**PURPOSE:** To provide a workflow and identification scheme for Gram-negative bacilli from clinical specimens.

## IDENTIFICATION OF ANAEROBIC GRAM-NEGATIVE BACILLI:

- Refer anaerobic organism(s) to DynaLIFE for identification and susceptibility testing if required.

**Some extra clues:**

| Identification | Brucella: Colonial morphology | Cell morphology | Indole |
|---|---|---|---|
| *Bacteroides fragilis* group | Large, convex | Regular | Not done |
| *Campylobacter ureolyticus* | Translucent, smooth, convex pinpoint (1mm), pitting or spreading (5mm); all colony types can occur in the same culture. | Slender rods/ coccobacilli | - |
| *Bilophila wadsworthia* | Tiny, translucent | Regular to filaments | - |
| *Fusobacterium nucleatum* | Opalescent, breadcrumb | Fusiform, thin pointed | + |
| *Porphyromonas* species | Small, translucent or opaque, fluoresce brick-red on BRU | Tiny coccobacilli | + |
| *Prevotella intermedia* | Small, translucent or opaque, fluoresce brick-red on BRU | Tiny coccobacilli | + |
| *Prevotella* species | Small, translucent or opaque, fluoresce brick-red on BRU | Tiny coccobacilli | - |

***Campylobacter ureolyticus*:**

- Formerly called *Bacteroides ureolyticus,* and before that, *Bacteroides corrodens* (due to the ability to pit the agar). Associated with polymicrobial oral and perianal abscesses. May be associated with enteric illness.

## IDENTIFICATION OF AEROBIC GRAM-NEGATIVE BACILLI:

| Non-fastidious, growth on MacConkey | Perform as required: |
|---|---|
| Oxidase negative, lactose fermenter<br>**PYR:** *Citrobacter* positive<br>*Salmonella* negative<br>*E.coli* negative | • Vitek 2 GN card, Vitek 2 AST-GN card.<br>• If no ID or for confirmation perform API 20E.<br>• If still no ID, refer to DynaLIFE. |
| Oxidase negative, lactose non-fermenter | • Vitek 2 GN card, AST-GN card.<br>• If no ID or for confirmation perform API 20 E.<br>• If still no ID, refer to DynaLIFE. |
| Oxidase positive, lactose non-fermenter<br>• *Pseudomonas aeruginosa:*<br>Green pigment, characteristic odor<br>• Other isolates | • Vitek 2 GN card, Vitek 2 AST-GN card<br>• If no ID or for confirmation perform API 20 E.<br>• If still no ID, refer to DynaLIFE. |

**Vitek 2 GN cards** are intended for the identification of most clinically significant fermenting and non-fermenting Gram-negative bacilli.

**NOTE:** These panels also identify several highly pathogenic organisms including: *Brucella melitensis, Burholderia mallei, Burkholderia pseudomallei, Escherichia coli O157, Francisella tularensis, and Yersinia pestis.* Always use universal precautions.

***Escherichia coli:***

- If β-hemolytic on BA, non-swarming, indole positive, oxidase negative, this is *E. coli.*
- If non-hemolytic, lactose fermenting, indole positive, PYR negative, this is *E. coli.*

**NOTE:** Rapid identification tests for E.coli should not be used for the reportable identification except on chromogenic media.

**Suspect *Escherichia coli* O157:** Refer to MIC31900 – Stool Culture.

***Pasteurella multocida*:**

- Catalase positive, oxidase positive, motility negative, indole positive, no growth on MAC, urea negative. Gram: plump almost coccoid, bipolar staining, singly, pairs, short chains.

***Proteus mirabilis*:** Swarming, indole negative, ampicillin susceptible or resistant.

***Proteus vulgaris*:** Swarming, indole positive.

***Pseudomonas aeruginosa*:**

- Oxidase positive, typical smell (fruity / grapes), recognizable morphology (metallic or pearlescent, rough, pigmented or extremely mucoid), often strong β-hemolysis on Blood agar.

**Suspect *Salmonella*:**

- Refer to MIC31900 – Stool Culture.

***Sphingomonas paucimobilis:***

- Catalase positive, oxidase weak positive, motile at RT, indole negative, no growth on MAC, slow growth on BA. Older colonies positive for yellow (mustard) pigment. Gram: medium to long, straight or slightly curved.

