PROCEDURE: **ORGANISM ID & AST**

I. Aerobic

A. Gram-Positive Cocci - *Refer to Appendix AP33 and Appendix AP34*

1. *Abiotrophia* species/ *Granuilcatella* species
   a. These are the nutritionally variant *Streptococci*. They usually satellite around a *Staphylococcus* streak. Gram stain should be performed on satellite colonies to rule out *Haemophilus* species
   b. If differentiation of genera is not achievable, report as *Abiotrophia/ Granuilcatella*

2. *Aerococcus* species
   a. *A. viridians* is alpha-hemolytic, LAP negative & PYR positive
   b. *A. urinae* is alpha-hemolytic, LAP positive & PYR negative
   c. Speciation performed by Vitek MS/ Vitek GP ID card
   d. Susceptibilities are not routinely performed on non-urine isolates.
      i. For isolates of *A urinae* from urine specimens add comment **&AERU**: "Isolates are typically susceptible to Beta-lactam antibiotics."

3. Alpha-Hemolytic/Non-hemolytic *Streptococcus* species (viridans group):
   a. Consists of *S.mitis* group, *S. anginosus* group (microaerophilic), *S. mutans* group, *S. salivarius* group, *S. bovis* group:
      i. Microaerophilic *Streptococci*
         a) Consists of *Streptococcus anginosus*, *Streptococcus constellatus*, and *Streptococcus intermedius*
         b) Identified group-wise by their atmospheric requirements
            1. Sub-culture suspected organism onto three BAP plates. Incubate one plate in ambient, another in CO2, and another in anaerobic conditions. If growth is observed on plates from only CO_2 and anaerobic condition, then this rules in microaerophilic *Streptococci*.
            2. Some strains may grow a little in ambient atmosphere
   b. LAP positive & PYR negative
   c. Speciation performed by Vitek MS/ Vitek GP ID card
   d. Susceptibility is performed by Kirby-Bauer disk diffusion method on Blood Mueller-Hinton
      i. Note: a penicillin E-test must be tested, since the penicillin disk method is unreliable
      ii. If unable to grow for susceptibilities add the isolate comment **&UNAB**:
         a) "Unable to perform susceptibility testing due to the atmospheric growth requirements of this organism."

4. *Streptococcus pneumoniae*
   a. Alpha-hemolytic, catalase negative
   b. Preliminary identification by optochin disk (PTAB) with a zone inhibition of ≥14mm
   c. Confirmation of identification by Vitek MS/Vitek GP ID
   d. Susceptibilities performed by MIC

5. Beta-Hemolytic *Streptococcus* species
   a. Speciation performed by strep grouping kit, including all sera types.
   b. Susceptibilities performed by Kirby-Bauer disk diffusion method on Blood Mueller Hinton agar, and incubated in CO_2 at 35^o

c. Susceptibilities are routinely performed on the following isolates:
   i. All Beta-Hemolytic *Streptococcus* isolated from sterile body sites
   ii. Beta-Hemolytic *Streptococcus* Group A and B isolated from wounds
   iii. Group A *Streptococcus* isolated from urine specimens
d. Susceptibilities are not routinely performed on Group B *Streptococcus* isolated from urine or genital specimens
   i. Add isolate comment **&GBS**:
      "Susceptibility testing not routinely performed. Group B Streptococci are predictably susceptible to ampicillin and penicillin. Call laboratory for further testing if patient is allergic to penicillin."
   ii. Susceptibilities performed upon physician request only.
e. Microaerophilic, minute Beta-Hemolytic *Streptococci* are identified group-wise by their atmospheric requirements
   i. Identified group-wise by their atmospheric requirements
      a) Sub-culture suspected organism onto three BAP plates. Incubate one plate in ambient, another in CO2, and another in anaerobic conditions. If growth is observed on plates from only CO_2 and anaerobic condition, then this rules in microaerophilic *Streptococci*.
      b) Some strains may grow a little in ambient atmosphere
   ii. Speciation is performed using Vitek MS/Vitek GP card
   iii. Susceptibility testing for microaerophilic, minute Beta-Hemolytic Streptococci is performed by Kirby-Bauer disk diffusion method on Blood Mueller-Hinton, including a penicillin E-test
   iv. Microaerophilic *Streptococci* can group serologically as A, C, F, or G
f. All Group F Beta-Hemolytic *Streptococcus* are microaerophilic

