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Isolate Notification and Freezing Table QPCMI16003 (Found in MSH Internal Manual)

(Found in MSH Internal Manual)

METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA)

I. Introduction

These specimens are submitted to identify carriers of methicillin-resistant *S. aureus* (MRSA). Swabs may be submitted from any body site, but the most common are nasal, rectal and wound, or the combined nasal/axilla/groin/perineum (NAGP).

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents/ Material/ Media

The OXOID Denim Blue Agar (DBLUE) contains a species-specific chromogen that turns *Staphylococcus aureus* colonies blue. As this chromogen is light sensitive, plates must be stored in their shipping boxes to protect them from unnecessary light exposure until use. After streaking, place directly into plastic bins inside the incubator shielded from light. No more than 4h light exposure by the final read is acceptable.
See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

A. Specimen Processing:
   a) Direct Examination: Not indicated
   b) Culture:

Media	Incubation
OXOID Denim Blue Agar (DBLUE)*	O_2, 37^{o}C x 24 h -in the dark

*If multiple swabs from a single patient are received individually, then process as separate specimens. If multiple swabs from a single patient are received as a "bundle" with a single label and order number, then process all swabs in the bundle on a single "DBLUE" plate.

Specimens are planted by WASP and incubated within the WAPlab system.

B. Workflow and Culture Interpretations

Images are taken by the WASPlab for a preliminary screening of blue colonies at 18hrs and a final read at 24hrs.

For small amount of plates requiring offline incubation, keep plates in covered container in the walk-in incubator (O_2, 37^{o}C) and screen plates at the beginning of your shift and at time of final incubation (end of your shift or earlier as applicable).
When significant downtime occurs, separate plates by shift planted into larger buckets for screening as above until final reads.

Screen remaining MRSA plates through Infection Control phenomatrix software in the WaspApp and offline incubation bucket for denim blue colonies (NOT blue hazes or dark blue pinpoint colonies)

No denim blue colonies:

For plates within the Wasplab system, workup will be done through the Waspapp phenomtrix software or IC screening as a backup.

- In MRSA phenomtrix application, 18hr and 24hr images will appear:
  - plates with no blue colonies will be reported automatically through segregation at 18hrs for re-incubation with a prelim status.
  - plates with no blue colonies will be reported automatically through segregation a 24hrs as Negative (see reporting section) and sent to Trash.
- In IC screening
  - At 18hrs: select 18hr-Reinc and send results to re-incubate and status to the MRSA test.
  - At 24hrs: select NO MRSA. Plate will be automatically reported as Negative (see reporting section) and sent to Trash.

For Screening offline document each reading within the LIS.

- For preliminary reads with no blue colonies document DBLUE reading as No Blue and status MRSA test as prelim.
- At the end of your shift or earlier as applicable, perform the 24hr final read. For negative plates at 24hrs, document in DBLUE media 24hr: No blue and report MRSA test as Negative (see reporting section). Trash plates.

Denim Blue colonies:

For each plate with blue colonies, plate with be sent for ID with an isolate quantitation (1-5, scant, light, moderate, heavy)

For plates within the Wasplab system, Reading and Pick plates to send for ID or a subculture.
For Screening offline document each reading within the LIS.

i. Send plate for colony identification:
   - If non-sufficient colonies for ID, document and make a subculture plate for next shift.

ii. For ID's achieved other than *S.aureus*, suppress the isolate and send out a negative MRSA report (See reporting section)

iii. For ID's of *S.aureus* check each patient's MRSA and VRE history.

Previous VRE history:
- Regardless of MRSA history (new or prev), if patient has had VRE history *within last 3 months* add "VANCS" before sending an interim result.

Previous Positive MRSA history: (within 3 months)
- If Vitek MS identified as *S. aureus*, or Pastorex Staph–Plus is positive, check patient VRE history. If patient has had any VRE, (within the last 3 months) and there is sufficient growth of blue colonies, set up VANCS. If no positive VRE history, report as "MRSA with quantitation"; assign "Interim" status for review.

- If Vitek MS identified as NOT *S. aureus*, suppress ID and finalize as "Negative - No methicillin-resistant Staphylococcus aureus (MRSA) isolated".

iii) If SUBBA grows **an organism other than staphylococcus**, document organism and supplementary tests performed and finalize as "Negative - No methicillin-resistant Staphylococcus aureus (MRSA) isolated".

New MRSA: follow NEW MRSA work up below

Check "New" MRSA worklist for outstanding specimens from the previous day and ask for replant if any are not accounted for.

Complete leftover old work from the previous day. Check "Old" MRSA worklist for outstanding workup needing completion.

i) **For NEW MRSA**

a) If Vitek MS is negative for *S.aureus*, result as "Negative – No methicillin-resistant Staphylococcus aureus (MRSA) isolated" and status as "Final".

b) If MS identified as *S.aureus*, perform DENKA (Denka Seiken PBP2a agglutination test).

c) If MS identified as *S.aureus* and DENKA+, <CTRL> "P" as "MRSA" and notify IC and ward as per Isolate Notification and Freezing Table QPCMI15003. Set up oxacillin screen (OXA), vancomycin screen (VANCS), Vitek GPAST and KB mupirocin (MUP_{200}) disc.
When complete, interim for review as "MRSA".
Set up MUP_{200} E-test if MUP_{200} zone <19mm.
Also set up BHIB/SUBBA for PFGE as appropriate (see Appendix II) and freeze (FRZ).

If VITEK SXT=R SUPPRESS SXT and confirm result by KB BEFORE reporting. A POP-UP will remind you: "Dsxt=R//uncommon susceptibility result. Suppress and verify w/ KB"

d) If MS identified as *S.aureus* but DENKA-negative, CTRL "P" as "MRSA presumptive identification, confirmation to follow" and notify IC/ward as per Isolate Notification and Freezing Table QPCMI16003, set up OXA/VANCS/MUP/VT GP- AST and set up KB (from 0.5 McFarland suspension) with cefoxitin disc.

e) After overnight incubation, record cefoxitin KB result and perform from colonies that grew closest to the cefoxitin disc.

If induced DENKA is positive, notify IC/ward of confirmed "MRSA". Document other test results, set up BHIB/SUBBA for PFGE as appropriate (see Appendix II) and freeze (FRZ).and status the test as "Interim" for review.

If induced DENKA is negative, refer to How to section in the susceptibility manual.

f) Send to NML in batches when requested by IC for CNISP surveillance

V. Reporting

Negative report: "Negative - No methicillin-resistant Staphylococcus aureus (MRSA) isolated"

Positive report: "Methicillin-Resistant *Staphylococcus aureus*" with quantitation and appropriate susceptibilities and comments for new cases ().

Positive reports for Sinai Health patients (MSH and Bridgepoint Health) should have the following comment automatically added **\ICPR** "THIS PATIENT IS TO BE MANAGED IN "CONTACT PRECUATIONS" UNTIL FURTHER NOTICE"

Scant growth (1-5 colonies) - Upon Infection Control request to replant into BHIB (2mL):

- Confirmed by replanting original specimen in broth
  Add ISOLATE Comment:
  "MRSA confirmed by broth enrichment culture."
  LIS Code: "\MRSc

- NOT confirmed by replanting original specimen in broth:
  1. Change original isolate to an alpha isolate
  2. Add TEST Comment
     "No MRSA isolated by broth enrichment culture. The previous report of "MRSA isolated" was not confirmed by broth enrichment culture suggesting that the previous report reflects contamination or a very low level positive result. Please send another screening swab as clinically indicated."
     LIS Code: "}MRSC"

VI. References

1. **Lo P, Small GW, Porter RC, Lai S, Willey BM, Wong K, Low DE, Mazzulli T, Skulnick M.** Evaluation of Four Rapid Agglutination Test Kits for the Identification of *Staphylococcus aureus* (SA) In Abstracts: *66th Conjoint Meeting on Infectious Diseases*, Toronto, Ontario, 1998

2. **Willey B. M., L. Pearce, D. Chen, T. C. Moore, B. Tennant, G. Ruzo, A. McGeer, D. E. Low, M. Skulnick** Evaluation of a PBP 2' Slide Agglutination Test for the Rapid Detection of Methicillin-Resistant *S. aureus*. In Abstracts: 99th American Society for Microbiology General Meeting, Chicago, 1999 (Abstract # C-233).

3. **Willey B. M., N, Kreiswirth, P. Akhavan, A. Tyler, S. Malek, V. Pong-Porter, G. Small, N. Nelson, A. McGeer, S. M. Poutanen, T. Mazzulli, D. E. Low, M. Skulnick.** Evaluation of four selective media for the detection of methicillin-resistant *Staphylococcus aureus* from surveillance specimens. In Abstracts from the 16th European Congress of Clinical Microbiology and Infectious Diseases, Nice, France 2006 (Abstract # O216)

4. **Willey B. M., N. Kreiswirth, P. Akhavan, A. Tyler, S. Malek, V. Pong-Porter, G. Small, N. Nelson, A. McGeer, S. Poutanen, T. Mazzulli, D. E. Low, M. Skulnick.** New laboratory approaches to the selective detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from surveillance specimens. In Abstracts from the AMMI-CACMID Annual Conference, Victoria, BC, 2006 (Abstract # B-4) published in *Can J Infect Dis & Med Microbiol* 2006;17:31

5. **N. Kreiswirth, V. Porter, A. Tyler, A. McGeer, T. Mazzulli, S.M. Poutanen, M. Skulnick, B.M. Willey.** Evaluation of Oxoid's *Denim Blue* Agar for Detecting methicillin-resistant *Staphylococcus aureus* (MRSA) from surveillance specimens. In Abstracts from the AMMI-CACMID Annual Conference, Halifax, NS, 2007 (Abstract #)

VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE)

Introduction

These specimens are submitted to identify carriers of vancomycin-resistant *E. faecium* and/or *E. faecalis* (VRE). Swabs may be submitted from any body site (other than nasal and axilla), but most commonly are collected from the rectum.
Some VRE are dependant on vancomycin to grow, these Vancomycin-dependant enterococci isolates (VDE) pose additional challenges to identification and should be considered if purple/blue are isolated on Brilliance VRE agar but do not grow on routine subculture.

Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

Specimen Rejection Criteria

Nasal and axilla swabs will not be processed for VRE. Refer to Reporting in **Section VI** for the appropriate reporting comment.

Reagents/ Material/ Media

See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

Procedure

A. Processing of Specimen:

Refer to Specimen Processing Procedure MI_SM_PROC

a) Direct Examination: Not indicated

b) Culture in non-outbreak setting:

Media	**Incubation**
Brilliance VRE Agar (BVRE)	O_2, 37°C x 36hrs in the dark

B. Culture for **VRE PCR positive samples** in outbreak setting:

Media	**Incubation time (all O_2 at 37°C)**
i) Place 500uL (0.5 mL mark of transfer pipette) of the eSwab transport medium into:	
- 2 mL Brain Heart Infusion broth (BHIB)	overnight on shaker
Place 30uL (1 drop from transfer pipette) of the eSwab transport medium onto:	
- Brilliance VRE Agar (BVRE)	36h in the dark
ii) If BVRE is no growth after overnight incubation, subculture 1 drop from BHIB to:	
- Brilliance VRE Agar (BVRE)	36h in the dark

C. Workflow and Interpretation of cultures:

Workflow is described in the Bench Workflow Manual.

Process specimens as per WASPLab Screening, Reading and Picking Manual.

For specimens processed offline:

a) Label new bin for Planting incubator
b) Read BVRE plates planted from the previous day, separating plates growing purple or blue colonies. Read 36 hrs. plates separating plates growing purple or blue colonies.

VRE cultures will be read at 18hrs, 30hrs and a final reading at 36hrs for VRE *faecium & faecalis*

Colonies on Briliance VRE Agar:

Isolate:	**Colony colour:**
Enterococcus faecium	Purple to Royal Blue colour on entire colony, moist
Enterococcus faecalis	Denim Blue
CNST	Blue (if grown)
Yeast	Light blue (if grown)
Enterococcus gallinarum	Blue (if grown)
Lactobaclli	Light blue/pink (if grown)

A. Royal Blue and Purple colonies:

Check history of patient whose specimens are growing purple colonies.

a) If patient is a **"New" positive purple colonies**; perform PCR and Vitek MS
    a) Inoculate a spot on Vitek MS slide for ID
    b) Pick purple colonies and emulsify them in 0.5 mL saline
    c) Using the same swab, inoculate a vial of PCR sample reagent and set up Cepheid PCR
    d) Using the 0.5mL emulsified saline, inoculate a SUBBA and ¼ BVRE (SBVRE).

Note: For isolates with unsuccessful ID results, report as Vancomycin-Resistant *E.faecium*, confirmation to follow (with vanA/B positive result)
For isolates that do not grow on SUBBA, consider vancomycin dependant enterococci (VDE) - subculture to BVRE/ vancscreen plate/ BA plate with vanco disc.

b) If patient is a "**Previous" positive (≤3 months)**
    - Set up Vitek MS, VANCS, PP,

- Do NOT report out isolate until VANCS results are know. (Refer to Antimicrobial Susceptibility Manual "How to detect VRE" section.

Follow : Table 1 VRE Workup Guide –PURPLE COLONIES for further work up.

B. Samples growing Denim/Light Blue colonies:

Observe quantity of suspect colony growth.

a) **Scant growth**: inoculate colonies into 0.5mL saline and onto ¼ BVRE (SBVRE)
b) **Moderate/Heavy growth**:
   - Inoculate a spot on Vitek MS for ID.
   - If *enterococcus faecium or faecalis* (or gpc chains when Vitek MS fails) emulsify colonies in 0.5 mL saline and use that swab to inoculate a vial of PCR sample reagent and set up Cepheid PCR.
   - Using the 0.5mL emulsified saline, inoculate a SUBBA and ¼ BVRE (SBVRE).

   Note: For isolates with unsuccessful ID results, report as Vancomycin-Resistant *E.faecium*, confirmation to follow (with vanA/B positive result)
   For isolates that do not grow on SUBBA, consider vancomycin dependant enterococci (VDE) -subculture to BVRE/ vancscreen plate/ BA plate with vanco disc.

Follow : Table 2 VRE Workup Guide – BLUE for further work up.

C. No Royal Blue and Purple or Denim/Light Blue colonies:

Re-incubate negative plates for further incubation as needed.

Enter "__hr: No purple or blue" and status as "Prelim".
Finalize 36 hr culture as negative. (See VRE reporting section)

Read and report old work. Communicate to ward and/or infection control if necessary as per

Continue to scan BVRE plates and process any that are now growing purple or blue colonies.

Check new VRE worklist after all plates are prelimmed for any missing plates. Document if plate is not found and ask for replant.

VRE Identification:
Rule out VRE as below:

Table 1 VRE Workup Guide –PURPLE COLONIES

NEW Purple/Royal Blue Colonies	PREVIOUS + (<3months) Purple cols
BVRE >5cols 24/48 hours	**BVRE (any amount)**
1. Set up vanA/vanB Cepheid PCRand MS and SBVRE and SUBBA	1. Set up MS and VANCS
2. **Cepheid – Positive** • Report according to ID as Entfar or Entfer with comment vanA gene positive OR vanB gene positive ▪ If Cepheid vanB Positive, Roche PCR must be done for MSH patients only (within 24 hours) • Notify ICP/ward • Set up Etest, VANCS ▪ Vanco Etest ≥8ug/mL, VANCS-growth, SBVRE-growth, ○ Report Entfar or Entfer with phenotype comment ▪ Vanco Etest ≤4ug/mL, VANCS - NG, SBVRE - NG, ○ Perform PCR again from etest plate to confirm presence of vanA ○ Report as vanco sensitive entvaa or entfva ○ Report with comment: "Vanco susceptible phenotype" • PFGE as required (refer to Appendix II) & FRZ 3. **Cepheid – Negative** • SBVRE - NG ▪ Report as No VRE • SBVRE - GROWTH	1. VANCS – Growth • Report Entfar or Entfer with comment 2. VANCS – NG • If "Previous" entfar or entfer: report as NO VRE • If "Previous" entvaa, perform Cepheid: ▪ Cepheid vanA Positive: report as entvaa ▪ Cepheid Negative: report as NO VRE *Set up etests for Vanco and Teico every 3 months from original isolate to confirm phenotype

▪ Set up Vanco/Teico Etests, VANCS ▪ Vanco Etest ≥8ug/mL, VANCS - growth, o Add comment non vanA/B to isolate o Send to NMLfor van genotyping & FRZ o Notify ICP	

Table 2 **VRE Workup Guide – BLUE COLONIES**

Blue Colonies (SCANT /LIGHT growth)			**Blue Colonies (HEAVY)**
Set up SBVRE on any amount of blue cols growing			Vitek MS ID of *Enterococcus faecium or Enterococcus faecalis.*
NG on SBVRE	**Scant Growth on SBVRE**	**Mod-Heavy Growth on SBVRE**	
Report – No VRE	Set up VANCS 'PP'	Set up Cepheid PCR& MS	Set up Cepheid PCR, SBVRE and SUBBA
	1. VANCS – No growth • Report No VRE 2. VANCS - Growth • Set up MS • Perform Cepheid PCR, Etests. ▪ Proceed as Mod-Heavy Growth • Notify ICP/ward	1. Cepheid – Positive • Follow NEW Purple >5 Cepheid positive workflow. 2. Cepheid – Negative • Set up Etests &VANCS • If Vanco Etest ≥8ug/mL, VANCS - growth, add comment: 'non vanA/B' to isolate • Send to NML for van genotyping & FRZ • Notify ICP	1. Cepheid – Positive • Follow NEW Purple >5 Cepheid positive workflow. 2. Cepheid – Negative • Set up Etests &VANCS • If Vanco Etest ≥8ug/mL, VANCS - growth, add comment: 'non vanA/B' to isolate • Send to NML for van genotyping & FRZ • Notify ICP

Table 3 VRE Workup Guide – Cepheid PCR + from E- swab directly

1. Phone/email ward and ICP as per Isolate Notification and Freezing table QPCMI15003.

2. For new or previous VRE patients where NO isolate has been isolated yet proceed as below:

Subculture to BHIB broth and BVRE and incubate overnight		
If BVRE is No growth, Subculture BHIB to BVRE		
NG	**Scant Growth (purple or blue colonies)**	**Mod-Heavy Growth (purple or blue colonies)**
Report as "No VRE isolated after broth enhancement"	1.Sub to SUBBA and SBVRE • Set up MS and Vanco/Teico etest, VANCS • Proceed as Mod-Heavy Growth	1. Set up MS and Vanco/Teico etest, VANCS *Do not set up Cepheid PCR from BVRE plate* • If Vanco R/Teico R report as vanA phenotype with comment • If Vanco R/Teico S report as vanB phenotype with comment • If Vanco S/Teico S, do Cepheid from Etest plate ▪ Cepheid Negative: report as No VRE after broth enhancement ▪ Cepheid Positive, vanA Positive: report as Entvaa with comment **}vaAi**

