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CRE PCR by Cepheid GeneXpert

Analytical Process - Bacteriology Reagents, Materials, Media List QPCMI10001

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:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXOID Denim Blue Agar (DBLUE)*</td>
<td>O₂, 37°C x 24 h -in the dark</td>
</tr>
</tbody>
</table>

*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 WASP lab 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 (O₂, 37°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:
  o plates with no blue colonies will be reported automatically through segregation at 18hrs for re-incubation with a prelim status.
  o 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
  o At 18hrs: select 18hr-Reinc and send results to re-incubate and status to the MRSA test.
  o 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 (MUP200) disc. When complete, interim for review as “MRSA”. Set up MUP200 E-test if MUP200 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 (5).

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


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:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brilliance VRE Agar (BVRE)</td>
<td>O₂, 37°C x 36hrs in the dark</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) Place 500uL (0.5 mL mark of transfer pipette) of the eSwab transport medium into:</td>
<td>overnight on shaker</td>
</tr>
<tr>
<td>- 2 mL Brain Heart Infusion broth (BHIB)</td>
<td></td>
</tr>
<tr>
<td>Place 30uL (1 drop from transfer pipette) of the eSwab transport medium onto:</td>
<td></td>
</tr>
<tr>
<td>- Brilliance VRE Agar (BVRE)</td>
<td>36h in the dark</td>
</tr>
<tr>
<td>ii) If BVRE is no growth after overnight incubation, subculture 1 drop from BHIB to:</td>
<td></td>
</tr>
<tr>
<td>- Brilliance VRE Agar (BVRE)</td>
<td>36h in the dark</td>
</tr>
</tbody>
</table>
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 \textit{faecium} \& \textit{faecalis}

<table>
<thead>
<tr>
<th>Isolate:</th>
<th>Colony colour:</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Enterococcus faecium}</td>
<td>Purple to Royal Blue colour on entire colony, moist</td>
</tr>
<tr>
<td>\textit{Enterococcus faecalis}</td>
<td>Denim Blue</td>
</tr>
<tr>
<td>CNST</td>
<td>Blue (if grown)</td>
</tr>
<tr>
<td>Yeast</td>
<td>Light blue (if grown)</td>
</tr>
<tr>
<td>\textit{Enterococcus gallinarum}</td>
<td>Blue (if grown)</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>Light blue/pink (if grown)</td>
</tr>
</tbody>
</table>

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).
      \textit{Note: For isolates with unsuccessful ID results, report as Vancomycin-Resistant \textit{E.faecium}, confirmation to follow (with vanA/B positive result)}
      \textit{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 (\textless;3 months)
      - Set up Vitek MS, VANCS, PP,
- Do NOT report out isolate until VANCS results are known. (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

<table>
<thead>
<tr>
<th>NEW Purple/Royal Blue Colonies</th>
<th>PREVIOUS + (&lt;3months) Purple cols</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVRE &gt;5cols 24/48 hours</td>
<td>BVRE (any amount)</td>
</tr>
</tbody>
</table>

1. Set up vanA/vanB Cepheid PCR and MS and SBVRE and SUBBA

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. Set up MS and VANCS

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,
  - Add comment non vanA/B to isolate
  - Send to NML for van genotyping & FRZ
  - Notify ICP
Table 2  VRE Workup Guide – BLUE COLONIES

<table>
<thead>
<tr>
<th>Blue Colonies (SCANT /LIGHT growth)</th>
<th>Blue Colonies (HEAVY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set up SBVRE on any amount of blue cols growing</td>
<td>Vitek MS ID of Enterococcus faecium or Enterococcus faecalis.</td>
</tr>
</tbody>
</table>

| NG on SBVRE | Scant Growth on SBVRE | Mod-Heavy Growth on SBVRE | Report – No VRE |
|-------------|-----------------------|---------------------------|----------------|-----------------|
| Report – No VRE | Set up VANCS ‘PP’ | Set up Cepheid PCR & MS | 1. VANCS – No growth | 1. Cepheid – Positive |
| 1. VANCS – No growth | | | • Report No VRE | • Follow NEW Purple >5 Cepheid positive workflow. |
| 2. VANCS - Growth | | | • Set up MS | 2. Cepheid – Negative |
| • Perform Cepheid PCR, Etests. | | | • Set up Etests & VANCS | • If Vanco Etest ≥8ug/mL, VANCS - growth, add comment: ‘non vanA/B’ to isolate |
| • Proceed as Mod-Heavy Growth | | | • Send to NML for van genotyping & FRZ | • Notify ICP |
| • 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:

<table>
<thead>
<tr>
<th>NG</th>
<th>Scant Growth (purple or blue colonies)</th>
<th>Mod-Heavy Growth (purple or blue colonies)</th>
</tr>
</thead>
</table>
| 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 automatically added: **ICPR** “THIS PATIENT IS TO BE MANAGED IN “CONTACT PRECAUTIONS” 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 vancomycin-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: \van1):
“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

UNIVERSITY HEALTH NETWORK/MOUNT SINAI HOSPITAL, DEPARTMENT OF MICROBIOLOGY
~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


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


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


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:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacConkey Agar (Mac) –no antibiotic</td>
<td>O₂, 35°C x 18 h</td>
</tr>
</tbody>
</table>

For *Enterobacterales* with fluoroquinolone and/or aminoglycoside resistance but susceptibility to cefpodoxime:

For *Serratia marcescens* outbreaks,

| CTCZ – with colistin | O₂, 35°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


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 QPCMI0001

IV. Procedure

A. Processing of Specimen:

a) Direct Examination: Not indicated

b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL Isolation Agar - MacConkey with 2 μg/ml cefpodoxime</td>
<td>O₂, 37°C x 18-24 hours</td>
</tr>
<tr>
<td>(Media code: MCPOD)</td>
<td></td>
</tr>
</tbody>
</table>

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

5. 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 **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 \VCPR “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”

NOTE: This document is Uncontrolled When Printed.
Any documents appearing in paper form that do not state “CONTROLLED COPY” in red print are not controlled and should be checked against the document (titled as above) on the server prior to use.
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

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

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Processing</th>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental swabs</td>
<td>Incubate the BHI BrothSubculture BHI broth after overnight incubation to MCPOD by the IC Bench tech using a new sterile swab</td>
<td>ESBL Isolation Agar – MacConkey with 2 μg/ml cefpodoxim (MCPOD)</td>
<td>O₂ at 35°C overnightO₂ at 35°C for 24 hours</td>
</tr>
<tr>
<td>Swabs from patients</td>
<td>Directly inoculate MCPOD plate with specimen</td>
<td>ESBL Isolation Agar – MacConkey with 2 μg/ml cefpodoxim (MCPOD)</td>
<td>O₂ at 35°C for 24 hours</td>
</tr>
</tbody>
</table>
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,
           o Report isolate with the following ISOLATE Comment: \CNML
           o Phone or e-mail IC and ward as per Isolate Notification Table. Send isolates to NML ASAP (Cannot send on Friday)
           o Order the LIS ISOLATE Breakpoint panel kpcrcon.
           o Freeze isolate

   b) **If βCARBA (BCARB) is positive:**
      i. **Previous CRE positive (≤ 6 months)**
         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:
         \upCRB
       - 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 kpccron
       - Set up Rosco with Temocillin (panel kpccros). For epidemiology purposes only. Record and suppress kpccros 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:**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Processing</th>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>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 the falcon tube containing the sediment</td>
<td>BHI Broth</td>
<td>O$_2$ at 35°C overnight</td>
</tr>
<tr>
<td></td>
<td>Subculture BHI broth after overnight incubation to MCPOD by the IC Bench technologist</td>
<td>MCPOD</td>
<td>O$_2$ at 35°C for 24 hours</td>
</tr>
<tr>
<td>Environmental swabs</td>
<td>Incubate the BHI Broth</td>
<td></td>
<td>O$_2$ at 35°C overnight</td>
</tr>
<tr>
<td></td>
<td>Subculture BHI broth after overnight incubation to MCPOD by the IC Bench tech using a new sterile swab</td>
<td>MCPOD</td>
<td>O$_2$ at 35°C for 24 hours</td>
</tr>
<tr>
<td>Patient pharmaceutical</td>
<td>≤1 mL</td>
<td>TH14 SD14</td>
<td>O$_2$ at 35°C for 14 days O$_2$ at RT° for 4 days</td>
</tr>
</tbody>
</table>
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.

