PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC31100 – VRE Screen	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s):	
Effective Date:	Effective Date:
Issuing Authority: Director, Health Services	Issuing Authority: Director, Health Services
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
VRE*Select* agar is a selective and differential chromogenic medium for the detection of gastrointestinal colonization of vancomycin-resistant *Enterococcus faecium* (VREfm) and *Enterococcus faecalis* (VREfs) and to aid in the prevention and control of VRE in healthcare settings.

The selectivity of VRE*Select* is based on the presence of an antifungal/antibiotic mixture that inhibits the growth of most yeasts, Gram-negative and Gram-positive bacteria, with the exception of vancomycin-resistant enterococci (VRE). Identification is based on the cleavage of chromogenic substrates by specific enzymatic activities of *Enterococcus faecium* which produces pink colonies, and *Enterococcus faecalis* which produces blue colonies.

Enterococcus gallinarum and *Enterococcus casseliflavus*, intrinsically resistant to vancomycin, when they are not inhibited, do not metabolize the chromogenic substrates, and appear as colourless or white colonies. Vancomycin-susceptible enterococci are inhibited.

PURPOSE/RATIONALE:
To screen for Vancomycin Resistant Enterococci (VRE) on admission and as part of Multi-Resistant Organism (MRO) screens.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLT) processing specimens for VRE screen.

SAMPLE INFORMATION:

Type	Swab • Amie's with or without charcoal

Source	• Rectum • Stool • MRO screen: any site
Stability	If the sample is received in the laboratory and processed greater than 48 hours from collection: • Add specimen quality comment: "Delayed transport may adversely affect pathogen recovery"
Storage Requirements	Room temperature
Criteria for rejection	1. Unlabeled/mislabeled swabs 2. Specimen container label does not match patient identification on requisition 3. Duplicate specimens obtained with same collection method from same collection location within 24 hours

REAGENTS and/or MEDIA:

- VRE*Select* agar (VRE), Blood agar (BA) and Muller Hinton agar (MH)
- Identification reagents: gram stain, catalase, gram stain, PYR and Vancomycin E-test

SUPPLIES:

- Disposable inoculation needles
- Wooden sticks

EQUIPMENT

- Biosafety cabinet
- 35° ambient air incubator
- Vitek 2 and supplies

SPECIAL SAFETY PRECAUTIONS:

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

- Ensure that appropriate hang 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.

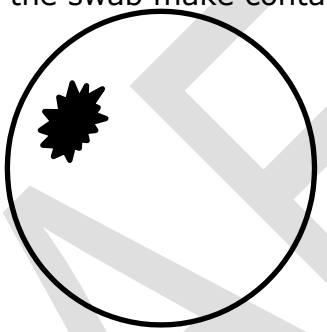
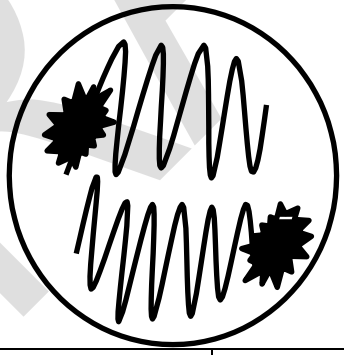
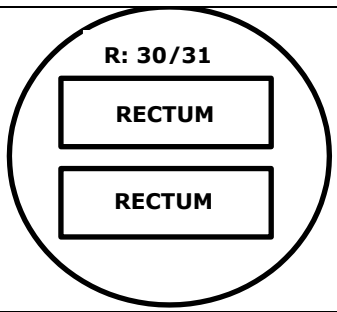
QUALITY CONTROL:

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

PROCEDURE INSTRUCTIONS:

- Monday to Friday: VRE swabs are processed by 15:00
- Saturday and Sunday: VRE swabs are processed by 15:00

PROCEDURE INSTRUCTIONS:

Step	Action
Processing swabs for VRE screen	
1	In the biosafety cabinet: • Inoculate top-left corner of VRE with the swab • Ensure all surfaces of the swab make contact with the agar:  • Streak for isolated growth using a disposable inoculation needle to cover half of the plate: 
2	Mark on VRE plate: • **R** (for read) followed by the read dates (24 hours and 48 hours from day of planting) **Reason:** Plates are read at 18 to 24 and 38 to 48 hours after incubation 
3	Incubate the media: • Place VRE in the O_2 incubator in appropriate tray

INTERPRETATION OF RESULTS:

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td>1</td><td colspan="2">• Observe VRE plate at 18 to 24 hours and 38 to 48 hours
• Examine for pink or blue colonies</td></tr>
<tr><td>2</td><td colspan="2">• Reject specimen if VRE was isolated from the patient in any other specimen collected within the past 2 weeks
• Use cancellation comment XVRD</td></tr>
<tr><td>3</td><td colspan="2">If no pink or blue colonies are seen at 18 to 24 hours:
• Record observations in the LIS
• Re-incubate plate in O_2 incubator on the “Old urine culture” shelf</td></tr>
<tr><td>4</td><td colspan="2">If no pink or blue colonies are seen at 38 to 48 hours:
• Record observations in the LIS
• Workup complete
• VRE not isolated</td></tr>
<tr><td rowspan="6">5</td><td colspan="2"><u>If blue colonies are seen</u>:
• Record observations in the LIS
• Perform catalase test</td></tr>
<tr><td>IF</td><td>THEN</td></tr>
<tr><td>Catalase
POSITIVE</td><td>• Record observations in the LIS
• Workup complete
• VRE not isolated
• Re-incubate plate for additional 38 to 48 hours</td></tr>
<tr><td>Catalase
NEGATIVE</td><td>• Record observations in the LIS
• Subculture to BA plate</td></tr>
<tr><td colspan="2">From the BA sub plate:
• Perform gram stain</td></tr>
<tr><td>IF</td><td>THEN</td></tr>
<tr><td>Gram stain
NOT GPC</td><td>• Record observations in the LIS
• Workup complete
• VRE not isolated</td></tr>
<tr><td>Gram stain
GRAM POSITIVE
COCCI</td><td>• Perform GPI
• Set up vancomycin E-test</td></tr>
<tr><td rowspan="4">6</td><td colspan="2"><u>If pink colonies are seen</u>:
• Record observations in the LIS
• Subculture colonies to BA sub plate
• From the BA sub plate, perform gram stain</td></tr>
<tr><td>IF</td><td>THEN</td></tr>
<tr><td>Gram stain
NOT GPC</td><td>• Record observations in the LIS
• Workup complete
• VRE not isolated</td></tr>
<tr><td>Gram stain
GRAM POSITIVE
COCCI</td><td>• Perform GPI
• Set up vancomycin E-test</td></tr>
</table>

REPORTING INSTRUCTIONS:

<table>
<tr><th>IF</th><th colspan="2">REPORT</th></tr>
<tr><td>No Pink or Blue colonies</td><td colspan="2">• Report:
“No Vancomycin Resistant Enterococci (VRE) isolated”</td></tr>
<tr><td>Pink or Blue colonies
-
Vitek ID:

E.gallinarum and/or
E.casseliflavus</td><td colspan="2">• Verify the organism ID
• Suppress GPI result in the isolates tab:
➢ Change the Isolate # to a letter
➢ Verify the result
• Enter and verify vancomycin E-test result
• Keep vancomycin E-test result suppressed
• Report:
“No Vancomycin Resistant Enterococci (VRE) isolated”</td></tr>
<tr><td rowspan="2">Pink and/or Blue colonies -
Vitek ID:

E.faecalis and/or
E.faecium</td><td>IF</td><td>REPORT</td></tr>
<tr><td>Vancomycin E-test MIC = <4 µg/mL</td><td>• Verify the organism ID
• Suppress GPI result in the isolates tab:
➢ Change the Isolate # to a letter
➢ Verify the result
• Enter and verify vancomycin E-test result
• Keep vancomycin E-test result suppressed
• Report:
“No Vancomycin Resistant Enterococci (VRE) isolated”</td></tr>
<tr><td rowspan="2">Pink and/or Blue colonies -
Vitek ID:

E.faecalis and/or
E.faecium</td><td>IF</td><td>REPORT</td></tr>
<tr><td>Vancomycin E-test MIC = 4 µg/mL</td><td>• Re-incubate vancomycin E-test
<u>If after 48 hours MIC is still 4:</u>
• Verify the organism ID
• Suppress GPI result in the isolates tab:
➢ Change the isolate # to a letter
➢ Verify the result
• Enter and verify vancomycin E-test result
• Keep vancomycin E-test result suppressed
• Report:
“No Vancomycin Resistant Enterococci (VRE) isolated”</td></tr>
</table>

		<u>If after 48 hours MIC ≥8 µg/mL:</u> • Verify the organism ID • List quantitation as **"Isolated"** • Enter and verify vancomycin E-test result • Keep vancomycin E-test result suppressed • Report organism with isolate comment **&VRE1** • Add test ?REFD and finalize with "." • In order entry, copy report to OCPHO (HPU1) • In order entry, copy report to appropriate IPAC ward • In order entry add ESO code "VRE" • Freeze and record in isolate log • Forward isolate to *Dyna*LIFE for vancomycin gene testing
	IF	**REPORT**
Pink and/or Blue colonies - Vitek ID: *E.faecalis* and/or *E.faecium*	Vancomycin E-test MIC = 8-16 µg/mL	• Repeat ID from vancomycin E-test plate • Verify the organism ID • List quantitation as **"Isolated"** • Enter and verify vancomycin E-test result • Keep vancomycin E-test result suppressed • Report organism with isolate comment **VRE2** • Add test ?REFD and finalize with "." • In order entry, copy report to OCPHO (HPU1) • In order entry, copy report to appropriate IPAC ward • In order entry add ESO code "VRE" • Freeze and record in isolate log • Forward isolate to *Dyna*LIFE for vancomycin gene testing

	IF	REPORT
Pink and/or Blue colonies - Vitek ID: *E.faecalis* and/or *E.faecium*	Vancomycin E-test MIC = ≥32 µg/mL	• Verify the organism ID • List quantitation as **"Isolated"** • Enter and verify vancomycin E-test result • Keep vancomycin E-test result suppressed • The following isolate comment will be added: **&VRE** • In order entry, copy report to OCPHO (HPU1) • In order entry, copy report to appropriate IPAC ward • In order entry add ESO code "VRE" • Freeze and record in isolate log

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

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*, intrinsically resistant to vancomycin, are inhibited or appear as colourless pinpoint colonies (blue or pink with tightly clustered colonies)
6. In some vancomycin-resistant *Enterococcus faecalis* strains such as ATCC 51299, which display low-level vancomycin resistance (MIC<32mg/L), the colonies may not develop the characteristic blue colour until 28h. If white colonies appear at 24h, a second reading must be performed at 28h.
7. Some Gram-negative bacteria may grow on VRE*Select,* but the size and colour of colonies enable differentiation from VREfm or VREfs.
8. Use of these plates may be difficult for individuals who have problems recognizing colours

CROSS-REFERENCES:

- MIC36400-Referral of Category B Specimens to *Dyna*LIFE
- MIC60040-Culture Media Quality Control

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4th 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, 11th edition*. Washington, D.C: ASM Press
3. Bio-Rad. (2016/03). *MRSASelect II* package insert

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
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 20	Procedure reviewed and added to NTHSSA policy template	L. Steven
4.0	31 Aug 22	Updated to reflect new VRE agar VRE*Select*	L. Steven