VRE PFGE:

Set up BHIB/SUBBA for PFGE as appropriate (see Appendix II)

VI. Reporting

Negative Report: "Negative - No Vancomycin-Resistant Enterococci (VRE) isolated"

Positive Report:

Note: Positive reports for Sinai Health patients (MSH and Bridgepoint Health) should have the following comment automaticallyadded ***\ICPR*** *"THIS PATIENT IS TO BE MANAGED IN "CONTACT PRECUATIONS" UNTIL FURTHER NOTICE"*

New Positive VRE Patients

Day 1

- **PCR direct from BVRE plate - with isolate**
  ISOLATE: "*Enterococcus (faecium or faecalis)*-vancomycin resistant" "isolated"
  ISOLATE COMMENT:
  "This organism is positive for the vanAorB gene as tested by the Cepheid vanA/B GenXpert Assay (for research only).
  ~Phenotypic confirmation to follow." Isolate Comment Code **\vaAg** or **\vaBg**

- **PCR direct from BVRE plate - no isolate, from sweep**
  ADD ISOLATE COMMENT:
  "PCR from a sweep of growth on the plate is positive
  for the vanA gene by the Cepheid vanA/B GenXpert Assay
  (for research use only) but a distinct
  vancomycin-resistant or vancomcyin-susceptible
  Enterococcus species that is vanA positive cannot be found."
  Isolate Comment Code **\vaAp**

Day 2

- Vancomycin=R, Teicoplanin=**R**:
  "*Enterococcus faecium or faecalis)* -vancomycin resistant" "isolated"
  ISOLATE COMMENT (Code **\vaA)**:

"This organism has a vanA phenotype."

- Vancomycin=R, Teicoplanin=**S**:
  "*Enterococcus faecium (or faecalis)* -vancomycin resistant"
  ISOLATE COMMENT (Code **\vaB)**:
  "This organism has a vanB phenotype."

Previous VRE Positive Patients:

Enterococcus (faecium or faecalis)–vancomycin-resistant isolated.
ISOLATE COMMENT (Code: **\vapr)**:
"The Cepheid vanA/B GenXpert Assay was not completed as this patient has had VRE isolated within the past 3 months that has had molecular characterization."

Vancomycin=S, *vanA gene*-positive VRE

- **Isolate from IC VRE Culture Screen**

1) Change previous isolate code of entfar to **entvaa** - "*Enterococcus faecium* - vanA gene positive" "isolated"
   ISOLATE COMMENT (Code: **\vaAi)** – "This organism is positive for vanA gene by the Cepheid vanA/B GenXpert Assay (for research use only)
   but has a vancomycin susceptible phenotype.
   The effectiveness of vancomycin in this setting is uncertain and is not recommended. Please contact Infectious Diseases or Medical Microbiology for treatment advice."
   Remove previous duplicated ISOLATE COMMENT.

2) Change previous isolate code of entfer to entfva - "*Enterococcus faecalis* - vanA gene positive" "isolated"

Vancomycin MIC =>8 by macro Etest, ***vanA/B*-negative by PCR**

"*Enterococcus faecium or faecalis*" "isolated"
ISOLATE COMMENT (Code: **\vanI**):
"This organism has reduced susceptibility to vancomycin but is negative for *vanA* and *vanB* genes as tested by the Cepheid vanA/B GenXpert Assay (for research use only).
~This organism has been sent to the National Microbiology
~Laboratory for further testing and results will be

~reported when available."

Confirmation from NML:

Negative – Add the following statement as an 'Updated Report': "The previously reported organism has no vancomycin resistance genes as tested by the National Microbiology Laboratory, …. Winnipeg, Specimen No. xxxx"

Positive – *Enterococcus faecalis or faecium* - vancomycin-resistant "isolated"
ISOLATE COMMENT (Code: vanE):
"This organism is positive for the *vanE* gene as reported by the National Microbiology Laboratory… NML Specimen No. xxx"

VII. References

1. **Facklam R. R., and J. A. Washington** *Streptococcus* and related Catalase-Negative Gram-Positive Cocci *Manual of Clinical Microbiology* 5th Edition ASM Washington, DC

2. **National Committee for Clinical Laboratory Standards** 2003 Performance Standards for Antimicrobial Susceptibility Testing; 13th Informational Supplement Document M100-S13 (M2) for use with M2-A8 – Disk Diffusion *NCCLS*, Wayne, PA

3. **National Committee for Clinical Laboratory Standards** 2003 Performance Standards for Antimicrobial Disk Susceptibility Tests 8th ed. Approved standard M2-A8 *NCCLS*, Wayne, PA

4. **National Committee for Clinical Laboratory Standards** Performance Standards for Antimicrobial Susceptibility Testing; 13th Informational Supplement Document M100-S13 (M7) for use with M7-A6 – MIC Testing NCCLS, Wayne, PA

5. **National Committee for Clinical Laboratory Standards** 2003 Methods for Dilution antimicrobial susceptibility tests for bacteria that grow aerobically 6th ed. Approved Standard M7-A5 NCCLS, Wayne, PA

6. **QMP-LS Committee Comments**, Survey B-0109 Patterns of Practice with VRE Surveillance Specimens *QMP-LS* Bacteriology; 3, Section 2.2: 663-669

7. **Willey B. M., B. N. Kreiswirth, A. E. Simor, G. Willaims, S. R. Scriver, A. Phillips, D. E. Low** Detection of vancomycin resistance in *Enterococcus* species *J Clin Microbiol* 1992; 30:1621-1624

8. **Willey B. M., R. N. Jones, A. McGeer, W. Witte, G. French, R. B. Roberts, S. G. Jenkins, H. Nadler, D. E. Low** Practical Approach to the Identification of Clinically Relevant *Enterococcus* Species *Diag Microbiol Infect Dis* 1999; 34:165-171.

9. **Chen D., L. Pearce, A. McGeer, D. E. Low, B. M. Willey** Evalution of D-Xylose and 1% Methyl-α-D-Glucopyranosidase Fermentation Tests for Distinguishing *Enterococcus gallinarum* from *Enterococcus faecium J Clin Microbiol* 2000; 38: 3652-3655

10. **Katz K. C., A. McGeer, D. E. Low, B. M. Willey** Laboratory Contamination of Specimens with Quality Control Strains of Vancomycin-Resistant Enterococci in Ontario *J Clin Microbiol* 2002; 40:2686-2688

11. **Woodford N., A. P. Johnson, D. Morrison, D. C. E. Speller** Current Perspectives on Glycopeptide Resistance *Clin Micobiol Reviews* 1995; 8:585-615

12. **Cetinkaya Y., P. Falk, C. G. Mayhall** Vancomycin-Resistant Enterococci *Clin Microbiol Reviews* 2000; 13: 686-707

13. **Depardieu F., P. E. Reynolds, P. Courvalin** VanD-Type Vancomycin-Resistance in *Enterococcus faecium* 10/96A *Antimicrob Agents Chemother* 2003; 47: 7-18

14. **Fines M., B. Perichon, P. Reynolds, D, F.. Sahm, P. Courvalin** VanE, a New Type of Acquired Glycopeptide Resistance in *Enterococcus faecalis* BM4405 *Antimicrob Agents Chemother* 1999; 43: 2161-2164

15. **McKessar S. J., A. M. Berry, J. M. Bell, J. D. Turnbridge, J. C. Paton** Genetic Characterization of *vanG*, a Noval Vancomycin Resistance Locus of *Enterococcus faecalis Antimicrob Agents Chemother* 2000; 44: 3224-3228

16. **Alam M. R., S. Donabedian, W. Brown, J. Gordon, J. W. Chow, E. Hershberger** Heteroresistance to Vancomycin in *Enterococcus faecium J Clin Microbiol* 2001; 39:3379-3381

RESISTANT GRAM NEGATIVE BACILLI including *Serratia marcescens*

I. Introduction

These specimens may be submitted to identify carriage of drug-resistant Gram negative bacilli, to determine cross-transmission between patients or to identify an environmental source of patient infection.

II. Specimen Collection and Transport

Any specimen may be submitted, although rectal swabs and environmental are the most common. Swabs should be transported in an Eswab or Amies transport medium. If a delay in transport or processing is anticipated, the swabs should be kept at 4°C.

III. Reagents/Materials/Media

See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

A. Processing of Specimen:

a) Direct Examination: Not indicated

b) Culture:

Media	**Incubation**
For *Enterobacterales* with fluoroquinolone and/or aminoglycoside resistance but susceptibility to cefpodoxime:	
MacConkey Agar (Mac) –no antibiotic	O_2, 35^{0}C x 18 h
For *Serratia marcescens* outbreaks,	
CTCZ – with colistin	O_2, 35^{0}C x 18 h

B. Interpretation of cultures:

1. Read cultures plates after 18 to 24 hours of incubation.

2. Workup requested organism as per Bacteria Workup Manual
3. Set up susceptibility as per
4. Communicate with requesting Infection Control Practitioner or Microbiologist as appropriate and freeze all positive isolates unless otherwise directed. PFGE will only be performed on request from Infection Control.

For *Serratia* Screen:

1. Read culture plates after 18 to 24 hours of incubation.
2. For *Serratia marcescens*, work-up NLF, LLF or orange-red pigmented colonies only. Perform Vitek MS.
    - **Phone ward and email ICP** if *Serratia marcescens* is isolated.
3. Set up susceptibility as per Susceptibility Manual.
4. Previously positive *Serratia marcescens* specimens only require a meropenem screen to be set up.
5. If *Serratia* is isolated, freeze and set up BHIB for PFGE as appropriate (see Appendix II).

