	267

[a] Colonies twice the size, or no growth on BAP.

Cryptococcus neoformans/gattii complexes

- Presumptive identification

  Spherical pleomorphic budding yeast with no hyphae

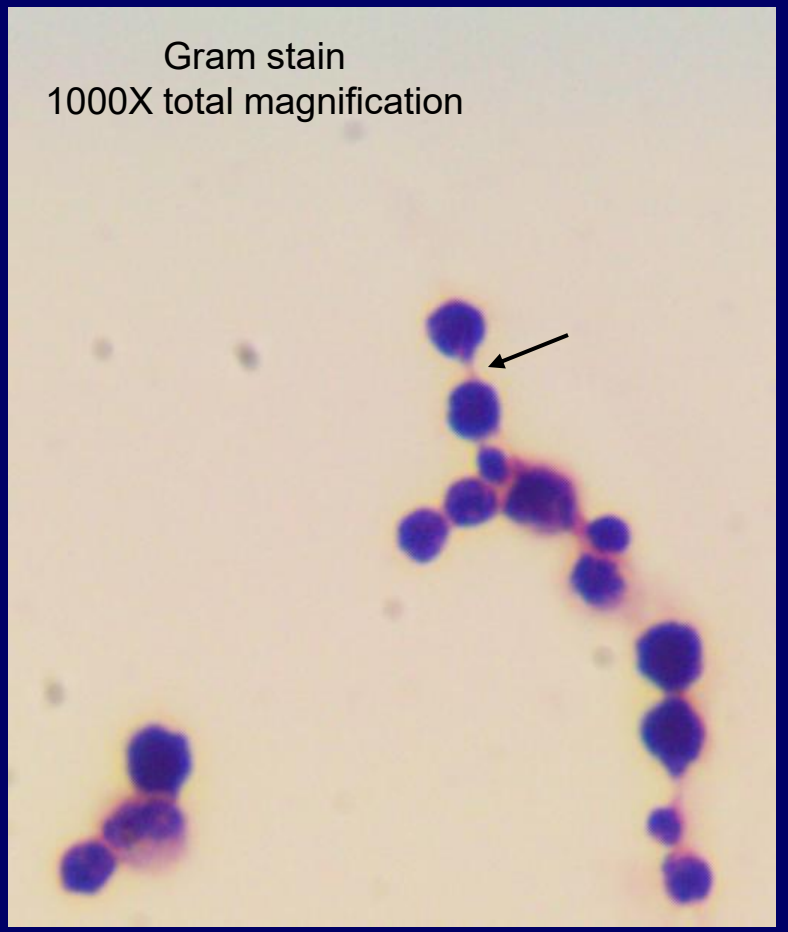
- Additional tests for definitive identification

  Urease-positive
  Phenol oxidase-positive (caffeic acid disk)

- Notes

  Phenotypic differentiation of complexes only by canavanine-glycine bromthymol blue medium (5 d)

