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:	Next Review Date:
Issuing Authority: Director, Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
Specimens are submitted to identify carriers of vancomycin resistant *Enterococcus*. Swabs may be submitted from any body site, but most common are rectal and wound swabs.

The selectivity of Colorex VRE agar 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* and *Enterococcus faecalis* which produce pink colonies. *Enterococcus gallinarum* and *Enterococcus casseliflavus*, intrinsically resistant to vancomycin, when they are not inhibited, do not metabolize the chromogenic substrates, and appear as blue 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 4. For swabs not visibly soiled with fecal matter, add specimen quality comment **VRE**

REAGENTS and/or MEDIA:

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

SUPPLIES:

- Disposable inoculation needles
- Wooden sticks
- Microscope slides

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.

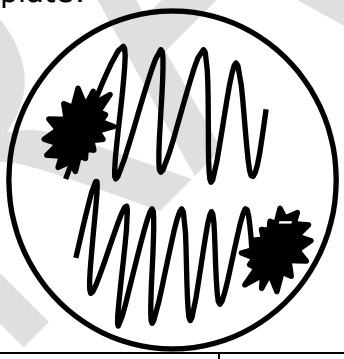
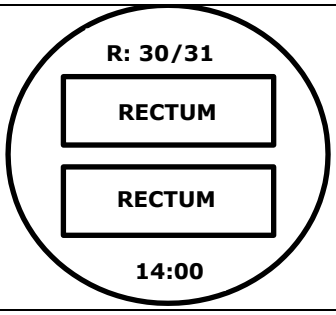
All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

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 at 14:00
- Saturday and Sunday: VRE swabs are processed at 14:00

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td colspan="2">Processing swabs for VRE screen</td></tr>
<tr><td>1</td><td>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:
  </td></tr>
<tr><td>2</td><td>Mark on VRE plate:
• R (for read) followed by the read dates (24 hours and 48 hours from day of planting)
• Time of planting (14:00)
Reason: Plates are read at 24 and 48 hours after incubation
  </td></tr>
<tr><td>3</td><td>Incubate the media:
• Place VRE in the O_2 incubator in appropriate tray, depending on time of incubation</td></tr>
</table>

INTERPRETATION OF RESULTS:

<table>
<tr><th>Step</th><th colspan="3">Action</th></tr>
<tr><td>1</td><td colspan="3">• Observe VRE plate at 24 hours and 48 hours
• Examine for pink/mauve colonies</td></tr>
<tr><td>2</td><td colspan="3">Reject specimen if VRE was isolated from the patient in any other specimen collected within the past 2 weeks. Use cancellation comment, in the resulting worklist screen, XVRD to state: “VRE was isolated from this patient within the past 2 weeks. Submit repeat specimens at least 2 weeks after previous positive culture”</td></tr>
<tr><td rowspan="4">3</td><td>IF</td><td colspan="2">THEN</td></tr>
<tr><td>No pink/mauve colonies seen at 24 hours</td><td colspan="2">• Record observations in the LIS
• Re-incubate plate in O_2 incubator on the “Old urine culture” shelf</td></tr>
<tr><td>No pink/mauve colonies seen at 38-48 hours</td><td colspan="2">• Record observations in the LIS
• Workup complete
• VRE not isolated</td></tr>
<tr><td>Pink/mauve colonies</td><td colspan="2">• Record observations in the LIS
• Subculture to BA plate
• From BA sub plate perform gram stain, catalase and PYR</td></tr>
<tr><td></td><td>IF</td><td>THEN</td></tr>
<tr><td></td><td>Gram stain
GRAM POSITIVE COCCI
Catalase
NEGATIVE
PYR POSITIVE</td><td>• Perform GPI
• Set up vancomycin E-test</td></tr>
</table>

REPORTING INSTRUCTIONS:

IF	REPORT
No Pink/Mauve colonies	• Report: **“No Vancomycin Resistant Enterococci (VRE) isolated”**
Pink/Mauve colonies - Vitek ID: *E.gallinarum* and/or *E.casseliflavus*	• 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”**

	IF	REPORT
Pink/Mauve colonies - Vitek ID: *E.faecalis* and/or *E.faecium*	Vancomycin E-test MIC = <4 µg/mL	• 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"**
Pink/Mauve colonies - Vitek ID: *E.faecalis* and/or *E.faecium*	**IF**	**REPORT**
	Vancomycin E-test MIC = 4 µg/mL	• 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"**
		<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 if ER or In-patient • 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/Mauve 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 if ER or In-patient • 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/Mauve 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 if ER or In-patient • In order entry add ESO code "VRE" • Freeze and record in isolate log

NOTE:

- Refer to MIC36400-Referral of Category B Specimens to *Dyna*LIFE and APL for sending isolates to *Dyna*LIFE
- 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. Fecal specimens may cause some localized discoloration in the primary area of inoculation and should not be confused with a true chromogenic reaction wherein colored colonies are visible. Interpret the color 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. Some rare strains of *Lactobacilli* and *Pediococcus* can sometimes appear as pinpoint mauve colonies.
5. Use of these plates may be difficult for individuals who have problems recognizing colors.

CROSS-REFERENCES:

- MIC36400-Referral of Category B Specimens to *Dyna*LIFE and APL
- 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. CHROMagar. (October 2014). *Colorex VRE Agar* package insert

APPROVAL:

_____
Date

_____

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