N.B. Susceptibilities can be referred for 3 months

V. Reporting

Negative report: "No <requested organism> isolated"

Positive report: "<requested organism> isolated"
Report their susceptibility results as per Susceptibility Manual.
Add Isolate comment: "Susceptibility testing results are provided for infection control purposes only." **\ICSN**

VI. References

1. **Clinical and Laboratory Standards Institute** 2016 Performance Standards for Antimicrobial Susceptibility Testing; Documents M100-S26, M2-A12, M7-A10 CLSI, Wayne, PA.

2. **Willey B. M., J. Bertolin, K. Schoer, G. Small, D. E. Low, A. McGeer** Evaluation of 2µg/mL Cefpodoxime screen plate for detection of 3rd generation cephalosporin resistance in *E. coli* and *Klebsiella* spp. In Abstracts: 99th American Society for Microbiology General Meeting, Chicago, 1999 (Abs# C-258).

3. **Livermore D. M.** β-Lactamases in Laboratory and Clinical Resistance *Clin Microbiol Reviews* 1995; 8:557-584.

4. **Livermore D. M. and D. F. J. Brown** Detection of β-lactamase-mediated resistance *J Antimicrob Chemother* 2001; 48: *Suppl. S1,* 59-64.

5. **Bradford P. A.** Extended-Spectrum β-Lacatmases in the 21st Century: Characterization, Epidemiology, and Detection of this important Resistance Threat *Clin Microbiol Reviews* 2001; 14: 933-951.

6. **Forward K. R., B. M. Willey, D. E. Low, A. McGeer, M. A. Kapala, M. M. Kapala, L. L. Burrows** Molecular mechanisms of cefoxitin resistance in Escerichia coli from the Toronto area hospitals *Diag Microbiol Infect Dis* 2001; 41:57-63.

7. **Courdron P. E., E. S. Moland, K. S. Thompson** Occurrence and Detection of AmpC Beta-Lactamases among *Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* Isolates at a Veterans Medical Center *J Clin Microbiol* 2000; 38:1791-1796.

8. **Pitout J. D. D., M. D. Reisberg, E. C. Venter, D. L. Church, N. D. Hanson** Modification of the Double-Disk Test for detection of *Enterobacterales* Producing Extended-Spectrum and AmpC β-Lactamases *J Clin Microbiol* 2003; 41: 3933-3935.

9. **Muller M., A. McGeer, B. M. Willey, D. Reynolds, R. Malanczyj, M. Silverman, M. Green, M. Culf** Outbreaks of multi-drug resistant Escherichia coli in long-term care facilities in the Durham, York and Toronto regions of Ontario, 2000-2002. *CCDR* 2002;28:113-8.

ESBL and Carbapenemase SCREEN

I. Introduction

These specimens are submitted to identify *Klebsiella* species (except *K. aerogenes*), *Escherichia coli* and *Proteus mirabilis* with acquired extended spectrum β-lactamases as well as carbapenemases from any *Enterobacterales*.

ESBL testing is only performed on specimens from pregnant patients, specimens originating from mothers and baby units or upon special request.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents/Materials/Media

See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

A. Processing of Specimen:

a) Direct Examination: Not indicated

b) Culture:

Media	Incubation
ESBL Isolation Agar - MacConkey with 2 µg/ml cefpodoxime (Media code: MCPOD)	O_2, 37°C x 18-24 hours

B. Interpretation of cultures:

1. Examine plates after 18-24 hours of incubation for any growth of an *Enterobacterales*.

2. If no *Enterobacterales* are isolated, report as "Negative - no ESBL or Carbapenemase producing organisms isolated."
3. For all LF and oxidase negative NLF *Enterobacterales* colony types, set up Vitek MS for identification.
4. Should an isolate ID as an *E.coli, Klebsiella spp. (except aerogenes), or P.mirabilis,* check patient history.
    - For a patient with no prior history or with "Previous" positive (>3months) history of *E.coli, Klebsiella spp., or P.mirabilis* in an IC sample set up 'KB IC ESBL '.
    - If a previous positive ESBL was isolated within the last 3 months, set up **Meropenem Screen,** only by disk diffusion. Refer to the previous sample's date that susceptibilities were reported. Report isolate with phrase

      "Phenotypic screening suggests this organism is ESBL POSITIVE as previously confirmed on "yyyy.mm.dd". LIS isolate comment code \ESBP

      Report with Test Comment:
      "Negative Carbapenemase screen - No cabapenemase producing organisms isolate. POSITIVE for ESBL screen".
5. For all other *Enterobacterales* set up **Meropenem Screen** only.
6. For CRE work up, refer to

V. Reporting

When **ESBL screen** is requested, report both ESBL and Carbapenemase comments where applicable.

Negative report for both ESBL and carbapenemase:
"Negative - No extended-spectrum-beta-lactamase producing (ESBL) or carbapenemase-producing organism isolated"

Positive reports:

Note: Positive reports for Sinai Health patients (MSH and Bridgepoint Health) should have the following comment automatically added ***\ICPR*** *"THIS PATIENT IS TO BE MANAGED IN "CONTACT PRECUATIONS" UNTIL FURTHER NOTICE"*

Positive for both ESBL and Carbapenemase:

At TEST Window:

POSITIVE for ESBL screen
POSITIVE Carbapenemase Screen

At ISOLATE Window:

"*Escherichia coli*" or "*Klebsiella* species" or "*Proteus mirabilis*" isolated **with** one of the following ISOLATE COMMENT:

"The susceptibility pattern suggests that this organism contains a class A extended spectrum beta-lactamase (ESBL)."

OR

"The susceptibility pattern suggests that this organism contains class A and C extended spectrum beta-lactamases (ESBL)."

OR

"The susceptibility pattern suggests that this organism contains a class C extended spectrum beta-lactamase (ESBL)."

OR

"The susceptibility pattern suggests that this organism contains an inducible class C extended spectrum beta-lactamase (ESBL)."

OR

"The susceptibility pattern suggests that this organism contains an extended spectrum beta-lactamase (ESBL) other than class A or C."

AND

From keypad: ESBLI: **\ICSN** "Susceptibility testing results are provided for infection control purposes only."

AND

Final Positive CRE Result by CARB-R PCR:

"_____ carbapenemase gene DETECTED by Cepheid Xpert CARBA-R Assay (for research use only). This assay

is able to detect NDM, KPC, OXA48, OXA181, OXA232, IMP-1, and VIM carbapenemase genes." Isolate Comment Code: **\CPC+**

OR

Preliminary CRE Result:

Isolate Comment: **\CNML**

AND

Send updated, Final Result once NML report is available

Negative report:

a. **Suppress the isolate**
b. Add the following comment in the TEST window for **NOT CONFIRMED** carbapenemase:
   Add TEST COMMENT code **}KPCN**
c. Enter the NML results to the LIS ISOLATE Breakpoint panel **kpcrcon.**
d. E-mail or call Infection Control Practitioner and ward as per.

Positive report:

a. "Updated Report"
b. Add the following isolate comment for **CONFIRMED** carbapenemase:
c. Add ISOLATE COMMENT code **\KPCP**
d. Enter the NML results to the LIS ISOLATE Breakpoint panel **kpcrcon.**
e. E-mail or call Infection Control Practitioner and ward as per Isolate Notification Table.

Negative report for carbapenemase but POSITIVE for ESBL:

At TEST Comment: "Negative Carbapenemase Screen - No carbapenemase-producing organism isolated"
POSITIVE ESBL Screen"

At ISOLATE Window:
"*Escherichia coli*" or "*Klebsiella* species" or "*Proteus mirabilis*" "isolated" **with** ISOLATE COMMENT: "The susceptibility pattern suggests that this organism contains a class A extended spectrum beta-lactamase (ESBL)." **OR** "The susceptibility pattern suggests that this organism contains class A and C extended spectrum beta-lactamases (ESBL)." **OR** "The susceptibility pattern suggests that this organism contains a class C extended spectrum beta-lactamase (ESBL)." **OR** "The susceptibility pattern suggests that this organism contains an inducible class C extended spectrum beta-lactamase (ESBL)." **OR** "The susceptibility pattern suggests that this organism contains an extended spectrum beta-lactamase (ESBL) other than class A or C."
Report appropriate sensitivity results as per

Previous ESBL Positive Patient:
Negative report for carbapenemase but POSITIVE for ESBL:

At TEST Comment: "Negative Carbapenemase Screen - No carbapenemase-producing organism isolated"
POSITIVE ESBL Screen"

At ISOLATE Window:
"*Escherichia coli*" or "*Klebsiella* species" or "*Proteus mirabilis*" "isolated" **with** ISOLATE COMMENT: "Phenotypic screening suggests this organism is ESBL POSITIVE as previously confirmed on "yyyy.mm.dd"."
LIS isolate comment code: \ESBP

Negative report for ESBL but POSITIVE for carbapenemase:

At TEST Comment: "Negative ESBL Screen- No extended spectrum beta-lactamase - producing organism (ESBL) isolated"
POSITIVE Carbapenemase Screen"

At ISOLATE Window:

Report isolate comment as per

Previous Carbapenemase Positive Patient:

At TEST Comment: "Negative ESBL Screen- No extended spectrum beta-lactamase –

producing organism (ESBL) isolated POSITIVE Carbapenemase Screen"

OR

"POSITIVE ESBL Screen and POSITIVE Carbapenemase Screen"

At ISOLATE Window: Report isolate along with Isolate Comment:

"Phenotypic testing suggests this organism is carbapenemase POSITIVE as previously confirmed on "yyyy.mm.dd"."
Isolate Comment code \CREP

VI. Reference

1. **Clinical and Laboratory Standards Institute** 2016 Performance Standards for Antimicrobial Susceptibility Testing; Documents M100-S26, M2-A12, M7-A10 CLSI, Wayne, PA.
2. **Willey B. M., J. Bertolin, K. Schoer, G. Small, D. E. Low, A. McGeer** Evaluation of 2µg/mL Cefpodoxime screen plate for detection of 3rd generation cephalosporin resistance in *E. coli* and *Klebsiella* spp. In Abstracts: 99th American Society for Microbiology General Meeting, Chicago, 1999 (Abs# C-258).
3. **Livermore D. M.** β-Lactamases in Laboratory and Clinical Resistance *Clin Microbiol Reviews* 1995; 8:557-584.
4. **Livermore D. M. and D. F. J. Brown** Detection of β-lactamase-mediated resistance *J Antimicrob Chemother* 2001; 48: *Suppl. S1*, 59-64.
5. **Bradford P. A.** Extended-Spectrum β-Lacatmases in the 21st Century: Characterization, Epidemiology, and Detection of this important Resistance Threat *Clin Microbiol Reviews* 2001; 14: 933-951.
6. **Forward K. R., B. M. Willey, D. E. Low, A. McGeer, M. A. Kapala, M. M. Kapala, L. L. Burrows** Molecular mechanisms of cefoxitin resistance in Escerichia coli from the Toronto area hospitals *Diag Microbiol Infect Dis* 2001; 41:57-63.
7. **Courdron P. E., E. S. Moland, K. S. Thompson** Occurrence and Detection of AmpC Beta-Lactamases among *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* Isolates at a Veterans Medical Center *J Clin Microbiol* 2000; 38:1791-1796.
8. **Pitout J. D. D., M. D. Reisberg, E. C. Venter, D. L. Church, N. D. Hanson** Modification of the Double-Disk Test for detection of *Enterobacterales* Producing Extended-Spectrum and AmpC β-Lactamases *J Clin Microbiol* 2003; 41: 3933-3935.

9. **Muller M., A. McGeer, B. M. Willey, D. Reynolds, R. Malanczyj, M. Silverman, M. Green, M. Culf** Outbreaks of multi-drug resistant Escherichia coli in long-term care facilities in the Durham, York and Toronto regions of Ontario, 2000-2002. *CCDR* 2002;28:113-8.

Carbapenemase (CRE) SCREEN (without ESBL Screen)

I. Introduction

These specimens are submitted to identify carbapenemases from any *Enterobacterales*.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents/Materials/Media

See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

A. Processing of Specimen:

a) Direct Examination: Not indicated
b) Culture

Specimen	Processing	Media	Incubation
Environmental swabs	Incubate the BHI Broth Subculture BHI broth after overnight incubation to MCPOD by the IC Bench tech using a new sterile swab	ESBL Isolation Agar – MacConkey with 2 µg/ml cefpodoxim (MCPOD)	O_2 at 35°C overnight O_2 at 35°C for 24 hours
Swabs from patients	Directly inoculate MCPOD plate with specimen	ESBL Isolation Agar – MacConkey with 2 µg/ml cefpodoxim (MCPOD)	O_2 at 35°C for 24 hours

B. Interpretation of cultures:

See IC Carbapenemase Testing Flowchart

1. Examine plate after 18-24 hours of incubation for any growth of an *Enterobacterales*.
2. If no *Enterobacterales* are isolated, report as negative for CRE.
3. For all *Enterobacterales* colony types, set up a meropenem screen disk diffusion test.
4. If isolates >25mm (susceptible) by "MEMS" disk diffusion, report as negative for CRE.
5. For all Meropenem Screen R (≤25mm) by disk diffusion, Set up Vitek MS
   - If the isolate is not identified as *Enterobacterales*, report as negative for CRE.
   - If the isolate is identified as *Enterobacterales*, suppress the isolate and set up βCARBA (BCARB)

a) **If βCARBA (BCARB) is negative**:

i. Set up Rosco with Temocillin (breakpoint panel kpcros)

- If **Temocillin = S** and Rosco disks show no potentiation, send out report as NO CRE.
- If **Temocillin = R** OR Rosco shows potentiation to MRBO or MRDP,
  - Report isolate with the following ISOLATE Comment: **\CNML**
  - Phone or e-mail IC and ward as per Isolate Notification Table. Send isolates to NML ASAP (Cannot send on Friday)
  - Order the LIS ISOLATE Breakpoint panel **kpcrcon.**
  - Freeze isolate

b) **If βCARBA (BCARB) is positive**:

– **Previous CRE positive (≤ 6 months)**

i. At TEST Comment: "POSITIVE Carbapenemase Screen"
At ISOLATE Window: Report (un-suppress) isolate along with Isolate Comment:
\CREP

- Phone or e-mail IC and ward as per.

– **New ?positive CRE**
Report (un-suppress) isolate. with comment **\PCRB**
Notify ICP

i. Set up Cepheid CARBA-R PCR (CARBR)

- If Cepheid CARBA-R PCR (CARBR) is negative
  - Report isolate with the following ISOLATE Comment: **\pCRB**
  - Phone or e-mail IC and ward as per Isolate Notification Table.
  - Send isolates of to NML ASAP (Cannot send on Friday)
  - Order the LIS ISOLATE Breakpoint panel **kpcrcon**
  - Set up Rosco with Temocillin (panel kpcros). For epidemiology purposes only. Record and suppress kpcros results.

- If Cepheid CARBA-R PCR (CARBR) is positive
  - Report gene identified by Cepheid using ISOLATE Comment: **\CPC+**
  - Phone or e-mail IC and ward as per Isolate Notification Table.
  - Send to NML and PHOL in batches when requested for and .
  - Freeze isolate (FRZ).
  - set up BHIB/SUBBA for PFGE as appropriate (see Appendix II)

V. Reporting

See Carbapenemase Testing Reporting.

VI. Reference

1. **Clinical and Laboratory Standards Institute** 2016 Performance Standards for Antimicrobial Susceptibility Testing; Documents M100-S26, M2-A12, M7-A10 CLSI, Wayne, PA.

2. **QMP-LS Bacteriology Consensus Practice Recommendations** – Antimicrobial Susceptibility Reporting Toronto ON: QMP-LS QView. c2011

RESISTANT *Pseudomonas aeruginosa* SCREEN

I. Introduction

Specimens are submitted for the screening of multi-drug resistant *Pseudomonas aeruginosa*.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents/Materials/Media

See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

1. Processing of Specimen:

Specimen	Processing	Media	Incubation
Water	Centrifuge the entire sample at 3500 rpm for 20 minutes. Pour off all supernatant. Transfer the contents of a 2 mL tube of BHI broth into in the falcon tube containing the sediment	BHI Broth	O_2 at 35°C overnight
	Subculture BHI broth after overnight incubation to MCPOD by the IC Bench technologist	MCPOD	O_2 at 35°C for 24 hours
Environmental swabs	Incubate the BHI Broth		O_2 at 35°C overnight
	Subculture BHI broth after overnight incubation to MCPOD by the IC Bench tech using a new sterile swab	MCPOD	O_2 at 35°C for 24 hours
Patient pharmaceutical	≤1 mL	TH14 SD14	O_2 at 35°C for 14 days O_2 at RT° for 4 days

Specimen	**Processing**	**Media**	**Incubation**
infusates/injectables (QC bench)	>1 mL	ETH14 ESD14	O_2 at 35°C for 14 days O_2 at RT° for 4 days
Swabs from patients	Directly inoculate MCPOD plate with specimen	MCPOD	O_2 at 35°C for 24 hours

2. Interpretation of Cultures:

For **water, environmental swabs, patient swabs**:
Work up these cultures on the IC Bench.
Work up oxidase-positive gram negative bacilli ONLY.
Set up Vitek MS
When identified as *P. aeruginosa* set up Vitek susceptibility card.
For patient samples, if resistant to all antimicrobials from the vitek card, set up colistin etest.
Freeze resistant strains of *Pseudomonas aeruginosa* into IGR boxes.

For **Patient pharmaceutical infusates/injectables**:
Work up these cultures on the QC/Sterility Bench.
Work up any growth as per Sterility Manual.

V. Reporting

For **water, environmental swabs, patient swabs**:

Negative Report: No resistant *Pseudomonas aeruginosa* isolated.

Positive (Resistant strains only) Report: *Pseudomonas aeruginosa* with susceptibility result.
Add Isolate comment: "Susceptibility testing results are provided for infection control purposes only." **\ICSN**

Email / Call ICP.

For **Patient pharmaceutical infusates/injectables**:

Negative Report: No growth.

Positive: Report *Pseudomonas aeruginosa* with susceptibility result. Call ICP
Add Isolate comment: "Susceptibility testing results are provided for infection control purposes only." **\ICSN**

VI. References

Clinical and Laboratory Standards Institute 2016 Performance Standards for Antimicrobial Susceptibility Testing; Documents M100-S26, M2-A12, M7-A10 CLSI, Wayne, PA.

Candida auris DIRECT-TO-AGAR TESTING

I. Introduction

Candida auris has been identified as an emerging multi-drug resistant organism, screening high-risk patients for resistant *C. auris* can prevent further spread in a hospital setting. The CHROMagar Candida Plus Agar contains a species-specific chromogen that turns *Candida species* different colors.