### Specimen Processing Media Incubation

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Processing</th>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>infusates/injectables (QC bench)</td>
<td>&gt;1 mL</td>
<td>ETH14</td>
<td>O₂ at 35°C for 14 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ESD14</td>
<td>O₂ at RT° for 4 days</td>
</tr>
<tr>
<td>Swabs from patients</td>
<td>Directly inoculate MCPOD plate with specimen</td>
<td>MCPOD</td>
<td>O₂ at 35°C for 24 hours</td>
</tr>
</tbody>
</table>

### 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:**

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

<table>
<thead>
<tr>
<th>Material</th>
<th>Vendor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromagar Candida Plus Agar</td>
<td>Micronostyx</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHROMagar Candida Plus Agar</td>
<td>O₂ 37°C x 48 h – in the dark</td>
</tr>
</tbody>
</table>

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 (02, 37°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.

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

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Colony colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida auris</td>
<td>White with blue halo, blue on reverse of the plate&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Green-blue</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>Dark pink</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>Metallic blue with pink halo</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Mauve</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>Pink and fuzzy</td>
</tr>
<tr>
<td>Candida lusitaniae</td>
<td>Purple</td>
</tr>
</tbody>
</table>

<sup>1</sup>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**

<table>
<thead>
<tr>
<th>Negative report:</th>
<th>“No Candida auris isolated.”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presumptive positive:</td>
<td>“Suspect <em>Candida auris</em> isolated, confirmation to follow.”</td>
</tr>
<tr>
<td>Positive report:</td>
<td>“<em>Candida auris</em> isolated.”</td>
</tr>
<tr>
<td></td>
<td>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”.</td>
</tr>
<tr>
<td></td>
<td>Communicate to ward and/or infection control if necessary as per Isolate Notification and Freezing Table QPCMI16003</td>
</tr>
</tbody>
</table>
Figure 1. Appearance of Common *Candida* species on CHROMagar Candida Plus Agar

24 h and 48 h incubation in O₂ 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 O₂ 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. Light blue colonies with blue halo at 48 h incubation

1. References

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


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:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNA (rectal/wound)</td>
<td>AnO₂, 35°C x 18-24 hours</td>
</tr>
<tr>
<td>Carrot Broth</td>
<td>O₂, 35°C x 18-24 hours</td>
</tr>
<tr>
<td>BA (for throat)</td>
<td>AnO₂, 35°C x 18-24 hours</td>
</tr>
</tbody>
</table>

If original plates are negative; Subculture the Carrot Broth to a second CNA plate and incubate overnight in AnO₂, 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°C freezer. Place a cover over the panel and place into the O₂ 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).

<table>
<thead>
<tr>
<th>Panel</th>
<th>Temp.</th>
<th>Atmosphere</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRE (vancomycin)</td>
<td>35°C</td>
<td>O₂</td>
<td>24 h</td>
</tr>
<tr>
<td>MRSA (oxacillin)</td>
<td>35°C</td>
<td>O₂</td>
<td>24 h</td>
</tr>
<tr>
<td>GNB</td>
<td>35°C</td>
<td>O₂</td>
<td>24 h</td>
</tr>
</tbody>
</table>

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


4. **Skov R., N., Frimodt-Moller, F. Esersen** 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