6. *Enterococcus* species
   a. Organism is PYR positive & LAP positive
   b. Speciate all *Enterococcus* isolates reported as pathogens
   c. Release Linezolid susceptibility results on vancomycin resistant isolates from blood cultures and sterile body sites – Do not release for urines.
   d. Add High Level Aminoglycoside Resistance statement to non-urine reports **&HLR**:
      "Synergy (from use of an aminoglycoside plus either Penicillin or Vancomycin) cannot be predicted unless both antibiotics used in combination are susceptible."
   e. If sensitivities are performed by Kirby-Bauer method, a synergy quad plate must be performed for non-urines.
   f. Add nitrofurantoin susceptibility disk to Kirby-Bauer performed on urines
   g. *E. raffinosus* susceptibilities must be performed by Kirby-Bauer
   h. *E. casseflavus* and *E. gallinarum* are intrinsically resistant to Vancomycin and are not considered VREs

7. *Staphylococcus* species/ *Micrococcus* species
   a. *Micrococcus* species
      i. Bacitracin (Atab) disk sensitive- demonstrating a zone of inhibition of ≥10mm
      ii. Speciation can performed by Vitek MS/Vitek GP ID, if necessary
   b. *Staphylococcus* species
      i. Sensitivities performed by Vitek GP AST. Alternatively, Kirby-Bauer may be performed.
      ii. Clindamycin inducible enzyme results may be reported from Vitek directly, otherwise, if a Kirby-Bauer is reported, save plates for 7 days in case a D-test is requested

iii. If sensitivities are performed by Kirby-Bauer method, set-up an E-Test for vancomycin. Performing vancomycin by disk diffusion is unreliable for *Staphylococcus* spp.
iv. Coagulase-Negative *Staphylococcus species*
    a) Latex agglutination can be performed on suspicious *Staphylococcus* species to r/o *S. aureus*
    b) Speciation can be performed by Vitek MS/Vitek GP ID, if required:
        1. *S. lugdunensis*
            a. PYR positive
            b. If isolate is reported as a pathogen with susceptibilities, speciation must be done due to differences in oxacillin interpretation between other Coagulase-negative *Staphylococcus* species
        2. *S. intermedius*
            a. May demonstrate delayed latex positivity. If there is a suspicion of *S. intermedius* identify by Vitek MS/Vitek GP ID card
        3. *S. saprophyticus*
            a. Speciation required when isolated from urine
            b. Susceptibilities not routinely performed
                i. Add isolate comment **&SAP**:
                "Routine susceptibility testing is not performed. Infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute uncomplicated urinary tract infections."

v. *Staphylococcus aureus*
    a) Circular, smooth, light-golden colonies, 1-4mm diameter
    b) Often Beta-hemolytic (some strains may appear non-hemolytic)
    c) Speciated by latex agglutination or Vitek MS/Vitek GP ID
    d) MRSA/MSSA may be determined by performing PBP2a, if requested or there is a discordance with sensitivities. Refer to ***Procedure: Alere PBP2a SA Culture Colony Test*** for further guidance.
    e) If vancomycin MIC is ≥ 2 by Vitek, an MIC must be done for confirmation – *Refer to Appendix AP41*

B. Gram-positive bacilli - *Refer to Appendix AP35*

1. *Arcanobacterium haemolyticum*
    a. Beta-hemolytic, irregular rod
    b. Catalase negative
    c. Esculin positive
    d. Identification performed by Vitek MS/Vitek ANC

2. *Bacillus* species
    a. Catalase positive
    b. *Bacillus anthracis* and *Bacillus cereus* biovar *anthracis* are potential bioterrorism agents and must be ruled out on all isolates
        i. *Bacillus anthracis* is non-motile, non-hemolytic and has a ground glass colony morphology.
        ii. *Bacillus cereus* biovar *anthracis* has variable motility, is non-hemolytic and has a ground glass colony morphology
    c. Potential bioterrorism isolates must be reported to RIDOH Epidemiology and an isolate sent to RIDOH laboratory for confirmation
    d. If *B. anthracis* and *B. cereus* biovar *anthracis* are ruled out, report generically as *Bacillus* species non-anthracis

e. Non-bioterrorism *Bacillus* isolates may be identified by Vitek MS upon request