Please refer to related document for Broth-to-Agar procedure

II. Specimen Collection and Transport

The most common specimens submitted to identify carriers of *C. auris* are nasal swab, axillary/groin swab or combined nasal/axillary/groin/perineum swab. Samples may be considered from any body site.
C. auris screening should include a single bilateral swab of a patient's axilla and groin. In addition, single swabs of previously colonized or clinically relevant sites may also be indicated (for example: wounds, exit sites of devices, external ear canal).
See Pre-analytical Procedure – Specimen Collection QPCMI02001?

III. Reagents/Materials/Media

Material	Vendor
Chromagar Candida Plus Agar	Micronostyx

IV. Procedure

A. Processing of Specimen:

a) Direct Examination: Not indicated

b) Direct-to-Agar Culture: Specimens are planted by WASP and incubated within the WASPlab system.

Media	Incubation
CHROMagar Candida Plus Agar	O_2 37°C x 48 h – in the dark

c) Broth-to-Agar Culture: See related document

B. Automated WASPLab culture reads - perform as per WASPLab Screening, Reading and Picking Manual.

Offline culture reads - For small amount of plates requiring offline incubation, keep plates in covered container in the walk-in incubator (O2, 37oC) and screen plates at the beginning of your shift and at time of final incubation (end of your shift or earlier as applicable).
When significant downtime occurs, separate plates by shift planted into larger buckets for screening as above until final reads.

C. Interpretation of cultures:

CHROMagar plates will be read at 36hrs and have a final read at 48hrs for *Candida auris.* Assess any growth on culture media based on the color of the colonies, as well as color of the halo around the colonies. The reverse of the plate can also help determine whether to suspect growth of C. auris

Colonies on CHROMagar Candida Plus Agar: (Refer to Figures 1 & 2)

Isolate	**Colony colour**
Candida auris	White with blue halo, blue on reverse of the plate[1]
Candida albicans	Green-blue
Candida parapsilosis	Dark pink
Candida tropicalis	Metallic blue with pink halo
Candida glabarta	Mauve
Candida krusei	Pink and fuzzy
Candida lusitaniae	Purple
[1] At 36 hours, *C. auris* colonies may appear light blue; colonies change to white after 48 hours incubation and may appear pale pink at 72 hours; halo may not be readily apparent until 48 hours.	

1. **Suspect C. auris colonies** (light blue/white colonies with blue halo)
    a. Report as presumptive positive and status as preliminary if isolated colonies matches description (See reporting section).
    b. If there are sufficient isolated colonies, set up MALDI to confirm.
        i. If identified as *Candida auris*, then send out a positive report (See reporting section) and status as preliminary.

ii. Then, sub-culture to sheep blood agar and send to PHOL the next day for susceptibility testing.

c. If colonies are not isolated or no I.D. on MALDI, sub-culture to sheep blood agar and repeat MALDI the next day.
   i. If identified as *Candida auris*, then send out a positive report (See reporting section) and status as preliminary. Then, send sheep blood agar plate to PHOL for susceptibility testing.
   ii. If still no I.D., OR I.D. other than *Candida auris*, then send sheep blood agar plate to PHOL for confirmation of I.D. and request for susceptibility testing if confirmed to be *Candida auris*
   iii. Freeze all isolates before sending out to PHOL

2. **No suspect *Candida auris* colonies**
   a. Re-incubate negative plates for further incubation as needed.
   b. Finalize as negative at 48 hours. (See reporting section)

D. Reporting

Negative report:	"No *Candida auris* isolated."
Presumptive positive:	"Suspect *Candida auris* isolated, confirmation to follow."
Positive report:	"*Candida auris* isolated." Positive reports for Sinai Health patients (MSH and Bridgepoint Health) should have the following comment automatically added **\ICPR** "THIS PATIENT IS TO BE MANAGED IN "CONTACT PRECUATIONS" UNTIL FURTHER NOTICE". Communicate to ward and/or infection control if necessary as per Isolate Notification and Freezing Table QPCMI16003

Figure 1. Appearance of Common *Candida* species on CHROMagar Candida Plus Agar

24 h and 48 h incubation in O_2 at 30-37°C
CHROMagar Candida Plus Agar

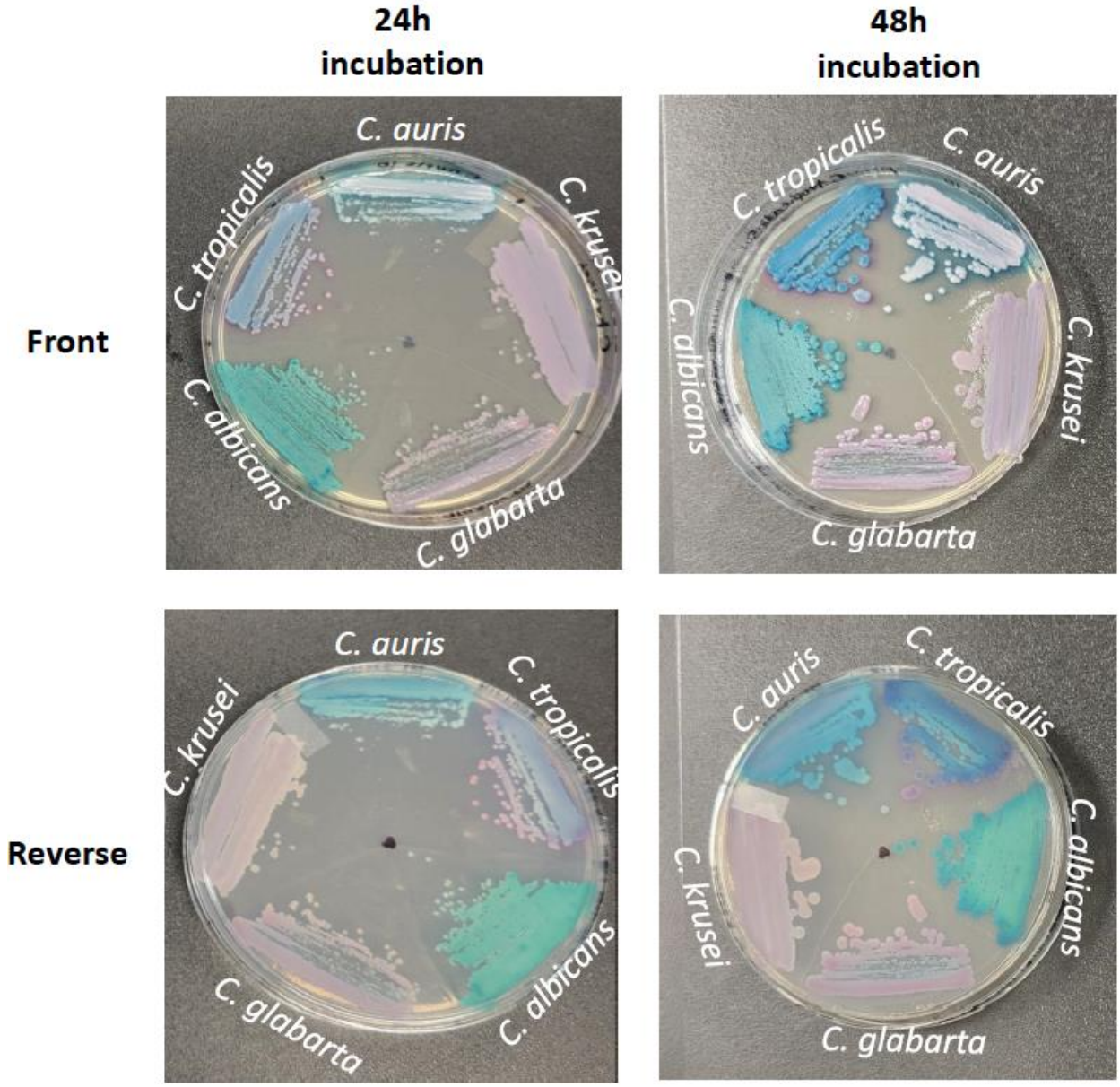


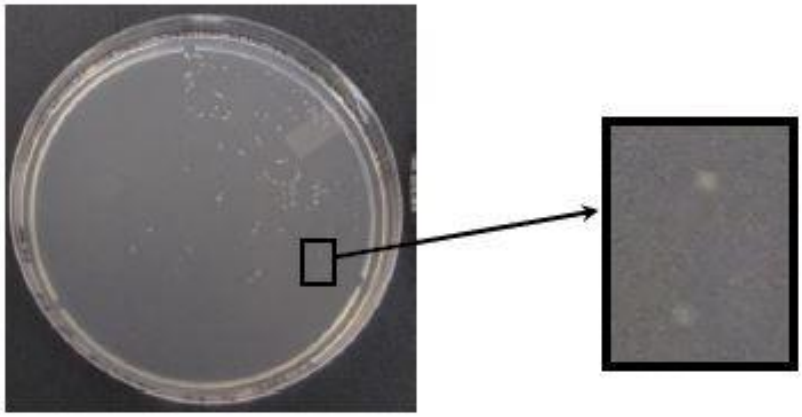
Figure 2. Appearance of direct to agar planting of nasal/bilateral axillary/groin eSwab spiked with *Candida auris*

48 h incubation in O_2 at 37°C
CHROMagar Candida Plus Agar

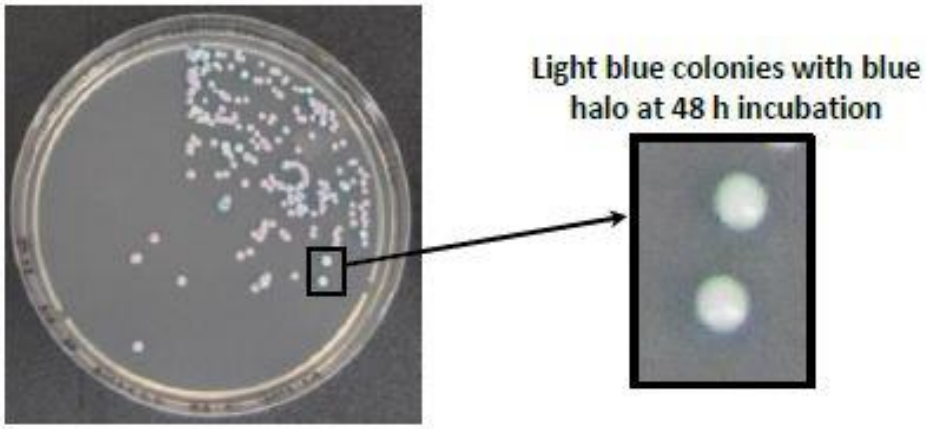
1. *Candida auris*
2. *Candida albicans*
3. *Candida parapsilosis*
4. *Candida lusitaniae*

Figure 3. *Candida auris* colonies after 24h and 48h incubation in O_2 at 37°C on CHROMagar Candida Plus Agar

A. *C. auris* colonies at 24 h incubation



B. *C. auris* colonies at 48 h incubation



E.