Gram stain
1000X total magnification

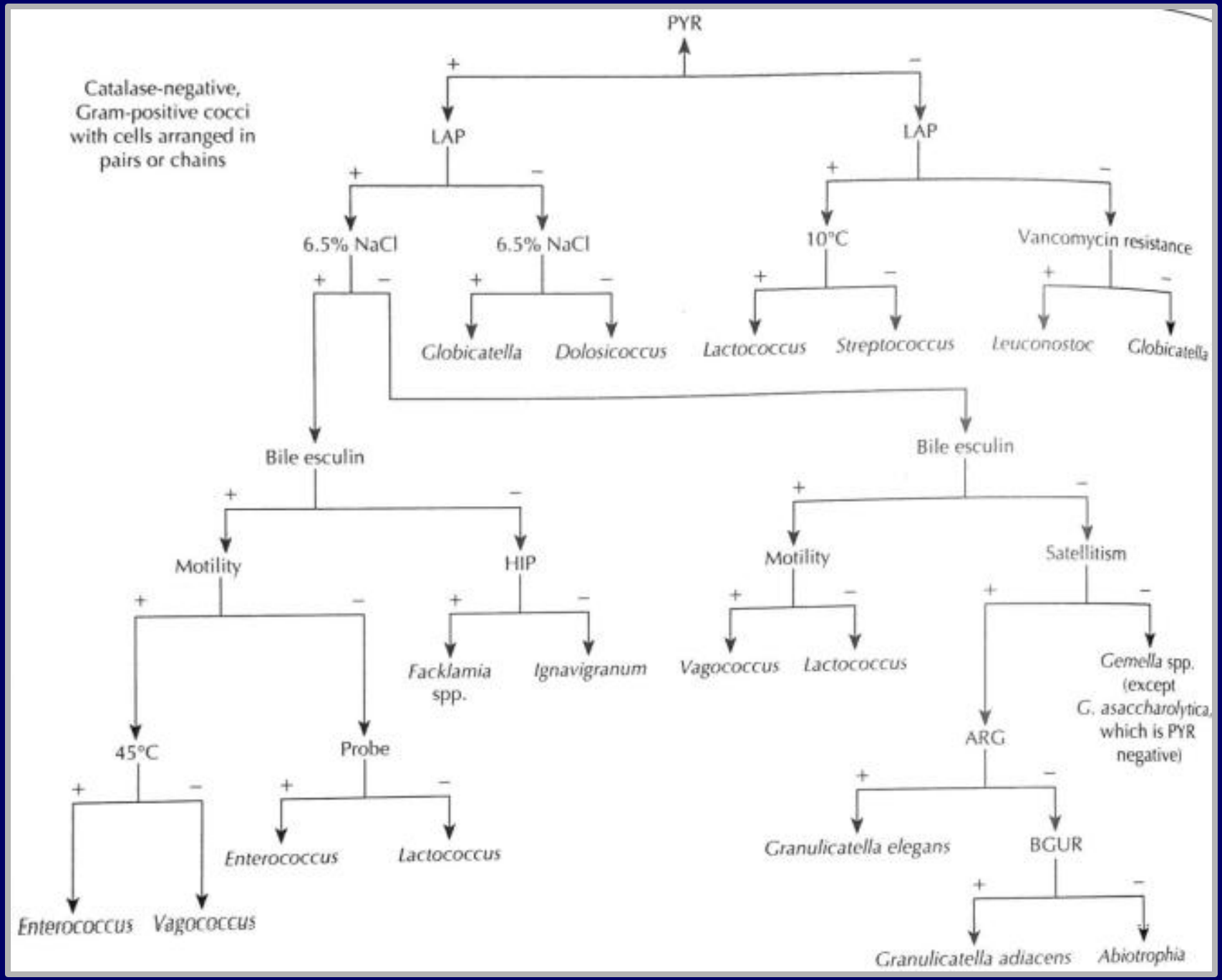


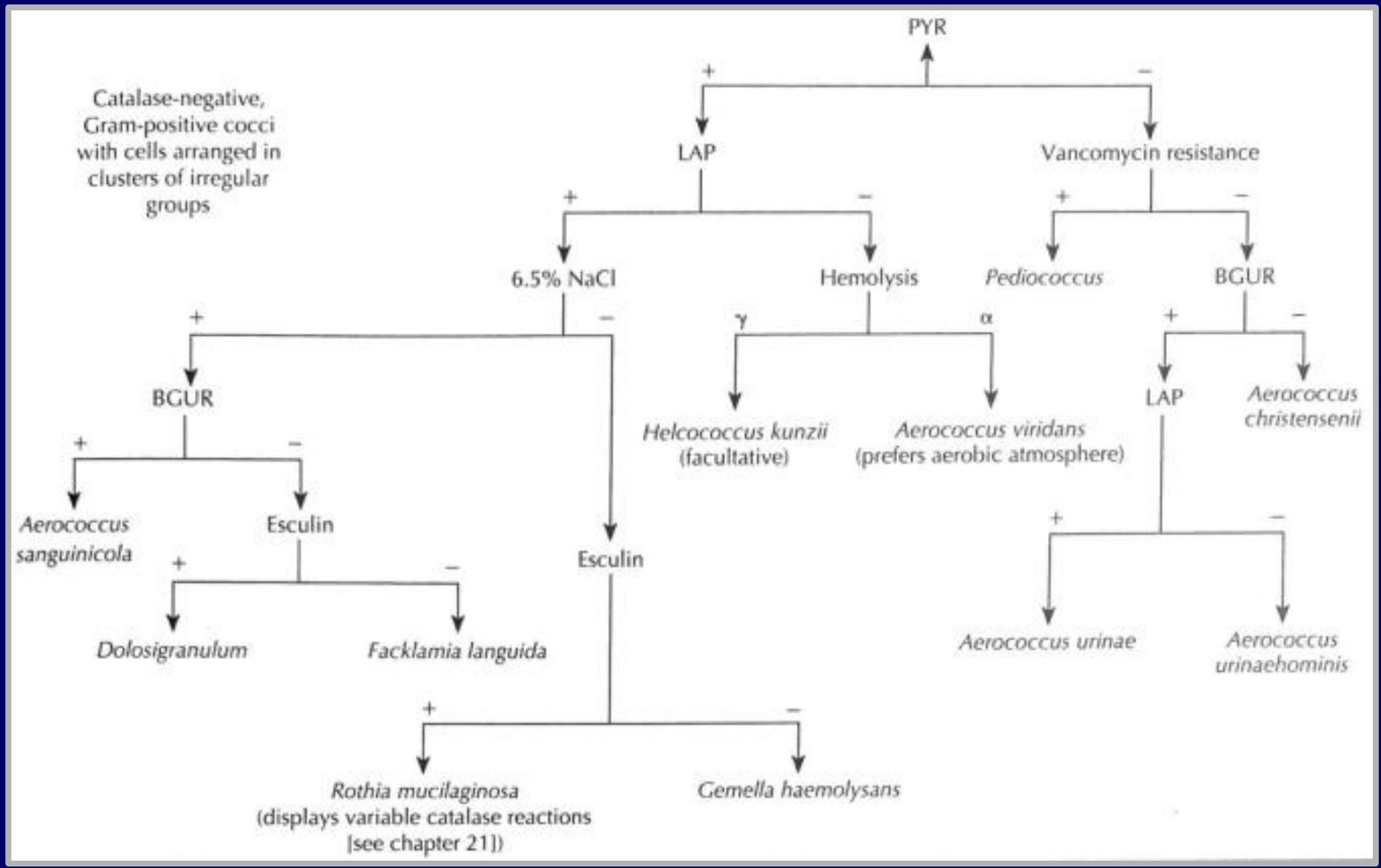

DETOUR


DÉTOUR
DÉTOUR
DÉTOU

GRAM-POSITIVE COCCI

Parameter	viridans group *Streptococcus*	*Aerococcus viridans*	*Enterococcus*	*Aerococcus urinae*	*Pediococcus*
catalase	negative	negative	negative	negative	negative
morphology	pairs, chains	clusters, tetrad	pairs, chains	clusters, tetrad	clusters, tetrad
hemolysis	α or γ	α	γ or α	α	α
PYR	negative	positive	positive	negative	negative
LAP	positive	negative	positive	positive	positive
fun fact(s)	bile solubility-negative		watch out for α VRE	Grandma's UTI	vancomycin-resistant


