

<b>PROGRAM Standard Operating Procedure – Laboratory Services</b>	
Title: MIC31100 – VRE Screen	Policy Number: 15-121-V2
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date: 06/05/2025	Next Review Date: 06/05/2027
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved: 06/05/2025
Accreditation Canada Applicable Standard: NA	

**GUIDING PRINCIPLE:**

Specimens are submitted to identify carriers of vancomycin-resistant *Enterococcus faecium* and/or *Enterococcus faecalis* (VRE). Swabs may be submitted from any body site (other than nasal and axilla), but most commonly are collected from the rectum and are used to aid in the prevention and control of VRE in healthcare settings.

**PURPOSE/RATIONALE:**

This standard operating procedure describes the screening for Vancomycin Resistant Enterococci (VRE) on admission and as part of Multi-Resistant Organism (MRO) screens.

**SCOPE/APPLICABILITY:**

This standard operating procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for VRE screen.

**SAMPLE INFORMATION:**

<b>Type</b>	Swab <ul style="list-style-type: none"> <li>• Amie’s with or without charcoal</li> </ul>
<b>Source</b>	<ul style="list-style-type: none"> <li>• Rectum</li> <li>• Stool</li> <li>• MRO screen: any site</li> </ul>
<b>Stability</b>	If the sample is received in the laboratory and processed greater than 48 hours from collection: <ul style="list-style-type: none"> <li>• Add specimen quality comment: “Delayed transport may adversely affect pathogen recovery”</li> </ul>

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<b>Storage Requirements</b>	Room temperature
<b>Criteria for rejection</b>	<ol style="list-style-type: none"><li>1. Unlabeled/mislabeled swabs</li><li>2. Specimen container label does not match patient identification on requisition</li><li>3. Duplicate specimens obtained with same collection method from same collection location within 24 hours</li><li>4. If no fecal matter is seen on swab, add the specimen quality comment "VRE" to state: "NO FECAL MATTER VISIBLE ON SWAB"</li></ol>

**REAGENTS and/or MEDIA:**

- VREselect agar (VRE), Blood agar (BA) and Mueller Hinton agar (MH)
- Identification reagents: gram stain, catalase and vancomycin ETEST

**SUPPLIES:**

- Disposable inoculation needles
- Wooden sticks
- Glass microscope slides

**EQUIPMENT:**

- Biosafety cabinet
- 35° ambient air incubator
- Microscope
- VITEK 2 and supplies

**SPECIAL SAFETY PRECAUTIONS:**

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures:

- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure of splashes
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC)
- The use of needles, syringes and other sharp objects should be strictly limited

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

**QUALITY CONTROL:**

- Refer to MIC60040-Culture Media Quality Control procedure
- Refer to Test Manual for reagent quality control procedures

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**PROCEDURE INSTRUCTIONS:**

Step	Action
<b>Processing swabs for VRE screen</b>	
<b>1</b>	In the biosafety cabinet: <ul style="list-style-type: none"> <li>• Inoculate VRE<i>Select</i> agar with the swab</li> <li>• Ensure all surfaces of the swab make contact with the agar</li> <li>• Streak for isolated growth using a disposable inoculation needle</li> </ul>
<b>2</b>	Label the VRE plate with: R (Date + 2 date).
<b>3</b>	Incubate the media: <ul style="list-style-type: none"> <li>• Place VRE in the O<sub>2</sub> incubator on the "VRE SCREEN" section on the old cultures shelf</li> </ul>

**INTERPRETATION OF RESULTS:**

Step	Action	
<b>1</b>	<ul style="list-style-type: none"> <li>• Observe VRE plate at 38 to 48 hours</li> <li>• Examine for blue or pink colonies</li> </ul>	
<b>3</b>	If no blue or pink colonies are seen at 38 to 48 hours: <ul style="list-style-type: none"> <li>• Record observations in the LIS</li> <li>• Workup complete</li> <li>• VRE not isolated</li> </ul>	
<b>4</b>	If blue colonies are seen: <ul style="list-style-type: none"> <li>• Record observations in the LIS</li> <li>• Subculture colonies to BA plate if no isolated colonies are present</li> <li>• If isolated colonies are present, perform catalase test</li> </ul>	
	<b>IF</b>	<b>THEN</b>
	Catalase POSITIVE	<ul style="list-style-type: none"> <li>• Record observations in the LIS</li> <li>• Workup complete</li> <li>• VRE not isolated</li> </ul>
	Catalase NEGATIVE	<ul style="list-style-type: none"> <li>• Record observations in the LIS</li> <li>• Subculture to BA plate</li> </ul>
	From the BA sub plate:	
	<b>IF</b>	<b>THEN</b>
	Gram stain NOT GPC	<ul style="list-style-type: none"> <li>• Record observations in the LIS</li> <li>• Workup complete</li> <li>• VRE not isolated</li> </ul>
Gram stain GRAM POSITIVE COCCI	<ul style="list-style-type: none"> <li>• Perform GPI</li> <li>• Set up vancomycin ETEST</li> </ul>	