1. References

1. Borman AM, Fraser M, Johnson EM. CHROMagar™ Candida Plus: A novel chromogenic agar that permits the rapid identification of Candida auris. Medical Mycology. 2021; 59:253-258.
2. Bayona JVM, Garcia CS, Palop NT, Cardona CG. Evaluation of a novel chromogenic medium for Candida spp. identification and comparison with CHROMagar™ Candida for the detection of

Candida auris in surveillance samples. Diagnostic Microbiology and infectious Disease. 2020; 98:1-5.

3. de Jong AW, Dieleman C, Carbia M, Tap RM, Hagen F. Performance of Two Novel Chromogenic Media for the Identification of Multidrug-Resistant Candida auris Compared with Other Commercially Available Formulations. Journal of Clinical Microbiology. 2021; 59:1-9.
4. Public Health Ontario. Interim Guide for Infection Prevention and Control of Candida auris. January 2019. https://www.publichealthontario.ca/-/media/documents/P/2019/pidac-ipac-candida-auris.pdf (accessed Sept. 8, 2021)
5. Public Health Agency of Canada. Candida autis interim recommendations for infection prevention and control. https://www.canada.ca/en/public-health/services/infectious-diseases/nosocomial-occupational-infections/notice-candida-auris-interim-recommendations-infection-prevention-control.html

GROUP A STREPTOCOCCUS SCREEN

I. Introduction

Throat, rectal or wound swabs are the most common that are submitted for the diagnosis of Group A streptococcal infection, to determine cross-transmission between patients or to identify an environmental source of patient infection.

II. Specimen Collection and Transport

See Pre-analytical Procedure - Specimen Collection QPCMI02001

III. Reagents / Materials/ Media

See Analytical Process - Bacteriology Reagents_Materials_Media List QPCMI10001

IV. Procedure

A. Processing of Specimens

See Specimen Processing Procedure

a) Direct Examination: Not routinely performed.
b) Culture:

Media	**Incubation**
CNA (rectal/wound)	AnO_2, 35°C x 18-24 hours
Carrot Broth	O_2, 35°C x 18-24 hours
BA (for throat)	AnO_2, 35°C x 18-24 hours

If original plates are negative;
Subculture the Carrot Broth to a second CNA plate and incubate overnight in AnO_2, 35°C x 18-24 hours

B. Interpretation of Cultures:
a) Examine the CNA/ BA plate after 18-24 hours incubation and identify all morphologically distinct beta haemolytic colonies by performing:
i) Catalase test
ii) Strep grouping

b) For all specimens processed after 1600 hours, re-incubate CNA/BA anaerobically for a further 24 hours.
c) Subculture the Carrot broth to a second CNA/BA plate and incubate overnight in anaerobic conditions
d) Examine the subculture CNA/BA plate after overnight incubation for distinct beta haemolytic colonies.
e) Perform catalase and strep grouping if any beta haemolytic colonies appear.
f) Freeze all isolates and prepare for PFGE (whether in house or to be sent to PHL)
g) No Susceptibility Testing Required
h) E-mail or call Infection Control Practitioner and ward as per.

V. Reporting

A. Culture:

Negative report: "No Group A streptococcus isolated".

Positive report: Report as isolate - "Group A streptococcus" with LIS ISOLATE COMMENT: "isolated"

E-mail or call all positive Group A streptococci isolates to ward / Infection Control Practitioners as per Isolate Notification Table.

.

VI. References

Clinical and Laboratory Standards Institute 2016 Performance Standards for Antimicrobial Susceptibility Testing; Documents M100-S26, M2-A12, M7-A10 CLSI, Wayne, PA

KLEBSIELLA OXYTOCA OR *KLEBSIELLA PNEUMONIAE* SCREEN

These specimens may be submitted to identify carriage of drug resistant ESBL producing *Klebsiella oxytoca* or *Klebsiella pneumoniae*, to determine cross-transmission between patients or to identify an environmental source of patient infection. See ESBL and CARBAPENEMASE SCREEN.

APPENDIX I - HOW TO SET UP AND INTERPRET A MIC PANEL

I. Materials

MIC panel
Panel inoculator set
Sterile distilled water
Sterile transfer pipettes
Blood agar plate
Sealable bag

II. Procedure

1. Remove the desired MIC panel from the -70^0C freezer. Place a cover over the panel and place into the O_2 incubator to thaw.

2. When thawed, label the panel and a blood agar plate with the LIS order number.

3. Make a suspension of the organism in saline to match a 0.5 McFarland standard.

4. Place 1.5 mL of organism into a 50mL tube. Add sterile distilled water to reach 40mL on same falcon tube (~38.5mL). Pour into the inoculator base. Gently mix by agitating slowly

5. Place the inoculator into the base making sure there are no bubbles and that all prongs are in contact with the bacterial suspension.

6. Align the left side (lettered) of the panel with the left side (lettered) of the inoculator.

7. Lift the inoculator straight up and place it, prong side down, into the wells of the MIC panel.

8. Using a transfer pipette, transfer 1 drop of suspension from within the inoculator base to a blood agar plate and streak for isolated colonies.

9. Pour the suspension into a sharps container containing hypochloride and discard the inoculator into a sharps disposal box.

10. Place a lid onto the panel and place into a sealable bag. Seal the bag and incubate the panel in the appropriate atmosphere and temperature (See below).

Panel	Temp.	Atmosphere	Incubation
VRE (vancomycin)	35^0C	O_2	24 h
MRSA (oxacillin)	35^0C	O_2	24 h
GNB	35^0C	O_2	24 h

III. Interpretation

Use a coordinating MIC panel sheet to record wells with any growth. Each panel contains a positive growth control well (no antibiotic) and a negative growth control well (no inoculum). The MIC for each drug is the lowest dilution showing no growth. Record results in the LIS.

Interpretation of MIC results is performed in accordance with NCCLS breakpoint criteria found in the Performance Standards for Antimicrobial Susceptibility Testing Informational supplement M100-S**. This informational supplement is updated annually and breakpoint criteria for all antibiotics used should be checked yearly for changes.

MIC breakpoints for antimicrobial agents tested in MIC panels that do not have NCCLS criteria available should be obtained from the literature (see references for agents such as mupirocin). When breakpoints are not available in the literature, no interpretation of MIC should be reported.

IV. References

1. **Clinical Laboratory Standards Institute** 2016. Performance Standards for Antimicrobial Susceptibility Testing; 26th ed. CLSI Approved Standard M100S, Clinical and Laboratory Standards Institute, Wayne, PA

2. **Clinical Laboratory Standards Institute** 2015. Methods for Dilution antimicrobial susceptibility tests for bacteria that grow aerobically 10th ed. CLSI Approved Standard M7A, Clinical and Laboratory Standards Institute Wayne, PA

3. **Fuchs P. C., R. N. Jones, A. L. Barry** Interpretive Criteria for Disk Diffusion Susceptibility Testing of Mupirocin, a Topical Antibiotic *J Clin Microbiol* 1990; 28: 608-609

4. **Skov R., N., Frimodt-Moller, F. Espersen** Correlation of MIC methods and tentative interpretive criteria for disk diffusion susceptibility testing using NCCLS methodology for fusidic acid *Diag Microbiol Infect Dis* 2001; 40: 111-116

APPENDIX II – Referring Isolates for PFGE testing

Organism	PFGE	BHIB	Frequency	Site	Notes
MRSA	Yes	Yes	New, then once every 3 months	MSH only **Excluding BPH**	UHN, BPH on request only
VRE	Yes	yes	New, then once every 12 months	MSH only **Excluding BPH**	UHN, BPH on request only
Serratia	Yes	Yes	All	MSH NICU only.	Others on request only.
Group A Streptococcus	On ICP request only	On ICP request only	On ICP request only		Inpatient only
CRE	On ICP request only	On ICP request only	On ICP request only		
Other organisms not listed	On ICP request only	On ICP request only	On ICP request only		