3. *Corynebacterium* species
   a. Club-shaped rods
   b. Catalase positive
   c. Specification performed by Vitek MS/ Vitek ANC, if appropriate:
      i. *Ex. Corynebacterium* species isolated in pure culture from multiple blood cultures, catheter tips, or multiple sterile body sites
   d. *C. ureolyticum* is ruled out in urine specimens if appropriate quantitation present
   e. Susceptibilities not routinely performed

4. *Listeria* species
   a. Coccobacillary rods, singly or in chains
   b. Catalase positive
   c. Demonstrates tumbling motility in wet prep, especially at 30°C
   d. Gray colonies exhibiting weak Beta-hemolysis
   e. Send to RIDOH for serotyping
   f. Susceptibilities not routinely performed

5. *Nocardia* /*Rhodococcus* / Aerotolerant *Actinomyces* / Rapid-Growing AFB
   a. Send to the Mycobacteriology/ Mycology laboratory for further workup
   b. *Nocardia* species are PAF positive & AFB negative
   c. *Mycobacterium* species are PAF positive & AFB positive
   d. Other species are PAF & AFB variable
   e. *Rhodococcus* is generally very mucoid and orange

C. Gram-negative bacilli - *Refer to Appendix AP36*

1. *Aeromonas/ Plesiomonas*
   a. Identification from non-stool specimens performed by Vitek MS/Vitek GN ID
   b. Oxidase positive
   c. Susceptibilities performed by MIC

2. *Acinetobacter* species
   a. Identification can be performed by Vitek MS/Vitek GN card
   b. For all *Acinetobacter* species perform a Kirby Bauer
   c. Add amox-clav comment to all *Acinetobacter* species on all benches **&ACIN**:
      "Amoxicillin-clavulanate is not active against *Acinetobacter*."

3. *Achromobacter* species
   a. If identified by MALDI as *Achromobacter dentrificans*/*xylosoxidans*, send organism to Vitek for GN card for better speciation
   b. Susceptibility testing performed by MIC

4. *Burkholderia cepecia*
   a. Identification performed by Vitek MS/Vitek GN card
   b. Susceptibility testing performed by MIC
   c. BCSA plates are inoculated for screening of cystic fibrosis (CF) patient respiratory specimens
      i. Plates must be incubated ambiently at 35°C for 72 hours
      ii. *B. cepacia* will grow well and have a yellow to pink-yellow zone on BCSA
      iii. For other species of *Burkholderia*, refer to miscellaneous GNRs

d. If *B. cepacia* or member of *B. cepacia complex* is isolated from a CF patient, isolate must be reported as probable *B. cepacia* (*B. cepacia* complex) then sent to reference for identification confirmation

5. *Campylobacter* species
   a. Curved, thin rods, usually “S” or sea-gull shaped rods
   b. Oxidase & catalase positive
   c. Grows best at 42°C in microaerophilic atmosphere
   d. Susceptibilities not routinely performed

6. *Eikenella corrodans*
   a. Oxidase positive, usually catalase negative
   b. Unable to grow on MacConkey agar
   c. Creates depressions or “pits” in the agar. There is a discoloration of a greenish pigment of the agar.
   d. Is part of the normal flora of the oral cavity, and therefore can be found in bites, clenched-fist wounds and subacute bacterial endocarditis
   e. Member of HACEK group
   f. Identification performed by Vitek MS/Vitek NH card
   g. Susceptibilities not routinely performed

7. *Enterobacteriaceae* species (*Escherichia, Klebsiella, Enterobacter, Citrobacter, Cronobacter, Serratia, Proteus,* and other *Enterobacteriaceae)*
   a. Identification performed by Vitek MS/Vitek GN ID
   b. Susceptibilities performed by Vitek GN AST, or MIC if necessary
      i. ESBL (Extended-Spectrum-Beta-Lactamases) – *Refer to Appendix AP39*
      ii. CRE (Carbapenem Resistant *Enterobacteriaceae)* – *Refer to Appendix AP40*

   c. Escherichia coli
      i. Spot indole can be performed for rapid identification for flat, dry, lactose fermenting colonies on the urine specimens only
      ii. If Ceftriaxone ≥ 4/R and spot indole was used for identification, confirmation of the identification must be performed by Vitek MS/Vitek GN card

   d. *Klebsiella* species
      i. Identification can be performed by Vitek MS/Vitek GN card
      ii. Susceptibilities are performed by Vitek GN AST card
      iii. *K. pneumoniae* and *K. oxytoca* can be differentiated by spot indole
         a) *K. pneumoniae* is indole positive and *K. oxytoca* is indole negative

   e. *Pantoea* species
      i. Identification can be performed by Vitek MS/Vitek GN card
      ii. Susceptibilities are performed by Vitek GN AST card

   f. *Serratia marcescens*
      i. A Pip/tazo disk must be performed on all in-house patients.