Catalase-negative, Gram-positive cocci with cells arranged in pairs or chains
PYR
LAP
LAP
6.5% NaCl
6.5% NaCl
10°C
Vancomycin resistance
Globicatella
Dolosicoccus
Lactococcus
Streptococcus
Leuconostoc
Globicatella
Bile esculin
Bile esculin
Motility
HIP
Motility
Satellitism
Facklamia spp.
Ignavigranum
Vagococcus
Lactococcus
45°C
Probe
ARG
Gemella spp. (except G. asaccharolytica, which is PYR negative)
Enterococcus
Vagococcus
Enterococcus
Lactococcus
Granulicatella elegans
BGUR
Granulicatella adiacens
Abiotrophia


Catalase-negative, Gram-positive cocci with cells arranged in clusters of irregular groups
PYR
+
−
LAP
Vancomycin resistance
+
−
+
−
6.5% NaCl
Hemolysis
Pediococcus
BGUR
+
−
γ
α
+
−
BGUR
LAP
Aerococcus christensenii
+
−
+
−
Helcococcus kunzii (facultative)
Aerococcus viridans (prefers aerobic atmosphere)
Aerococcus sanguinicola
Esculin
+
−
Esculin
+
−
+
−
Dolosigranulum
Facklamia languida
Aerococcus urinae
Aerococcus urinaehominis
+
−
Rothia mucilaginosa (displays variable catalase reactions [see chapter 21])
Gemella haemolysans

Escherichia coli

- Presumptive identification

  Gram-negative bacilli via Gram or growth on selective medium

  Oxidase-negative

  Indole-positive

- Additional tests for definitive identification

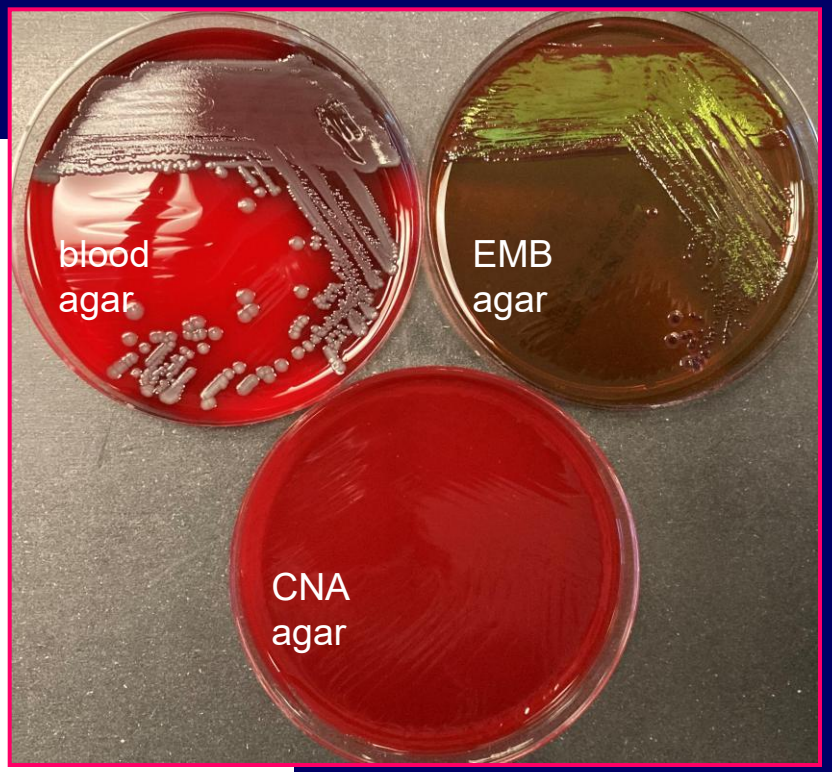
  Hemolytic OR lactose-positive AND PYR-negative
  OR lactose-negative AND MUG-positive

