Overview of CLSI Document M35-A2 For Bench-level Identification of Clinically-significant Microorganisms

Erik Munson
Clinical Microbiology
Wheaton Franciscan Laboratory
Milwaukee, Wisconsin

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

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OUTLINE

I. Introduction of concept

II. Major players

III. Application one
   A. CLSI M35-A2
   B. Perhaps why you are here

IV. Application two
   A. Routine (but possibly covert) bench dealings
   B. Help out WSLH

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Introduction

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INTRODUCTION TO CLSI M35-A2

- Goes way back to the introduction of commercial identification systems

  Laboratories lacking confidence, resources in validation of alternative methods

- Utilization of these methods has resulted in greater standardization and more accuracy
- In some cases, may have resulted in cost and turnaround time increases

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INTRODUCTION TO CLSI M35-A2

- Laboratorians have used rapidly-determined characteristics for years; this document seeks to standardize this

  Odor
  Immediate enzymatic reactions (spot testing)

- Cost savings associated with using rapid methods or overall patient care benefits

Chromogenic medium	Slide	Fluorogenic
Agglutination	Spot	Enzymatic
Macroscopic, microscopic	Disk	Single-tube

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BENEFITS OF RAPID RESULTS

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
[4]Role of rapid susceptibility testing also factored into this calculation
[5]Not significant
[6]Not determined

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WARNING

- Laboratory directors, managers, and supervisors responsible for ensuring appropriate use of rapid methods

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

CLSI M35-A2; 2008

- Competency assessment
  - Colony, Gram stain
  - Smell (when safe)

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CAVEATS

- Isolates conforming to reactions described in M35-A2 identify organism with >95% accuracy; identification can be reported without qualification
- "Confirmation by additional procedures is unnecessary for many of the species described in this document."

CLSI M35-A2; 2008

- Lack of a positive result does not rule out the identification of an isolate; just signifies need for additional testing

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MORE WARNINGS

- Employ standard precautions
  - Cover transmission of all infectious agents
  - Universal precautions cover blood-borne pathogens
  - Refer to CLSI M29
- Sniffing/wafting <u>can</u> be dangerous
  - Once mold colony is ruled out, opening of plates from non-invasive sources (i.e., urine, sputum) is common & relatively safe
  - *P. aeruginosa*, *H. influenzae*, *Eikenella*, *S. anginosus* group odor can be detected by opened plates "not sniffed purposely"

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EXCLUSIONS????

- Rapid results may need to be validated
  - Certain microbes from normally-sterile sites
  - Potential agents of bioterrorism
  - Microbes important to infection control practitioners
  - Microbes implicated in nosocomial outbreaks
- Risk (being wrong) vs. benefit (patient care/safety)
  - M35-A eliminated certain organism groupings
  - However, possibility of serious pathogen or ability to rule out potential agent of bioterrorism has resulted in M35-A2 including additional organisms

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Major Players

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CLSI M35-A2 PROTOCOLS

- Test descriptions
  - Principle
  - Reagents
  - Procedure
  - Interpretation
  - Limitations (precautions)
  - Quality control
- Thirteen reagents/tests
  - Bile solubility
  - Rapid spot CAMP
  - Catalase (aerobes)
  - Catalase (anaerobes)
  - Germ tube
  - Rapid hippurate hydrolysis
  - Spot indole
  - Indoxyl acetate
  - MUG test
  - Spot oxidase
  - δ-aminolevulinic acid
  - Rapid trehalose
  - Urea/phenylalanine deaminase

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CATALASE

- 3% hydrogen peroxide
- Touch center of isolated colony with stick
  - Transfer to clean glass slide
  - Place drop of hydrogen peroxide onto cell paste
- "Immediate" bubbling (< 20 seconds) is positive
- No non-platinum loops; don't grab blood agar
- If slow-growing isolate on blood agar (no growth MacConkey) or small GNR, perform test in biological safety cabinet

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SPOT INDOLE

- 5% (w/v) *p*-dimethylaminobenzaldehyde
  1% paradimethylaminocinnamaldehyde in HCl
- Moisten piece of filter paper with reagent
  - Rub portion of CFU from blood agar onto paper
  - Growth medium must contain sufficient tryptophan
- Pigment formation (< 20 seconds) is positive
- Cannot use media containing dyes
  Cannot use Mueller-Hinton or high-glucose agar
  Detectable indole diffuses to adjacent colonies within 5 mm (false-positive results)