   g. *Salmonella* species
      i. Speciation performed by Vitek MS/ Vitek GN card
      ii. Susceptibilities may be done by Kirby-Bauer
         a) If Levofloxacin is needed, a MIC must be performed for lack of interpretations by Kirby-Bauer method
      iii. Send isolates to RIDOH for typing.

h. *Shigella* species
    i. Identification performed by Vitek GN ID card
    ii. Vitek MS should not be performed because of its inability to differentiate from clear colonies that could be *E.coli*
    iii. Susceptibilities performed by Kirby-Bauer
    iv. Send to RIDOH for confirmation

8. *Vibrio* species
    a. Identification from non-stools specimens performed by Vitek MS/Vitek GN ID
    b. TCBS media and *Vibrio* species colony morphology:
        i. V. cholerae - large yellow colonies
        ii. V. parahaemolyticus - colonies with blue to green centers
        iii. V. alginolyticus - Large yellow colonies
    c. Send to RIDOH for confirmation if possible *V. cholera*, *V. parahemolyticus*, or *V. vulnificus*
    d. Sensitivities are not routinely performed from stool isolates
    e. Susceptibilities are performed by MIC when recovered from non-stool specimens

9. *Yersinia* species
    a. Identification is performed by Vitek MS/ Vitek GN ID card
    b. On CIN media *Y. enterocolitica* is characterized as “bull’s eye”, with a deep-red center, surrounded by transparent border
    c. Sensitivities are done by Kirby-Bauer method
    d. Send to RIDOH for confirmation

10. *Haemophilus* species
    a. Identification performed by Vitek MS/ Vitek NH ID
    b. Member of HACEK group
    c. Identified by Gram-stain, colony morphology and satellites around *S. aureus*
    d. A Haem-Quad plate can be performed to rule in certain *Haemophilus* spp. Refer to *Haemophilus ID Agar Procedure*.
    e. If isolate is *Haemophilus influenzae*, perform B-lactamase and result accordingly using the following isolate comments:
        i. B-Lactamase Positive- (&HBLP) “*Haemophilus isolates producing beta lactamase are resistant to Amoxicillin.”*
        ii. B-Lactamase Negative- (&HBLN) *“Haemophilus isolates negative for beta-lactamase are likely to be susceptible to Amoxicillin, Macrolides and Cephalosporin antibiotics.”*

11. *Moraxella* species/ *Neisseria* species
    a. *N. meningitidis* or *Moraxella catarrhalis* can colonize the nasopharynx and is generally considered as part of normal flora. Work-up only if it is the predominant organism and/ or the Gram-stain demonstrates intracellular Gram-negative diplococci.
    b. Bring up any *N. meningitides* isolates on ROUNDS
    c. Susceptibilities are not performed
    d. Molecular confirmation is required on isolates from patients ≤ 17 years old for *Neisseria gonorrhoeae* and any legal cases
    e. *Moraxella catarrhalis* is always considered beta-lactamase positive.

12. *Pasteurella* species
    a. Oxidase positive, indole positive
    b. Unable to grow on MacConkey agar
    c. Identification performed by Vitek MS/ Vitek GN ID card
    d. Add isolate comment (&NOSU), *“Susceptibilities not routinely performed.”*

13. *Pseudomonas aeruginosa*
    a. Identification may be determined if organism is oxidase positive, demonstrates a green pigment, and exhibits as a flat, fuzzy colony
    b. If mucoid, supplement report with isolate comment &MUC *"Mucoid isolate"*
    c. Mucoid *P. aeruginosa* can be identified if oxidase positive, green pigment is variable, oxidative metabolism of glucose, and grows at 42°C
    d. Susceptibilities are performed by Kirby-Bauer with Muller-Hinton at 35°C, in ambient atmosphere
    e. A MIC can be performed on resistant isolates or for additional antibiotic requests