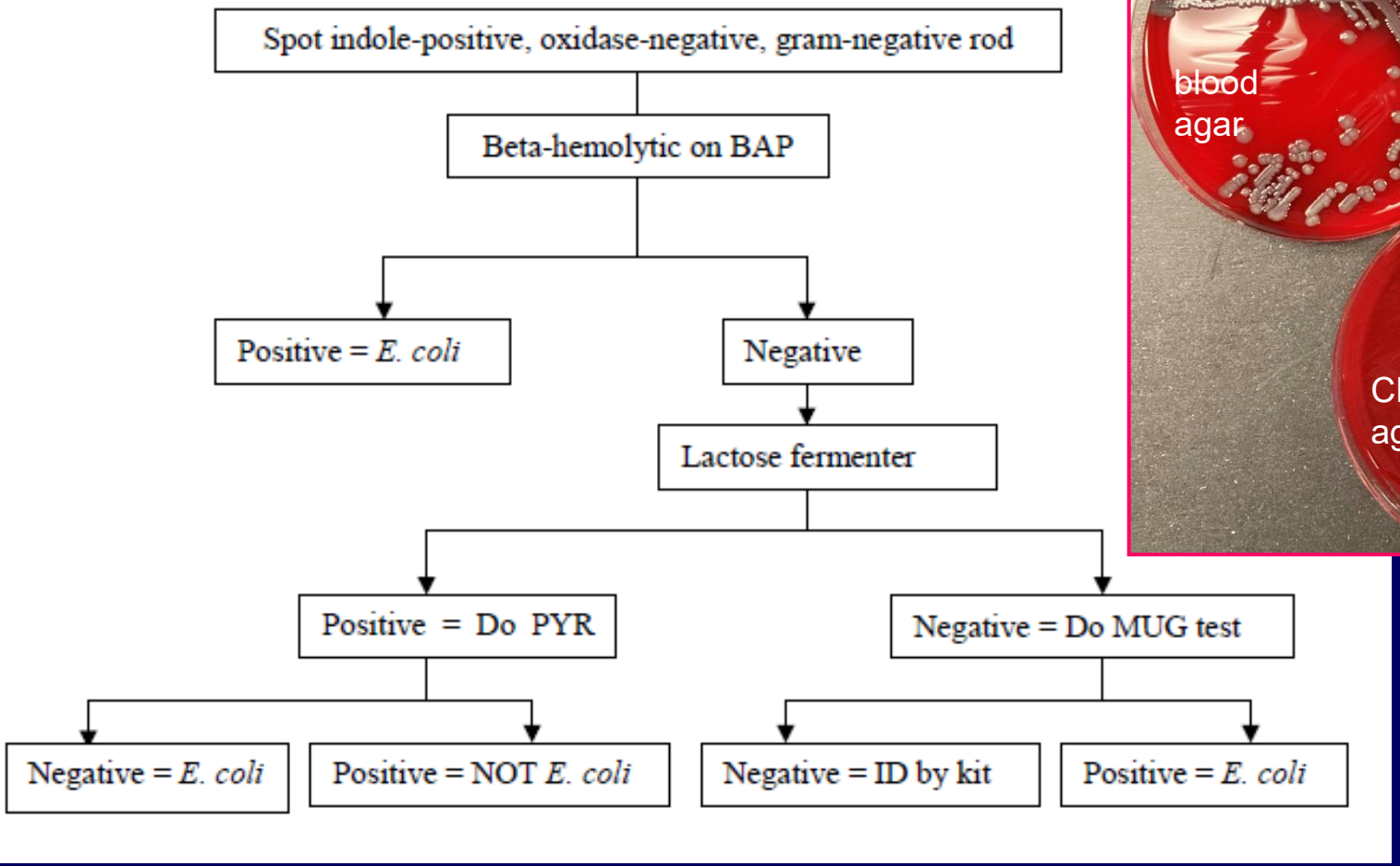
- Notes

  Isolate must be growing as large colonies

  Isolate cannot be derived from gastrointestinal source (*Shigella* spp.)

Escherichia coli





CLSI M35-A2; 2008

Let's (finally) Finish this Up

Neisseria meningitidis

- Presumptive identification

  Gram-negative diplococci Oxidase-positive
  Glistening non-hemolytic growth on blood agar

- Additional tests for definitive identification

  γ-glutamyl-aminopeptidase-positive

- Notes

  Highly infectious; work in biological safety cabinet

Neisseria lactamica

- Presumptive identification

  Gram-negative diplococci Oxidase-positive
  Graying non-hemolytic growth on blood agar

- Additional tests for definitive identification

  β-galactosidase-positive

- Notes

Neisseria gonorrhoeae

- Presumptive identification

  Gram-negative diplococci Oxidase-positive
  Strongly catalase-positive with 30% H_2O_2

- Additional tests for definitive identification

  β-galactosidase-negative AND
  γ-glutamyl-aminopeptidase-negative

- Notes

  Growth on *N. gonorrhoeae*-selective medium OR
  No growth on Mueller-Hinton or tryptic soy media

Moraxella catarrhalis

- Presumptive identification

  Gram-negative diplococci — Oxidase-positive
  Hockey puck on blood agar — Catalase-positive

- Additional tests for definitive identification

  Butyrate esterase-positive OR
  Indoxyl acetate-positive

- Notes

  Broth microdilution and disk diffusion susceptibility testing guidelines described in M45-A2 (Mueller-Hinton media)

Moraxella catarrhalis

- Presumptive identification

  Gram-negative diplococci — Oxidase-positive
  Hockey puck on blood agar — Catalase-positive