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SPOT OXIDASE

- 1% tetramethyl-*p*-phenylenediamine dihydrochloride
- Moisten piece of filter paper with reagent
  - Rub portion of CFU from blood agar onto paper
  - Use wooden stick or platinum bacteriological loop
- Blue/purple pigment (< 10 seconds) is positive
- Cannot use MacConkey or other purple agar
  Nickel-based alloy wires containing chromium or iron may yield false-positive results upon organism transfer

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UREASE TUBE

- 20 g/L urea
- Inoculate with large loopful of growth;
  Incubate 35˚ C ambient air for two hours
- Deep pink color of broth indicates urease-positive organisms; timing of reaction is important
- Positive reactions can be followed up with by phenylalanine deaminase testing

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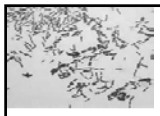
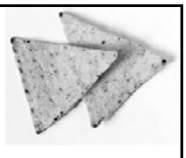
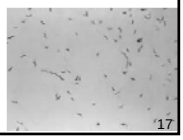




M35-A2 Rapid Identification



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Brucella spp.

- Presumptive identification
  - Tiny Gram-negative coccobacillus — Oxidase-positive
  - No growth on MacConkey — Catalase-positive
- Additional tests for definitive identification
  - Urease-positive (quick with disk) — Indole-negative
  - Non-hemolytic on blood agar
- Notes
  - Highly infectious; work in biological safety cabinet
  - Sterile tissues and fluids
  - Refer to Laboratory Response Network laboratory

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Brucella spp.

- Presumptive identification

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

- Additional tests for definitive identification

  Urease-positive (quick with disk) — Indole-negative
  Non-hemolytic on blood agar

- Notes

LIMITATIONS/additional factors

Oligella ureolytica, Bordetella bronchiseptica, and *Haemophilus influenzae* can resemble *Brucella* spp.

Brucella spp. are phenylalanine deaminase-negative

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Campylobacter jejuni/coli

- Presumptive identification

  Gram-negative bacilli (gull wing) — Oxidase-positive
  Darting motility — Catalase-positive

- Additional tests for definitive identification

  Hippurate-positive — *Campylobacter jejuni*
  Indoxyl acetate-positive — *Campylobacter jejuni/coli*

- Notes

  Isolated colonies on *Campylobacter*-selective medium incubated in microaerophilic 42° C

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Cardiobacterium hominis

- Presumptive identification

  Pleomorphic, thin GNR — Oxidase-positive
  No growth on MacConkey — 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

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Cardiobacterium hominis

- Presumptive identification

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

- Additional tests for definitive identification

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

- Notes

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

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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

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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

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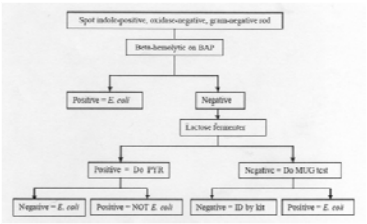
Escherichia coli



CLSI M35-A2; 2008

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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

LIMITATIONS/additional factors

Occasional *Shigella* spp. can be indole-positive and MUG-positive; therefore, no rapid identification of *E. coli* from blood, fecal, GI sources

Positive MUG test may be preliminary rule-out test for O157:H7

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Francisella tularensis

- Presumptive identification

  Tiny GNR or coccobacillus (sand) — Oxidase-negative
  Growth on chocolate (48-72h) — Catalase-neg/wk

- Additional tests for definitive identification

  β-lactamase-positive

- Notes

  Highly infectious; work in biological safety cabinet
  No satellitism on blood agar around *Staph* streak
  Refer to Laboratory Response Network laboratory

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Haemophilus influenzae

- Presumptive identification

  Gram-negative coccobacillus
  Good growth on chocolate; not blood, MacConkey

- Additional tests for definitive identification

  δ-aminolevulinic acid-negative

- Notes

  Satellitism on blood agar around *Staphylococcus* streak separates *Haemophilus* from *Brucella* and *Francisella*

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Haemophilus influenzae

- Presumptive identification

  Gram-negative coccobacillus
  Good growth on chocolate; not blood, MacConkey

- Additional tests for definitive identification

  δ-aminolevulinic acid-negative

- Notes

LIMITATIONS/additional factors

Rapid identification algorithm applies to CSF and respiratory isolates

Haemophilus haemolyticus cannot be differentiated from *H. influenzae*, with exception of hemolysis characterization on horse blood agar