14. *Stenotrophomonas maltophilia*
    a. Identification performed by Vitek MS/ Vitek GN ID card
    b. Susceptibility testing is performed by MIC method
        i. *S. maltophilia* should be susceptible to SXT. When reading the MIC for SXT, choose the well where the growth is 80-90% inhibited compared to the positive control well

15. Miscellaneous Gram-negative Bacilli/ Non-Fermenting Gram-negative Bacilli - *Refer to Appendix AP37*
    a. Identification performed by Vitek MS/ Vitek GN ID card
    b. Susceptibilities are performed by the MIC
    c. *Burkholderia species* (not *B. cepacia*)
        i. *B. cenocepacia* and *B. multivorans* grow well with pink to red zone on BCSA

II. Anaerobic - *Refer to Appendix AP38*

A. Cocci
1. Speciation is done by Vitek MS/ Vitek ANC ID card
    a. Vitek MS result is acceptable if it is consistent with colony morphology, fluorescence (if applicable), Gram-stain, or any rapid biochemicals.
    b. If a Vitek ANC ID card is used, perform Gram-stain, fluorescence, and any rapid biochemicals, aerotolerance plate and special potency disk diffusion by using: kanamycin, vancomycin, and colistin for affirmation

B. Bacilli
1. Speciation by Vitek MS/ Vitek ANC ID card
    a. Vitek MS result is acceptable if it is consistent with colony morphology, fluorescence (if applicable), Gram-stain, or any rapid biochemicals.
    b. If a Vitek ANC card is used, perform Gram-stain, fluorescence, and any rapid biochemicals, aerotolerance plate and special potency disk diffusion by using: kanamycin, vancomycin, colistin, and bile (for Gram-negative) disks for affirmation
    c. *Cutibacterium* (formerly Propionibacterium) *acnes*
        i. Gram positive rods
        ii. Catalase positive
        iii. Spot indole variable (most are positive)
    d. *Actinomyces* species
        i. Gram positive rods
        ii. Catalase negative
            a) Note: rare *Actinomyces* species are catalase positive
    e. *Clostridium* species
        i. Gram positive bacillus with spores
        ii. Catalase negative

III. Fungi

A. Yeast
1. Generally considered part of mixed respiratory flora, and should not be reported, unless it is *Cryptococcus neoformans*
2. Urine isolates are speciated if isolated in significant amounts. See urine protocol
   a. Urine isolates showing “feet” can be reported as Probable *C. albicans*
3. PNA FISH smear must be performed on a patient’s first positive blood culture when Gram-stain shows yeast
4. Isolates from catheter tips are speciated, regardless of quantity. Correlate with blood culture results
5. Sensitivities are performed on select Candida spp. by MIC
6. *Candida auris*
   a. Can be identified by Vitek YST card
   b. Currently is difficult to identify due to its lack of distinct phenotypic characteristics from other *Candida* species
   c. Budding yeast, which almost never forms short pseudohyphae and does not form germ tube
   d. May be misidentified as:
      i. *C. haemulonii (Vitek MS)*
      ii. *C. haemulonii* or *C. duobushaemulonii,(Vitek YST card)*
      iii. Repeat testing using opposite method for confirmation
   e. Report out *C. auris* if identified
   f. If *C. auris* is not identified, then it must be sent to RI Department of Health for further identification
   g. Multidrug resistant
   h. Isolate must be reported to Infection Control and Department of Health if *C. auris* is either identified or not ruled out.

B. Filamentous fungi
1. Refer to AFB/ Mycology laboratory for workup
   a. If only one colony grows, do not report. Refer culture to AFB/ Mycology laboratory for its significance.

IV. Attachments

A. Appendix AP33 – Catalase Negative, GPC Flowchart
B. Appendix AP34 – Catalase Positive, GPC Flowchart
C. Appendix AP35 – Aerobic Gram-Positive Rods Flowchart
D. Appendix AP36 – Aerobic GNR, Good Growth on BAP
E. Appendix AP37 – Aerobic GNR, Poor Growth on BAP
F. Appendix AP38 – Anaerobe Identification Flowchart

V. Revisions

A. 01/29/20 Alterations to testing for organisms to accommodate changes to accessible testing materials or changes to procedures. Addition of Candida auris.
B. 02/03/2025 Updated testing protocols for *Enterobacterales, Salmonella/Shigella sp.,* and *Acinetobacter sp.* resulting from Vitek AST-N812 implementation.