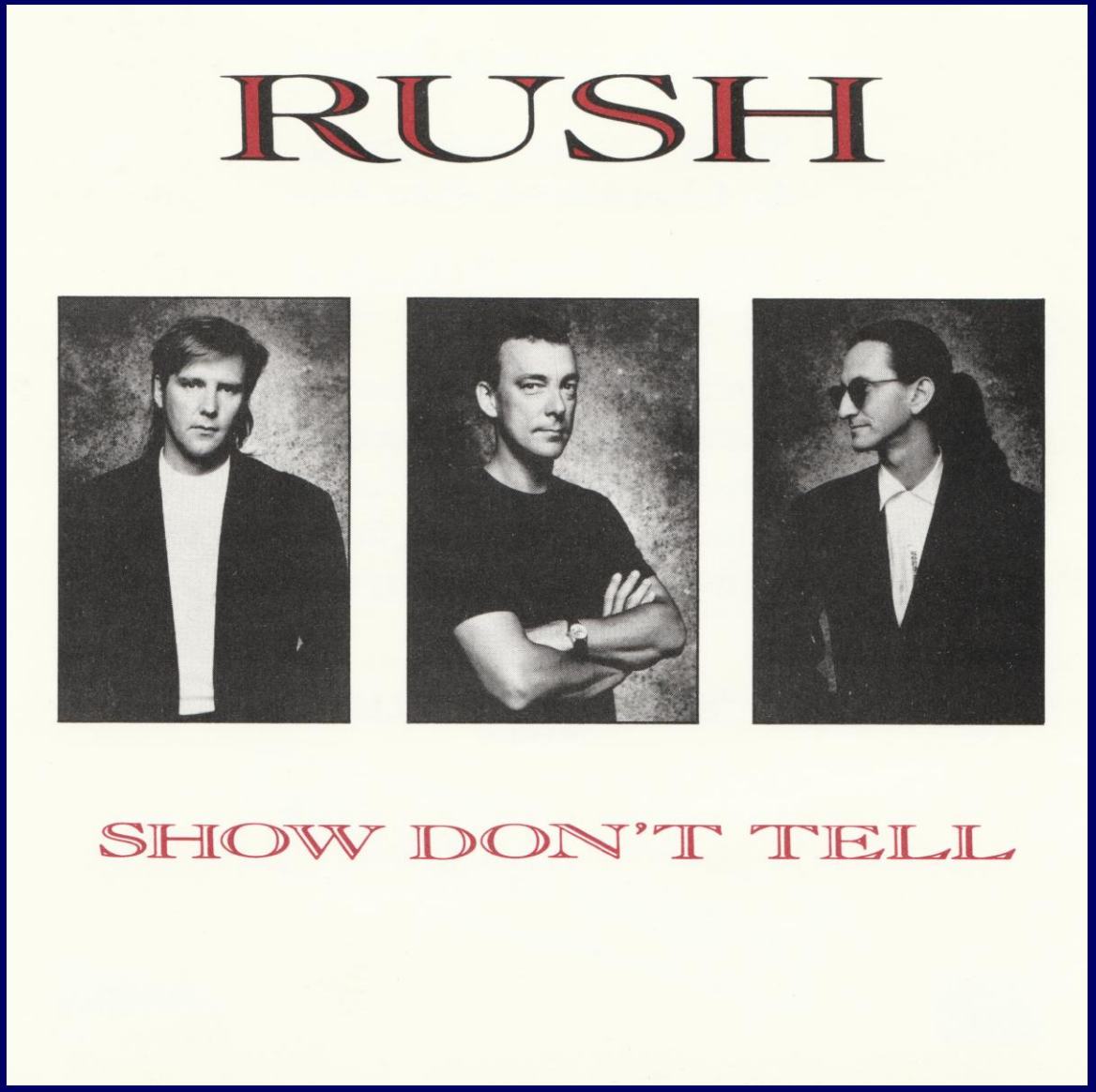
- Additional tests for definitive identification

  Butyrate esterase-positive
  Indoxyl acetate-positive

- Not

LIMITATIONS/additional factors

Most other *Moraxella* spp. are positive for butyrate esterase, but are coccobacilli rather than diplococci


RUSH
SHOW DON'T TELL

GRAM-NEGATIVE COCCOBACILLI

Genus	Growth Rate	Blood agar	Chocolate agar	MacConkey agar	Fun Fact(s)
Haemophilus	overnight	no growth	growth	no growth	M35-A2
Pasteurella	overnight	growth	growth	no growth	sick TSI, penicillin
Gardnerella	overnight	growth	growth	no growth	teeny tiny
Brucella (nasty one)	two days	growth	growth	no growth	BSC, rule-out
Francisella	two days	no growth*	growth	no growth	BSC, rule-out
Acinetobacter	overnight	growth	growth	growth	non-motile, K/NC

GRAM-NEGATIVE COCCOBACILLI

Genus	Growth Rate	Blood agar	Chocolate agar	MacConkey agar	Fun Fact(s)
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Brucella (nasty one)	two days	growth	growth	no growth	BSC, rule-out
Francisella	two days	no growth*	growth	no growth	BSC, rule-out
Acinetobacter	overnight	growth	growth	growth	non-motile, K/NC
HACEKs	overnight	WE	WILL	SEE	SOON

Cardiobacterium hominis

- Presumptive identification

  Pleomorphic, thin GNR
  No growth on MacConkey
  Oxidase-positive
  Catalase-negative

- Additional tests for definitive identification

  Indole-positive
  Non-hemolytic on blood agar; may pit

- Notes

  Isolate must be derived from blood culture
  Confirm with negative nitrate test
  CLSI M45; CAMHB with LHB for all of the ACEKs

Cardiobacterium hominis

- Presumptive identification

  Pleomorphic, thin GNR
  No growth on MacConkey
  Oxidase-positive
  Catalase-negative

- Additional tests for definitive identification

  Indole-positive
  Non-hemolytic on blood agar; may pit

- [illegible]