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<b>5</b>	<u>If pink colonies are seen:</u>	
	<ul style="list-style-type: none"> <li>Record observations in the LIS</li> <li>Subculture colonies to BA sub plate</li> <li>From the BA sub plate, perform gram stain</li> </ul>	
	IF	THEN
	Gram stain NOT GPC	<ul style="list-style-type: none"> <li>Record observations in the LIS</li> <li>Workup complete</li> <li>VRE not isolated</li> </ul>
	Gram stain GRAM POSITIVE COCCI	<ul style="list-style-type: none"> <li>Perform GPI</li> <li>Set up vancomycin ETEST</li> </ul>

**REPORTING INSTRUCTIONS:**

IF	REPORT
VRE not isolated	<ul style="list-style-type: none"> <li>Report: <b>"No Vancomycin Resistant Enterococci (VRE) isolated"</b></li> </ul>

IF	REPORT
VITEK ID:  <i>E. gallinarum</i> and/or <i>E. casseliflavus</i>	<ul style="list-style-type: none"> <li>Suppress GPI result in the isolates tab:                             <ul style="list-style-type: none"> <li>➤ Change the Isolate # to a letter</li> <li>➤ Verify the result</li> </ul> </li> <li>Enter and verify vancomycin ETEST result</li> <li>Keep vancomycin ETEST result suppressed</li> <li>Report: <b>"No Vancomycin Resistant Enterococci (VRE) isolated"</b></li> </ul>

IF	AND	REPORT
Blue and/or Pink colonies - VITEK ID:  <i>E. faecalis</i> and/or <i>E. faecium</i>	Vancomycin ETEST MIC = <4 µg/mL	<ul style="list-style-type: none"> <li>Suppress GPI result in the isolates tab:                             <ul style="list-style-type: none"> <li>➤ Change the Isolate # to a letter</li> <li>➤ Verify the result</li> </ul> </li> <li>Enter and verify vancomycin ETEST result</li> <li>Keep vancomycin ETEST result suppressed</li> <li>Report: <b>"No Vancomycin Resistant Enterococci (VRE) isolated"</b></li> </ul>

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IF	AND	REPORT
<p>Blue and/or Pink colonies - VITEK ID:  <i>E. faecalis</i> and/or <i>E. faecium</i></p>	<p>Vancomycin ETEST MIC = 4 µg/mL</p>	<ul style="list-style-type: none"> <li>• Re-incubate vancomycin ETEST</li> </ul> <p><u>If after 48 hours MIC is still 4:</u></p> <ul style="list-style-type: none"> <li>• Suppress GPI result in the isolates tab:                             <ul style="list-style-type: none"> <li>➢ Change the isolate # to a letter</li> <li>➢ Verify the result</li> </ul> </li> <li>• Enter and verify vancomycin ETEST result</li> <li>• Keep vancomycin ETEST result suppressed</li> <li>• Report: <b>"No Vancomycin Resistant Enterococci (VRE) isolated"</b></li> </ul> <hr/> <p><u>If after 48 hours MIC ≥8 µg/mL:</u></p> <ul style="list-style-type: none"> <li>• List quantitation as <b>"Isolated"</b></li> <li>• Enter and verify vancomycin ETEST result</li> <li>• Keep vancomycin ETEST result suppressed</li> <li>• Report organism with isolate comment <b>&amp;VRE1</b></li> <li>• Add test ?REFD and finalize with "."</li> <li>• In order entry, copy report to OCPHO (HPU1)</li> <li>• In order entry, check the home address of the patient. If from Nunavut, copy report to the applicable NU CPHO</li> <li>• In order entry, copy report to appropriate IPAC ward</li> <li>• In order entry, add ESO code "VRE"</li> <li>• Freeze and record in isolate log</li> <li>• Refer isolate to APL for vancomycin gene testing</li> </ul>