<table>
<thead>
<tr>
<th>Organism</th>
<th>PFGE</th>
<th>BHB</th>
<th>Frequency</th>
<th>Site</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MRSA</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>New, then once every 3 months</td>
<td>MSH only <strong>Excluding BPH</strong></td>
<td>UHN, BPH on request only</td>
</tr>
<tr>
<td>VRE</td>
<td>Yes</td>
<td>Yes</td>
<td>New, then once every 12 months</td>
<td>MSH only <strong>Excluding BPH</strong></td>
<td>UHN, BPH on request only</td>
</tr>
<tr>
<td>Serratia</td>
<td>Yes</td>
<td>Yes</td>
<td>All</td>
<td>MSH NICU only</td>
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<td>Group A Streptococcus</td>
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### Record of Edited Revisions

**Manual Section Name: Infection Control Manual**

<table>
<thead>
<tr>
<th>Page Number / Item</th>
<th>Date of Revision</th>
<th>Signature of Approval</th>
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<tr>
<td>Annual Review</td>
<td>March 13, 2002</td>
<td>Dr. T. Mazzulli</td>
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<td>October 25, 2003</td>
<td>Dr. T. Mazzulli</td>
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<td>May 26, 2004</td>
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<td>July 23, 2006</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Link to <a href="#">IC\Infection Control Pulsed-field Gel Electrophoresis.doc</a> added</td>
<td>January 30, 2007</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Link to <a href="#">IC\VRE PCR Procedure.doc</a> added</td>
<td>January 30, 2007</td>
<td>Dr. T. Mazzulli</td>
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<td>Enter the no. of pink colonies grown on MRSA-Select if &lt;5</td>
<td>January 30, 2007</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Added quantitation for MRSA</td>
<td>February 28, 2007</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Change to Denim Blue plates for MRSA Screen</td>
<td>March 13, 2007</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Change negative resulting phrases for MRSA, VRE and ESBL screen</td>
<td>March 13, 2007</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Included P. mirabilis for ESBL screen</td>
<td>March 13, 2007</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>March 13, 2007</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Revised VRE Identification Procedure</td>
<td>March 22, 2008</td>
<td>Dr. T. Mazzulli</td>
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<td>VRE – VANCS resistant <em>E. faecium</em> or <em>E. faecalis</em> report to MSH ICP if it is MSH patient; change to report as Presumptive VRE to all ICP</td>
<td>September 20, 2008</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Pseudo screen, patient swabs – change incubation period from 48 hours to 24 hours</td>
<td>September 20, 2008</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>September 20, 2008</td>
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<tr>
<td>Annual Review</td>
<td>September 20, 2010</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>ESBL screen updated to include KPC and NDM screen</td>
<td>November 10, 2010</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Removed send by taxi for carbapenemase PCR send out for Monday, Wednesday and Thursday</td>
<td>January 20, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Modified carbapenemase screening procedure to match Susceptibility manual</td>
<td>April 04, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Change VRE screening to Brilliance VRE Agar</td>
<td>April 04, 2011</td>
<td>Dr. T. Mazzulli</td>
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<th>Page Number / Item</th>
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<tr>
<td>Removed VRE Table 3; added link to Susceptibility manual</td>
<td>May 11, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>VRE Screen, added VANCS back to heavy growth from BVRE or SBVRE</td>
<td>May 31, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>VRE Screen – modified, finalized all day 2 reading in the morning of day 2</td>
<td>October 17, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>October 17, 2011</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Modified Serratia screen</td>
<td>November 25, 2011</td>
<td>Dr. T. Mazzulli</td>
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<td>Modified VRE resulting phrases</td>
<td>December 13, 2011</td>
<td>Dr. T. Mazzulli</td>
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<td>Added CRE only screen</td>
<td>December 13, 2011</td>
<td>Dr. T. Mazzulli</td>
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<td>Modified VRE reporting for vanA gene positive but phenotype vancomycin=S strains</td>
<td>February 1, 2012</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Added link to VRE PCR by Cephied</td>
<td>July 16, 2012</td>
<td>Dr. T. Mazzulli</td>
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<td>Modified planting volume into BHI broth for VRE/MRSA</td>
<td>August 28, 2012</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>May 31, 2013</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Manual updates in each section (Maldi procedure review)</td>
<td>October 10, 2013</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Changed CRE screen from ERTA to MERO discs</td>
<td>October 10, 2013</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Update VRE Identification</td>