LIMITATIONS/additional factors

Pasteurella bettyae has same rapid biochemical profile as *C. hominis*, but is not known to cause endocarditis

Rosette-forming, thin GNR (with above biochemicals) in adult blood cultures

Eikenella corrodens

- Presumptive identification

  Small GNR; pits blood, chocolate Oxidase-positive
  No growth on MacConkey Catalase-negative

- Additional tests for definitive identification

  Indole-negative Distinct odor of bleach
  Non-hemolytic on blood agar

- Notes

  Capnophilic
  The only MacConkey-negative, catalase-negative, oxidase-positive GNR that is ornithine-positive

Kingella kingae

- Presumptive identification

  Gram-negative coccoid bacillus — Oxidase-positive
  No growth on MacConkey — Catalase-negative

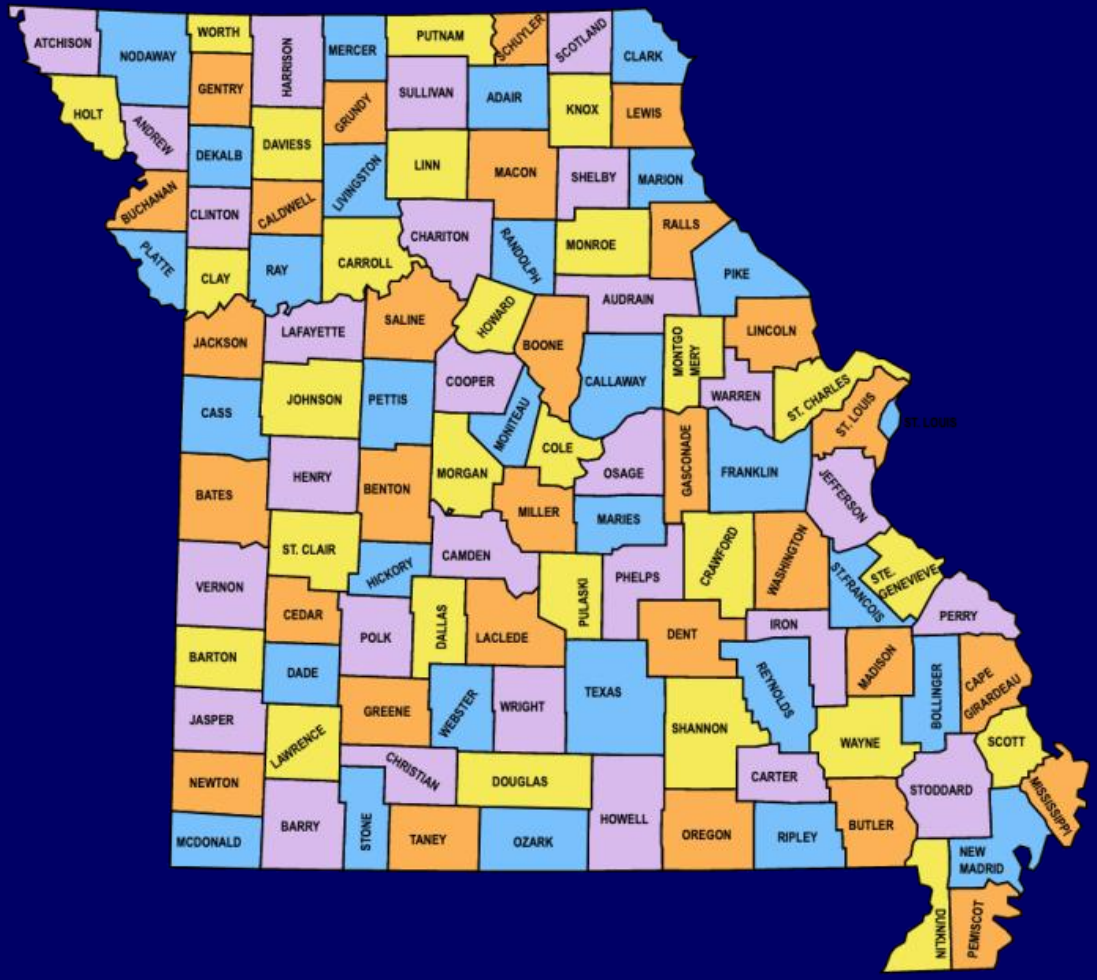
- Additional tests for definitive identification

  Hemolytic colonies on blood agar

- Notes

  Joint fluids, blood cultures, other sterile body sites
  Early growth confused for β-hemolytic streptococci