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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

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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

- Notes

  Broth microdilution and disk diffusion susceptibility testing guidelines described in M45-A2

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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

- Notes

LIMITATIONS/additional factors

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

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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

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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

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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
  γ-gluatmyl-aminopeptidase-negative

- Notes

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

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Proteus mirabilis

- Presumptive identification

  Spreading colony — Indole-negative

- Additional tests for definitive identification

  None, if susceptible to ampicillin

- Notes (if resistant to ampicillin)

Proteus spp.	Ornithine decarboxylase	Maltose fermentation
P. mirabilis	positive	negative
P. penneri	negative	positive

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Proteus mirabilis

- Presumptive identification

  Spreading colony Indole-negative

- Additional tests for definitive identification

  None, if susceptible to ampicillin

- Notes (if resistant to ampicillin)

LIMITATIONS/additional factors

Instead of pursuing maltose or ornithine decarboxylase testing, one could report ampicillin-resistant (indole-negative) Proteae as "indole-negative *Proteus*" or *P. mirabilis*/*P. penneri*

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Proteus vulgaris

- Presumptive identification

  Spreading colony Indole-positive

- Additional tests for definitive identification

- Notes

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Pseudomonas aeruginosa

- Presumptive identification

  Indole-negative Oxidase-positive
  Metallic/pearlescent, rough, pigmented, mucoid

- Additional tests for definitive identification

  Grape-like odor; corn tortilla

- Notes

  Most often strongly β-hemolytic on blood agar

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Pseudomonas aeruginosa

- Presumptive identification

  Indole-negative Oxidase-positive
  Metallic/pearlescent, rough, pigmented, mucoid

- Additional tests for definitive identification

  Grape-like odor; corn tortilla

- Notes

LIMITATIONS/additional factors

Rare *Aeromonas* spp. may resemble *P. aeruginosa*, but can be differentiated by positive spot indole test

Burkholderia cepacia isolates from CF patients can resemble *P. aeruginosa*

40

Staphylococcus aureus

- Presumptive identification

  Catalase-positive
  Tube/slide coagulase- or latex agglutination-positive

- Additional tests for definitive identification

  Typically β-hemolytic colonies on blood agar

- Notes

  Tube coagulase required if non-hemolytic isolates from urine

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Staphylococcus aureus

- Presumptive identification

  Catalase-positive
  Tube/slide coagulase- or latex agglutination-positive

- Additional tests for definitive identification

  Typically β-hemolytic colonies on blood agar

- Notes

LIMITATIONS/additional factors

S. schleiferi and *S. lugdunensis* may be slide coagulase-positive (clumpy, rather than complete agglutination); aforementioned species PYR-positive

S. saprophyticus may yield positive latex agglutination results

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Staphylococcus lugdunensis

- Presumptive identification

  Gram-positive cocci — Catalase-positive
  Tube coagulase-negative

- Additional tests for definitive identification

  PYR-positive (deep ruby red);
  Polymyxin B-resistant; ornithine-positive

- Notes

  May be slide coagulase-positive or clumpy

43

Enterococcus spp.

- Presumptive identification

  GPC prs, chains (no clusters) — Catalase-negative
  Non-β-hemolytic on blood agar (>1 mm diameter)

- Additional tests for definitive identification

  PYR-positive

- Notes

  Demonstrate an LAP-positive reaction with
  α-hemolytic *Enterococcus* spp. (improved specificity)

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Enterococcus spp.

- Presumptive identification

  GPC prs, chains (no clusters) — Catalase-negative
  Non-β-hemolytic on blood agar (>1 mm diameter)

- Additional tests for definitive identification

  PYR-positive

- Notes

LIMITATIONS/additional factors

Lactococcus garvieae may be misidentified as *Enterococcus* spp.