Record of Edited Revisions

Manual Section Name: Infection Control Manual

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	March 13, 2002	Dr. T. Mazzulli
Annual Review	October 25, 2003	Dr. T. Mazzulli
Annual Review	May 26, 2004	Dr. T. Mazzulli
Annual Review	May 12, 2005	Dr. T. Mazzulli
Annual Review	July 23, 2006	Dr. T. Mazzulli
Link to IC\Infection Control Pulsed-field Gel Electrophoresis.doc added	January 30, 2007	Dr. T. Mazzulli
Link to IC\VRE PCR Procedure.doc added	January 30, 2007	Dr. T. Mazzulli
Enter the no. of pink colonies grown on MRSA-*Select* if <5	January 30, 2007	Dr. T. Mazzulli
Added quantitation for MRSA	February 28. 2007	Dr. T. Mazzulli
Change to Denim Blue plates for MRSA Screen	March 13, 2007	Dr. T. Mazzulli
Change negative resulting phrases for MRSA, VRE and ESBL screen	March 13, 2007	Dr. T. Mazzulli
Included P. mirabilis for ESBL screen	March 13, 2007	Dr. T. Mazzulli
Annual Review	March 13, 2007	Dr. T. Mazzulli
Revised VRE Identification Procedure	March 22, 2008	Dr. T. Mazzulli
VRE – VANCS resistant *E. faecium* or *E. faecalis* report to MSH ICP if it is MSH patient; change to report as Presumptive VRE to all ICP	September 20, 2008	Dr. T. Mazzulli
Pseudo screen, patient swabs – change incubation period from 48 hours to 24 hours	September 20, 2008	Dr. T. Mazzulli
Annual Review	September 20, 2008	Dr. T. Mazzulli
Annual Review	September 20, 2009	Dr. T. Mazzulli
Annual Review	September 20, 2010	Dr. T. Mazzulli
ESBL screen updated to include KPC and NDM screen	November 10, 2010	Dr. T. Mazzulli
Removed send by taxi for carbapenemase PCR send out for Monday, Wednesday and Thursday	January 20, 2011	Dr. T. Mazzulli
Modified carbapenemase screening procedure to match Susceptibility manual	April 04, 2011	Dr. T. Mazzulli
Change VRE screening to Brilliance VRE Agar	April 04, 2011	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
Removed VRE Table 3; added link to Susceptibility manual	May 11, 2011	Dr. T. Mazzulli
VRE Screen, added VANCS back to heavy growth from BVRE or SBVRE	May 31, 2011	Dr. T. Mazzulli
VRE Screen – modified, finalized all day 2 reading in the morning of day 2	October 17, 2011	Dr. T. Mazzulli
Annual Review	October 17, 2011	Dr. T. Mazzulli
Modified Serratia screen	November 25, 2011	Dr. T. Mazzulli
Modified VRE resulting phrases	December 13, 2011	Dr. T. Mazzulli
Added CRE only screen	December 13, 2011	Dr. T. Mazzulli
Modified VRE reporting for vanA gene positive but phenoptype vancomycin=S strains	February 1, 2012	Dr. T. Mazzulli
Added link to VRE PCR by Cephied	July 16, 2012	Dr. T. Mazzulli
Modified planting volume into BHI broth for VRE/MRSA	August 28, 2012	Dr. T. Mazzulli
Annual Review	August 28, 2012	Dr. T. Mazzulli
Annual Review	May 31, 2013	Dr. T. Mazzulli
Manual updates in each section (Maldi procedure review)	October 10, 2013	Dr. T. Mazzulli
Changed CRE screen from ERTA to MERO discs	October 10, 2013	Dr. T. Mazzulli
Update VRE Identification	April 19, 2014	Dr. T. Mazzulli
Annual Review	April 19, 2014	Dr. T. Mazzulli
CRE reporting changes (Mero screen I/R)	June 27, 2014	Dr. T. Mazzulli
VITEK SXT=R SUPPRESS SXT confirm result by KB BEFORE reporting.	June 27, 2014	Dr. T. Mazzulli
vanB gene detected by Cepheid Xpert vanA/vanB Assay reporting	August 6, 2014	Dr. T. Mazzulli
Insert proper headers/footers, UHN/MSH Logo Fix broken link to appendix 2	August 12, 2014	Dr. T. Mazzulli
Added Group A Strep Screen and Klebsiella Screen	September 30, 2014	Dr. T. Mazzulli
Reviewed and updated procedure steps in all sections	September 30, 2014	Dr. T. Mazzulli
Added MRSA scant growth repeat broth culture comments	December 10, 2014	Dr. T. Mazzulli
Annual Review MRSA Removed MACRO use p.6 add "Scant growth (1-5 colonies) **Upon Infection Control request to replant into BHIB (2mL**):"	May 19, 2015	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
p.19 changed serratia media to new ctcz media' p.22 ESBL testing is only performed on specimens from pregnant patients, specimens originating from mothers and baby units or upon special request. p.35 BA (for throat) added to GAS screen procedure culture media Changed Mac with cefpodoxime to ESBL isolation agar with cefpodoxime p.39 added ESBL comments to reporting comments p.24: added suscep. comment: \MRSS		
Specimen collected and transport for each section transferred to Specimen collection manual QPCMI02001 And replaced with link to specimen collection manual	May 26, 2015	Dr. T. Mazzulli
VRE outbreak: Temporary Procedure change in effect :VRE PCR on **any** amount of purple colonies	June 11, 2015	Dr. T. Mazzulli
VRE outbreak: Temporary Procedure change ended. Section removed.	July 15, 2015	Dr. T. Mazzulli
Prev + ESBL and Prev + CRE new statements	August 20, 2015	Dr. T. Mazzulli
p.23 Previously ESBL reporting phrased changed from "Susceptibility not done, please refer to sample collected on __Date_" to "Phenotypic screening suggests this organism is ESBL POSITIVE as previously confirmed on "yyyy.mm.dd"." LIS isolate comment code: \ESBP	December 2, 2015	Dr. T. Mazzulli
Updated ESBL+CRE and CRE sections with new reporting phrases Updated CRE section with new BCARB/CARB-R/ROSCO procedure	December 21, 2015	Dr. T. Mazzulli
MRSA reporting section: added link for susceptibility comments to MRSA reporting phrases in susceptibility manual. Resistant GNB reporting section & Pseudomonas screen section added for Positive reports: Add comment: "Susceptibility testing results are provided for infection control purposes only." **\ICSN**	April 4, 2016	Dr. T. Mazzulli
Added links in TOC to CNISP Surveillance Study for MRSA, VRE, and CRE and PHOL CRE Surveillance Study as well as in the CRE procedure section.	April 12, 2016	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	May 19, 2016	Dr. T. Mazzulli
VRE outbreak: Temporary Procedure change in effect :VRE PCR on **any** amount of purple colonies	June 13, 2016	Dr. T. Mazzulli
MRSA for new MRSA added step "Send to NML in batches when requested by IC for CNISP surveillance" VRE - added HEAVY growth workup	July 29, 2016	Dr. T. Mazzulli
VRE: Updated commetnb \vaAi to include : "The effectiveness of vancomycin in this setting is uncertain and is not recommended. Please contact Infectious Diseases or Medical Microbiology for treatment advice."	December 1, 2016	Dr. T. Mazzulli
Annual Review	May 20, 2017	Dr. T. Mazzulli
Updated Direct VRE PCR results with instructions to phone/email as per Isolate Notification and freezing table.	February 2, 2018	Dr. T. Mazzulli
Annual Review Temporary Procedure change in effect:VRE PCR on **any** amount of purple colonies. PFGE for all new VRE from MSH NOT UHN. Instructions to release ID once PCR is done of *E. faecium or faecalis* for suspect colonies if Vitek MS fails, confirmation of ID to follow. WASPLAB screening/incubation time changes: • MRSA 12hr & 24hrs modified to one 24hr read on Wednesday May 18th, 2018 evening. • MRSA 24 hr read changed to 18 & 24 hr read to Monday May 21, 2018 evening. • VRE changed from 12 & 36 to 18, 30, 36 on Monday May 21, 2018 evening.	May 22nd, 2018	Dr. T. Mazzulli
Minor format change	September 14, 2018	Dr. T. Mazzulli
Addition of Wasplab changes to MRSA section.	December 11, 2018	Dr. T. Mazzulli
Annual Review Update of MUP to MUP_{200}	November 11, 2019	Dr. T. Mazzulli
Updated CPO flowchart. Removed reporting and notification for BCARB negative Enterobacterales (}NCRB), and icp notification of Enterobacterales that are BCARB & Rosco negative reported as No CPO.	December 19, 2019	Dr. T. Mazzulli

Full document review included in all updates. Bi-annual review conducted when no revision had been made within 2 years.

Page Number / Item	Date of Revision	Edited by:
Removed temporary VRE procedure to test any new purp by PCR – inserted into permanent procedure	February 02, 2021	Dorna Zareianjahromi
Minor formatting change	April 11, 2021	Jessica Bourke
Nomenclature update – Enterobacterales	April 19, 2021	Wayne Chiu
Added info regarding Vanco dependant enterococci	May 25, 2021	Wayne Chiu
Updated positive reporting comments for VRE, MSRA, ESBL, CPE for Sinai Health to include contact precautions comment.	May 26, 2021	Jessica Bourke
Added C auris direct to agar section	Nov 5, 2021	Wayne Chiu
Clarified ESBL isolation agar – mac with cefpod	Nov 16, 2021	Wayne Chiu
Minor formatting changes, clarified appendices	Nov 22, 2021	Wayne Chiu
Added appendix II – referring isolate for PFGE Referred instructions for PFGE to appendix II	April 1, 2022	Wayne Chiu
Added environmental swab processing for CRE Removed fusidic acid testing	July 31, 2022	Wayne Chiu
Added specimen collection details from PHAC reference	Dec 28, 2022	Wayne Chiu