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IF	AND	REPORT
<p>Blue and/or Pink colonies -                      VITEK ID:  <i>E. faecalis</i>                      and/or  <i>E. faecium</i></p>	<p>Vancomycin ETEST MIC =                      8-16 µg/mL</p>	<ul style="list-style-type: none"> <li>Repeat ID from vancomycin ETEST plate</li> <li>If repeat ID from vancomycin ETEST plate is the same:</li> <li>List quantitation as <b>"Isolated"</b></li> <li>Enter and verify vancomycin ETEST result</li> <li>Keep vancomycin ETEST result suppressed</li> <li>Report organism with isolate comment <b>&amp;VRE2</b></li> <li>Add test ?REFD and finalize with "."</li> <li>In order entry, copy report to OCPHO (HPU1)</li> <li>In order entry, check the home address of the patient. If from Nunavut, copy report to the applicable NU CPHO</li> <li>In order entry, copy report to appropriate IPAC</li> <li>In order entry, add ESO code "VRE"</li> <li>Freeze and record in isolate log</li> <li>Refer isolate to APL for vancomycin gene testing</li> </ul>

IF	AND	REPORT
<p>Blue and/or Pink colonies -                      VITEK ID:  <i>E. faecalis</i>                      and/or  <i>E. faecium</i></p>	<p>Vancomycin ETEST MIC =                      ≥32 µg/mL</p>	<ul style="list-style-type: none"> <li>Verify the organism ID</li> <li>List quantitation as <b>"Isolated"</b></li> <li>Enter and verify vancomycin ETEST result</li> <li>Keep vancomycin ETEST result suppressed</li> <li>The following isolate comment will be added: <b>&amp;VRE</b></li> <li>In order entry, copy report to OCPHO (HPU1)</li> <li>In order entry, check the home address of the patient. If from Nunavut, copy report to the applicable NU CPHO</li> <li>In order entry, copy report to appropriate IPAC ward</li> <li>In order entry, add ESO code "VRE"</li> <li>Freeze and record in isolate log</li> </ul>

**NOTE:** STH IPAC ward is **SIPAC**. IRH IPAC ward is **IIPAC**

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### LIMITATIONS:

1. Organisms with atypical enzyme patterns may give anomalous results. The growth requirements of certain VRE can lead to their partial or total inhibition in culture.
2. Faecal specimens may cause some localized discolourization in the primary area of inoculation and should not be confused with a true chromogenic reaction wherein coloured colonies are visible. Interpret the colour of the isolate on well isolated colonies.
3. Strains of *E. faecalis* or *E. faecium* with intermediate resistance to vancomycin are infrequently encountered and may yield positive results.
4. Tightly clustered colonies of *Enterococcus gallinarum* and *Enterococcus casseliflavus* could appear as gray, slightly blue, or pink.
5. *Leuconostoc*, *Pediococcus* and *Lactobacillus* are intrinsically resistant to vancomycin, are inhibited or appear as colourless pinpoint colonies (blue or pink with tightly clustered colonies).
6. Some Gram-negative bacteria may grow on VRESelect, but the size and colour of colonies enable differentiation from Vancomycin-resistant *Enterococcus faecium* or Vancomycin-resistant *Enterococcus faecalis*.

### CROSS-REFERENCES:

- MIC36300-Referral of Category B Specimens to APL
- MIC60040-Culture Media Quality Control

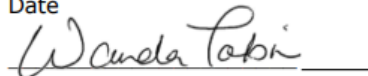
### REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook*. (4<sup>th</sup>ed.) Washington, D.C.: ASM Press
2. Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. (2015). *Manual of Clinical Microbiology*, 11<sup>th</sup> edition. Washington, D.C: ASM Press
3. Bio-Rad. (2016/03). VRESelect package insert

### APPROVAL:

May 06, 2025

Date



Acting Director, Laboratory and Diagnostic Imaging Services

**REVISION HISTORY:**

<b>REVISION</b>	<b>DATE</b>	<b>Description of Change</b>	<b>REQUESTED BY</b>
1.0	26 Apr 17	Initial Release	L. Steven
2.0	30 Nov 18	Updated to include new VITEK 2 instrument and two specimens per plate	L. Steven
3.0	30 Dec 21	Procedure reviewed and added to NTHSSA policy template	L. Steven
4.0	31 Aug 22	Updated to reflect new VRE agar VRESelect	L. Steven
5.0	25 Mar 25	Updated to reflect removal of 24 hour read	L. Steven

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