45

Aerococcus viridans

- Presumptive identification

  GPC (tetrads, clusters) — Catalase-negative
  α-hemolytic

- Additional tests for definitive identification

  PYR-positive
  LAP-negative

- Notes

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Listeria monocytogenes

- Presumptive identification

  Small Gram-positive bacillus — Catalase-positive
  Usually tumbling motility — Small β-hemolysis

- Additional tests for definitive identification

  Hippurate-positive

- Notes

  Isolate should be from blood culture or CSF culture
  Listeria can be non-motile after 35-37˚ C incubation

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Listeria monocytogenes

- Presumptive identification

  Small Gram-positive bacillus — Catalase-positive
  Usually tumbling motility — Small β-hemolysis

- Additional tests for definitive identification

  Hippurate-positive

- Notes

LIMITATIONS/additional factors

Presence of catalase is not a required factor for identification in an otherwise typical isolate

Species confirmation not necessary; 95% of clinical isolates *monocytogenes*

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Streptococcus agalactiae

- Presumptive identification
  - GPC (pairs, chains) Catalase-negative
  - Small zone of β-hemolysis on blood agar
- Additional tests for definitive identification
  - Hippurate-positive OR CAMP-positive
  - OR Lancefield group B via latex agglutination
- Notes
  - Hippurate method not to be used on non-hemolytic colonies

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Streptococcus agalactiae

- Presumptive identification
  - GPC (pairs, chains) Catalase-negative
  - Small zone of β-hemolysis on blood agar
- Additional tests for definitive identification
  - Hippurate-positive OR CAMP-positive
  - OR Lancefield group B via latex agglutination
- Notes

LIMITATIONS/additional factors

β-hemolytic *Enterococcus* spp. can be hippurate-positive

Many viridans group streptococci and non-hemolytic *S. agalactiae* are hippurate-positive

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Streptococcus anginosus group

- Presumptive identification
  - GPC (pairs, chains) Catalase-negative
  - CFU (<0.5 mm diameter); variable hemolysis
- Additional tests for definitive identification
  - Odor of butterscotch or vanilla OR
  - Lancefield group F by latex agglutination
- Notes
  - May be Lancefield group A, C, F, or G by latex agglutination

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Streptococcus pneumoniae

- Presumptive identification
  - GPC (lancet-shaped in pairs) Catalase-negative
  - α-hemolysis on blood agar
- Additional tests for definitive identification
  - Bile solubility-positive
- Notes
  - ~1% of *S. pneumoniae* with typical colony morphology may not be bile soluble

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Streptococcus pyogenes

- Presumptive identification
  - GPC (pairs, chains) Catalase-negative
  - CFU (>0.5 mm diameter); sharp β-hemolysis
- Additional tests for definitive identification
  - PYR-positive OR
  - Lancefield group A by latex agglutination
- Notes
  - Careful observation of size and hemolysis, as *Enterococcus* spp. can demonstrate β-hemolysis

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Viridans group *Streptococcus*

- Presumptive identification
  - GPC (pairs, chains) Catalase-negative
  - Non-hemolytic or α-hemolysis
- Additional tests for definitive identification
  - PYR-positive LAP-positive
  - Bile solubility-negative if α-hemolytic
- Notes
  - *Aerococcus urinae* are cocci in clusters/tetrads
  - *Pediococcus* spp. are cocci in clusters/tetrads

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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*

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Candida glabrata

- Presumptive identification

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

- Additional tests for definitive identification

  Better growth on EMB agar than blood agar
  Rapid trehalose-positive at 42° C

- Notes

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Cryptococcus neoformans

- Presumptive identification

  Spherical pleomorphic budding yeast with no hyphae

- Additional tests for definitive identification

  Urease-positive
  Phenol oxidase-positive

- Notes

  Cannot differentiate from *C. gattii*

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ANAEROBIC GRAM-NEGATIVES

Table 4. Abbreviated Identification of Anaerobic Gram-Negative Bacteria

Identification	sBAP/LKV Colony Morphology	Cell Morphology	Bacteroides, Rile-Escutia 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, broad-crumb	Fusiform, thin pointed	No growth	Positive
Porphyromonas spp.	Small, translucent or opaque, fluoresce brick-red on sBAP	Tiny coccobacilli	No growth	Positive
Prevotella intermedia	Small, translucent or opaque, fluoresce brick-red on LKV or sBAP	Tiny coccobacilli	No growth	Positive
Prevotella spp.				Negative
Veillonella spp.	Small, transparent or opaque, fluoresce bricked on sBAP	Tiny diplococci	No growth	Negative