<td>April 19, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>April 19, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>CRE reporting changes (Mero screen I/R)</td>
<td>June 27, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>VITEK SXT=R SUPPRESS SXT confirm result by KB BEFORE reporting.</td>
<td>June 27, 2014</td>
<td>Dr. T. Mazzulli</td>
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<td>vanB gene detected by Cepheid Xpert vanA/vanB Assay reporting</td>
<td>August 6, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Insert proper headers/footers, UHN/MSH Logo</td>
<td>August 12, 2014</td>
<td>Dr. T. Mazzulli</td>
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<td>Fix broken link to appendix 2</td>
<td>August 12, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Added Group A Strep Screen and Klebsiella Screen</td>
<td>September 30, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Reviewed and updated procedure steps in all sections</td>
<td>September 30, 2014</td>
<td>Dr. T. Mazzulli</td>
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<td>Added MRSA scant growth repeat broth culture comments</td>
<td>December 10, 2014</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>May 19, 2015</td>
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<tr>
<td>p.19 changed serrata media to new ctcz media’</td>
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<td>p.22 ESBL testing is only performed on specimens from pregnant patients, specimens originating from mothers and baby units or upon special request.</td>
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<td>p.35 BA (for throat) added to GAS screen procedure culture media</td>
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<tr>
<td>Changed Mac with cepfodoxime to ESBL isolation agar with cepfodoxime</td>
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<td>p.39 added ESBL comments to reporting comments</td>
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<td>p.24: added suscep. comment: MRSS</td>
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<td>Specimen collected and transport for each section transferred to Specimen collection manual QPCMI02001 And replaced with link to specimen collection manual</td>
<td>May 26, 2015</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>VRE outbreak: Temporary Procedure change in effect VRE PCR on any amount of purple colonies</td>
<td>June 11, 2015</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>VRE outbreak: Temporary Procedure change ended. Section removed.</td>
<td>July 15, 2015</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Prev + ESBL and Prev + CRE new statements</td>
<td>August 20, 2015</td>
<td>Dr. T. Mazzulli</td>
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<td>p.23 Previously ESBL reporting phrased changed from “Susceptibility not done, please refer to sample collected on _<em>Date</em> ” to “Phenotypic screening suggests this organism is ESBL POSITIVE as previously confirmed on “yyyy.mm.dd”.” LIS isolate comment code: ESBP</td>
<td>December 2, 2015</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Updated ESBL+CRE and CRE sections with new reporting phrases</td>
<td>December 21, 2015</td>
<td>Dr. T. Mazzulli</td>
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<td>Updated CRE section with new BCARB/CARB-R/ROSCO procedure</td>
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<tr>
<td>MRSA reporting section: added link for susceptibility comments to MRSA reporting phrases in susceptibility manual. Resistant GNB reporting section &amp; Pseudomonas screen section added for Positive reports: Add comment: “Susceptibility testing results are provided for infection control purposes only.” ICSN</td>
<td>April 4, 2016</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>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.</td>
<td>April 12, 2016</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>VRE outbreak: Temporary Procedure change in effect: VRE PCR on <strong>any</strong> amount of purple colonies</td>
<td>June 13, 2016</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>MRSA for new MRSA added step “Send to NML in batches when requested by IC for CNISP surveillance” VRE - added HEAVY growth workup</td>
<td>July 29, 2016</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>VRE: Updated comment 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.”</td>
<td>December 1, 2016</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>May 20, 2017</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Updated Direct VRE PCR results with instructions to phone/email as per Isolate Notification and freezing table.</td>
<td>February 2, 2018</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>May 22nd, 2018</td>
<td>Dr. T. Mazzulli</td>
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| 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. | | |
| Minor format change                                                        | September 14, 2018| Dr. T. Mazzulli        |
| Addition of Wasplab changes to MRSA section.                               | December 11, 2018 | Dr. T. Mazzulli        |
| Annual Review                                                               | November 11, 2019 | Dr. T. Mazzulli        |
| Update of MUP to MUP<sub>200</sub>                                         |                   |                       |
| 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.

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