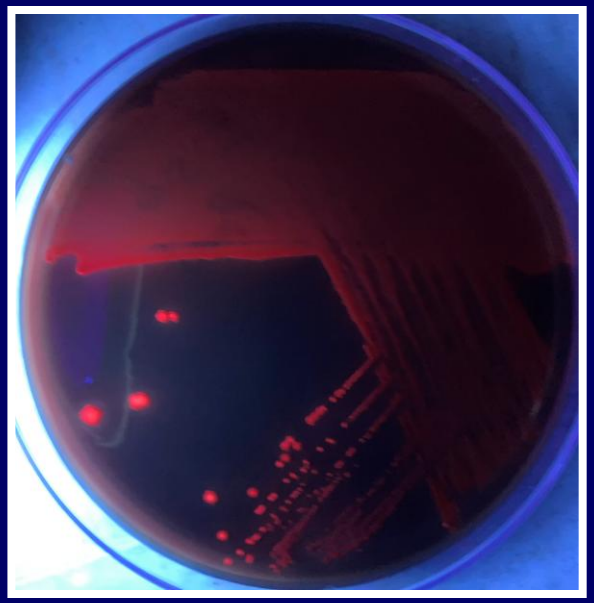
MISSOURI


ATCHISON
NODAWAY
WORTH
HARRISON
MERCER
PUTNAM
SCHUYLER
SCOTLAND
CLARK
HOLT
ANDREW
GENTRY
GRUNDY
SULLIVAN
ADAIR
KNOX
LEWIS
DEKALB
DAVIESS
LIVINGSTON
LINN
MACON
SHELBY
MARION
BUCHANAN
CLINTON
CALDWELL
CHARITON
RANDOLPH
MONROE
RALLS
PLATTE
CLAY
RAY
CARROLL
AUDRAIN
PIKE
JACKSON
LAFAYETTE
SALINE
HOWARD
BOONE
LINCOLN
COOPER
CALLAWAY
MONTGOMERY
WARREN
ST. CHARLES
ST. LOUIS
ST. LOUIS
CASS
JOHNSON
PETTIS
MONITEAU
COLE
OSAGE
GASCONADE
FRANKLIN
JEFFERSON
BATES
HENRY
BENTON
MORGAN
MILLER
MARIES
ST. CLAIR
CAMDEN
CRAWFORD
WASHINGTON
VERNON
HICKORY
PULASKI
PHELPS
ST. FRANCOIS
STE. GENEVIEVE
PERRY
CEDAR
POLK
DALLAS
LACLEDE
DENT
IRON
BARTON
DADE
MADISON
BOLLINGER
CAPE GIRARDEAU
JASPER
GREENE
WEBSTER
WRIGHT
TEXAS
REYNOLDS
SHANNON
WAYNE
SCOTT
LAWRENCE
CHRISTIAN
CARTER
NEWTON
DOUGLAS
HOWELL
STODDARD
MISSISSIPPI
BARRY
STONE
TANEY
OZARK
OREGON
RIPLEY
BUTLER
MCDONALD
NEW MADRID
DUNKLIN
PEMISCOT

ANAEROBIC GRAM-NEGATIVES

Table 4. Abbreviated Identification of Anaerobic Gram-Negative Bacteria

Identification	aBAP/LKV Colony Morphology	Cell Morphology	Bacteroides Bile Esculin Colony Morphology	Indole Reaction
Bacteroides fragilis group	Large, convex	Regular	Large, convex, gray-black	Not Done
Bacteroides ureolyticus	Translucent, pitting the agar (catalase-negative with 15% H_2O_2)	Tiny rods, coccobacilli	No growth	Negative
Bilophila wadsworthia	Tiny, translucent (catalase +++ with 15% H_2O_2)	Regular to filaments	Translucent with black center at 72 hours	Negative
Fusobacterium nucleatum	Opalescent, breadcrumb	Fusiform, thin pointed	No growth	Positive
Porphyromonas spp.	Small, translucent or opaque, fluoresce brick-red on aBAP	Tiny coccobacilli	No growth	Positive
Prevotella intermedia	Small, translucent or opaque, fluoresce brick-red on LKV or aBAP	Tiny coccobacilli	No growth	Positive
Prevotella spp.				Negative
Veillonella spp.	Small, transparent or opaque, fluoresce brick-red on aBAP	Tiny diplococci	No growth	Negative



CLSI M35-A2; 2008

ANAEROBIC GRAM-POSITIVES

Table 5. Abbreviated Identification of Anaerobic Gram-Positive Bacteria

Identification	aBAP Colony Morphology	Cell Morphology	Indole Reaction
C. difficile	Large, flat colonies; barnyard (cow manure) smell; chartreuse fluorescence	Thin rods, rare spores	Negative
C. perfringens	Large, irregular-shaped, double zone beta-hemolysis	Boxcar, large, square rods	Not done
C. septicum	Smoothly swarming	Thin rods, subterminal spores	Negative
C. sordellii	Very large, lobate, irregular, flat	Thin rods, subterminal spores	Positive
C. tetani	Smoothly swarming but slow growing	Swollen terminal spores	Positive
Peptostreptococcus spp.	Small, peaked, circular	Cocci, pairs and chains	Not done
Propionibacterium acnes	Small, opaque, enamel-white, circular (catalase+ with 15% H_2O_2)	Coryneform rods	Positive

CLSI M35-A2; 2008


Miller Park
TOYOTA
Fisher Nuts
Froedtert & Medical College of Wisconsin
An Academic Medical Center
Roll out the barrel
We'll have a barrel of fun
Roll out the barrel
We've got the blues on the run
We're working to make the Ballpark as GREEN as the field.
Johnson Controls
brewers.com
where baseball is always on
GREAT TASTE.
Miller Lite
LESS FILLING.
M&I BANK


BEERS ABOUT NOTHING
INNOVATION SERIES
ZING, BOOM, TARARREL
DARK LAGER
MALTY - TOASTY - CRISP

CLSI M35-A2, BIOTHREAT AGENTS

- Changes in recommendations for handling cultures on the basis of unsuspected exposure to agents of bioterrorism
- Colonial growth examined in biological safety cabinet (while wearing gloves) until agents of bioterrorism or highly-pathogenic agents ruled out