CLSI M35-A2; 2008

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ANAEROBIC GRAM-POSITIVES

Table 5. Abbreviated Identification of Anaerobic Gram-Positive Bacteria

Identification	sBAP Colony Morphology	Cell Morphology	Indole Reaction
C. difficile	Large, flat colonies, bunyond (cow manure) smell; chartreuse fluorescence	Thin rods, rare spores	Negative
C. perfringens	Large, irregular-edged, 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)	Corynetform rods	Positive

CLSI M35-A2; 2008

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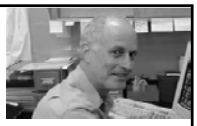

A Second Application

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CLSI M35-A2 AND BIOTERRORISM

- 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

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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

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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

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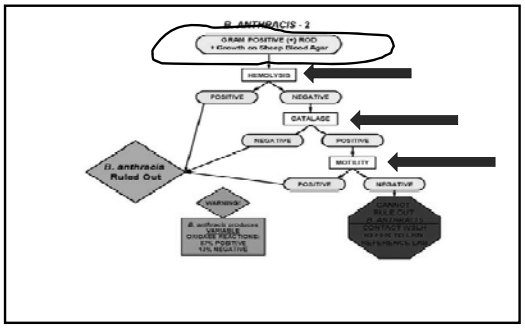
ONE RULE-OUT ALGORITHM



Wisconsin State Laboratory of Hygiene Bench Guide

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VITAL STATISTICS--2009 EXERCISE

- Two samples sent to 116 Wisconsin laboratories

  101 (87%) reported data
  15 did not report results

- Sample BPE 09-2-1 *Bacillus megaterium*
  Sample BPE 09-2-2 *Bacillus licheniformis*
- Samples intended to simulate *Bacillus anthracis* in the context of rule-out testing

Wisconsin State Laboratory of Hygiene Audioconference; 120909

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RULE-OUT EXERCISE--I

Test	Sample 1: Expected Result	% Correct (Reporting Labs)	Sample 2: Expected Result	% Correct (Reporting Labs)
α-hemolysis	Negative	89.4% (85)	Negative	96.3% (82)
β-hemolysis	Negative	98.9% (87)	Positive	38.4% (86)
Catalase	Positive	97.8% (89)	Positive	91.0% (89)
Indole	Negative	100% (42)	Negative	100% (44)
Oxidase	Negative	85.2% (54)	Negative	64.8% (54)
Urease	Positive	53.8% (13)	Negative	76.9% (13)
Motility	Negative	98.1% (54)	Positive	94.3% (53)
Growth on Mac/EMB	Negative	90.9% (77)	Negative	98.7% (77)

Wisconsin State Laboratory of Hygiene; Fall 2009

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RULE-OUT EXERCISE--II

Test	Sample 1: Expected Result	% Correct (Reporting Labs)	Sample 2: Expected Result	% Correct (Reporting Labs)
α-hemolysis	Negative	89.4% (85)	Negative	96.3% (82)
β-hemolysis	Negative	98.9% (87)	Positive	38.4% (86)
Catalase	Positive	97.8% (89)	Positive	91.0% (89)
Indole	Negative	100% (42)	Negative	100% (44)
Oxidase	Negative	85.2% (54)	Negative	64.8% (54)
Urease	Positive	53.8% (13)	Negative	76.9% (13)
Motility	Negative	98.1% (54)	Positive	94.3% (53)
Growth on Mac/EMB	Negative	90.9% (77)	Negative	98.7% (77)

Wisconsin State Laboratory of Hygiene; Fall 2009

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RULE-OUT EXERCISE--III

Test	Sample 1: Expected Result	% Correct (Reporting Labs)	Sample 2: Expected Result	% Correct (Reporting Labs)
α-hemolysis	Negative	89.4% (85)	Negative	96.3% (82)
β-hemolysis	Negative	98.9% (87)	Positive	38.4% (86)
Catalase	Positive	97.8% (89)	Positive	91.0% (89)
Indole	Negative	100% (42)	Negative	100% (44)
Oxidase	Negative	85.2% (54)	Negative	64.8% (54)
Urease	Positive	53.8% (13)	Negative	76.9% (13)
Motility	Negative	98.1% (54)	Positive	94.3% (53)
Growth on Mac/EMB	Negative	90.9% (77)	Negative	98.7% (77)

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THE END

- Rapid identification algorithms can benefit both the laboratory and patient care
- Requires sufficient knowledge base in terms of knowing principles and limitations
- Can (importantly) be applied to rule-out situations
- Use an algorithm-based approach for bioterrorism rule-out exercises

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