***Vibrio* species:**

- Oxidase positive, glucose fermented.

| *Pseudomonas* and other genera | Oxidase | Growth on Mac | Growth at 42˚C | Motile | Indole production |
|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | + | + | + | + | |
| *Pseudomonas* spp. | + | + | - | + | |
| *Stenotrophomonas maltophilia* | - | + | Varies | + | |
| *Acinetobacter* spp. | - | Most + | Varies | - | |
| *Alcaligenes xylosoxidans* | + | + | 84% | + | - |
| *Aeromonas hydrophila* | + | + | + | + | + |
| *Plesiomonas shigelloides* | + | + | + | + | + |

**API NH tests** are a standardized system for the identification of *Neisseria*, *Haemophilus* (and related genera) and *Moraxella catarrhalis (Branhamella catarrhalis).*

**Some extra clues:**

| | Gram stain | Satellitism | ALA | Catalase | Oxidase |
|---|---|---|---|---|---|
| *Haemophilus influenzae* | Small coccobacilli | + | - | + | Varies |
| *Aggregatibacter aphrophilus* | Small coccobacilli | + | + | - | |
| *Francisella tularensis* | Tiny coccobacilli | Ng on BA | - | weak | - |
| *Brucella abortus* | Tiny coccobacilli | Ng on BA | n/a | + | + |

Refer to Primary Specimen Handling Flow Chart if Risk Group 3 organisms are suspected. All work and processing should be performed in BSC.

***Haemophilus influenzae* isolates from sterile sites must be sent to ProvLab immediately after identification is confirmed. Ensure there is a purity plate made that can be used for this purpose.**

***Haemophilus influenzae:***

- From blood and sterile sites, perform susceptibility testing as per ASTM and send to ProvLab for serotyping and NML for the International Circumpolar Surveillance Program.
- Requires X and V factors (*Aggregatibacter aphrophilus* requires V only).
- Can be confused with *Francisella tularensis* and *Brucella: Haemophilus* grows well on Chocolate agar at 24 hours, *Francisella* does not. Growth on Blood agar only around *Staphylococcus* separates *Haemophilus* from *Francisella* and *Brucella,* if necessary.

| HACEK organisms | Growth Mac | Oxidase | Catalase | Indole | Gram stain |
|---|---|---|---|---|---|
| *Aggregatibacter actinomycetemcomitans* | - | V | + | - | |
| *Capnocytophaga* | - | V | V | - | Slender almost filamentous. Curved, spindle, coccoid forms may be observed |
| *Cardiobacterium hominis* **NOTE:** isolate MUST be from blood culture | - | + | - | + | Pleo, thin, bulbous ends, occurring singly, in pairs, short chains or rosettes/clusters |
| *Eikenella corrodens* | - | + | - | - | Small slender straight |
| *Aggregatibacter aphrophilus* | - | V | - | - | |
| *Kingella denitrificans* | - | + | - | - | Short, coccoid |
| *Kingella kingae* | 65% - | + | - | - | Coccoid |

**Aggregatibacter actinomycetemcomitans:**

- Renamed Aggregatibacter actinomycetemcomitans.

***Eikenella corrodens:***

- Often first recognized on Chocolate agar where it tends to form flat spreading edges that extend out from the edge of the colonies. On Blood agar, colonies are non-hemolytic, pit or adhere to the agar, and give off a distinct odor of bleach when the plate is first opened.

***Haemophilus parainfluenzae:***

- Has been renamed *Aggregatibacter aphrophilus*.

***Kingella kingae:***

- Gram-negative coccoid rods, found in sterile tissues and fluids.
- Early growth can be confused with beta-hemolytic Streptococci, but Streptococci are not oxidase positive.

## PROCEDURE NOTES:

- If identification is problematic and the isolate is clinically significant, refer isolate to DynaLIFE for further identification and susceptibility testing (if required).
- Refer the following to DynaLIFE or ProvLab as applicable for further testing:
  - Unusual or uncommon isolates for confirmation.
  - *Haemophilus influenzae* from blood or sterile sites, for typing and serotyping. Report this isolate to Chief Medical Officer of Health and Infection Control Nurse if inpatient.
  - *Salmonella, Shigella, Escherichia coli* O157 and *Yersinia,* for confirmation and typing. Report these isolates to Chief Medical Officer of Health and Infection Control Nurse if inpatient.
  - Potential agents of bioterrorism: upon first suspicion of a possible *Francisella tularensis, Brucella* species, or *Yersinia pestis* all further handling and processing must take place in the Biological safety cabinet in the Microbiology Laboratory. Refer to the Primary Specimen Handling Flow Chart. Report these isolates to Chief Medical Officer of Health and Infection Control Nurse if inpatient.

## REFERENCES:


- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. 2015. Manual of Clinical Microbiology, 11th edition, ASM Press, Washington, D.C.
- Vitek 2 Systems product information
- CLSI. *Abbreviated Identification of Bacteria and Yeast; Approved Guideline—Second Edition.* CLSI document M35-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008


## REVISION HISTORY:

| REVISION | DATE | Description of Change | REQUESTED BY |
|---|---|---|---|
| 1.0 | | Initial Release | L. Steven |
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