  Blood cultures
  CSF cultures
  Lymph node cultures

CLSI M35-A2; 2008

MORE…

- Unidentified Gram-negative, Gram-variable bacillus, or Gram-negative coccobacillus that only grows on blood and chocolate agar only (not MacConkey) is handled with extreme caution until rule-out

- Gram staining and wet mount preparation takes place in biological safety cabinet; wear gloves

CLSI M35-A2; 2008

THIS IS SERIOUS

- Procedures known to create aerosols (catalase, others) should be confined to biological safety cabinet with extra care or avoided all together until rule-out
- Automated systems may pose danger with respect to these organisms; may not generate accurate identification or susceptibility data in the first place

Should not be performed until bioterrorism agent possibility has been eliminated

J Hosp Infect 22:159-162; 1992

Diagn Microbiol Infect Dis 60:241-246; 2008

Brucella spp.

- Presumptive identification

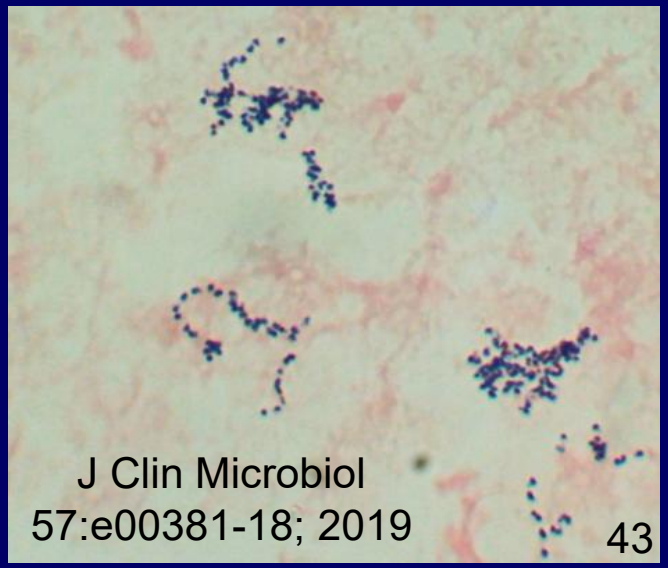
  Tiny Gram-negative coccobacillus Oxidase-positive
  No growth on MacConkey Catalase-positive

- Additional tests for definitive identification (R/O)

  Urease-positive Indole-negative
  Non-hemolytic on blood agar

- Notes

  Phenylalanine-negative
  Sterile tissues and fluids
  Growth on CNA agar



J Clin Microbiol
57:e00381-18; 2019

Francisella tularensis

- Presumptive identification

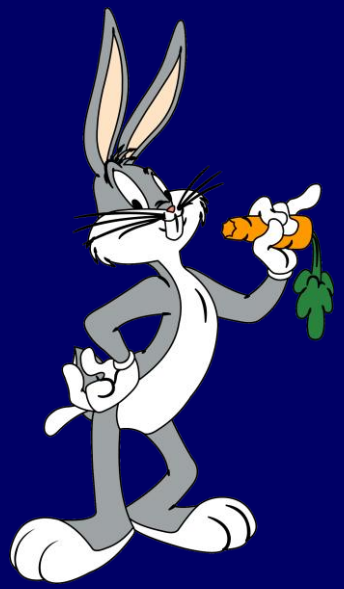
  Tiny GNR or coccobacillus (sand) Oxidase-negative
  Slow on chocolate; no satellite Catalase-neg/wk

- Additional tests for definitive identification (R/O)

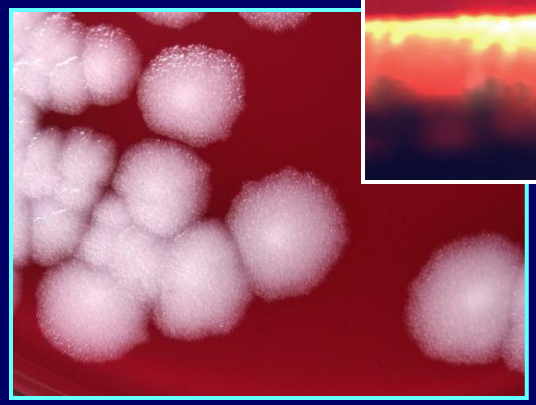
  β-lactamase-positive

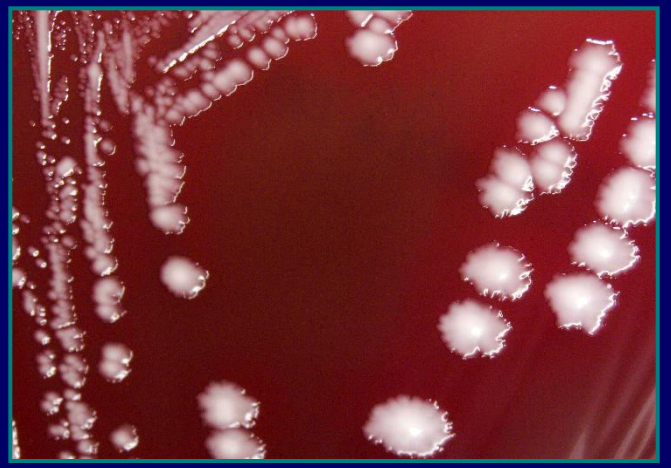
- Notes

  L-cysteine
  On the rise?



STAY TUNED





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