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

SAMPLE INFORMATION:

Type	Swab • Amies Stool specimen
Source	• VRE admission screen: rectum or 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 and follow up action	1. Unlabeled/mislabeled swabs 2. Dry swab 3. Nasal and axilla swabs will not be processed for VRE 4. For swabs not visibly soiled with faecal matter, add order comment **IOCLN** to state: **"No faecal matter visible on swab. Interpret results with caution."**

REAGENTS and/or MEDIA:

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

SUPPLIES:

- Wooden sticks,
- Disposable inoculation needles
- Microscope slides
- 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 potentially infectious materials or cultures.

- 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 where there is a known or potential risk of exposure to 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. Universal precautions 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 MIC60100 Non-Exempt Media Quality Control procedure

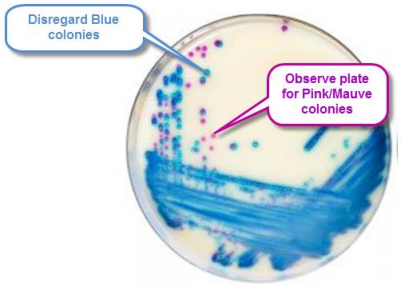
Refer to Quality Control manual for reagent quality control procedures

PROCEDURE INSTRUCTIONS:

*Note: VRE swabs are set up twice a day, Monday → Friday at noon and 17:00. On weekends, they are set up once a day before 15:00.

Step	Action
Processing Swabs for VRE Culture	
1	In the biosafety cabinet, inoculate Colorex VRE agar from the swab
2	Streak for isolated growth using a disposable inoculation needle  Streak out to cover the whole plate
3	Incubate plate in O_2 incubator at 35° for 24 hours in separate batches depending on time of incubation.

INTERPRETATION OF RESULTS:

Step	Action	
1	Remove culture plates after 24 hours incubation.	
2	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."**	
3	Observe plates for pink/mauve colonies. Note: Blue colonies are typical *Enterococcus gallinarum* or *Enterococcus casseliflavus* and should be ignored. Phenotypically, the definition of VRE includes those *Enterococcus faecalis* and *Enterococcus faecium* organisms that are resistant to vancomycin. It does not include motile enterococci, *Enterococcus gallinarum* and *Enterococcus casseliflavus*. These organisms do not account for the spread of vancomycin resistance and are not an infection control concern.  	
4	**If:**	**Then:**
	No pink/mauve colonies seen at 24 hours	• Record observations in the LIS. • Re-incubate plates in O_2 incubator with urine bench old culture specimens.
	No pink/mauve colonies seen at 38-48 hours	• Record observations in the LIS. • No workup required. • Report: **"No Vancomycin Resistant Enterococcus (VRE) isolated"**
	Pink/mauve colonies seen at 24 or 38 hours	• Record observations in the LIS. • Subculture to BA plate • From BA plate, perform gram stain to confirm colonies are Gram-positive cocci. Perform Catalase (negative) and PYR (positive) • Set up GPI to identify species level of Enterococcus • Set up Vancomycin E-test to determine vancomycin MIC • Refer to table below for interpretation

INTERPRETATION OF GPI AND VANCOMYCIN E-TEST MIC:

<table>
<tr><th>If:</th><th colspan="2">Then:</th></tr>
<tr><td>Vitek ID:
E.gallinarum
and/or
E.casseliflavus</td><td colspan="2">• Verify the organisms ID.
• Suppress GPI result in the isolates tab:
➢ Change the Isolate # to a letter
➢ Verify the result even though it will be hidden from final report
• Enter and verify vancomycin E-test result even though it will not appear on the final report.
• Report: ““No Vancomycin Resistant Enterococcus (VRE) isolated”</td></tr>
<tr><td rowspan="3">Vitek ID:
E.faecalis
and/or
E.faecium</td><td>If:</td><td>Then:</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 even though it will not appear on the final report
• Enter and verify vancomycin E-test result even though it will not appear on the final report.
• Report: “No Vancomycin Resistant Enterococcus (VRE) isolated”</td></tr>
<tr><td>Vancomycin E-test MIC=4 µg/mL</td><td>Re-incubate vancomycin E-test for additional 24 hours.
<u>If after 48 hours MIC is still 4 µg/mL:</u>
• Verify the organism ID.
• Suppress GPI result in the isolates tab:
➢ Change the isolate # to a letter
➢ Verify the result even though it will be hidden from final report
• Enter and verify vancomycin E-test result even though it will not appear on the final report
• Report: “No Vancomycin Resistant Enterococcus (VRE) isolated”</td></tr>
</table>

	If:	Then:
Vitek ID: *E.faecalis* and/or *E.faecium*	Vancomycin E-test MIC=4 µg/mL	<u>If after 48 hours MIC ≥ 8 µg/ML:</u> • Verify the organism ID. List quantitation as "Isolated". • Enter and suppress vancomycin E-test result • Report organism with isolate comment **VRE1** to state: **"Preliminary test indicates this isolate may be resistant to vancomycin and has been sent to referral laboratory for Van gene testing."** • Verify the result → set the Status to Final • Add test ?REFD and send to Prov. Lab for Van gene testing • In order entry, copy report to Chief Medical Officer of Health (HPU1) and Infection Control (SOHS) if in-patient. • Freeze organism and record in patient isolate log
	Vancomycin E-test MIC= 8-16 µg/mL	• Check purity plate carefully • Repeat ID from Vanc E-test plate • Verify the organism ID. List quantitation as "Isolated" • Enter and suppress vancomycin E-test result • Report organism with isolate comment **VRE2** to state: **"Presumptive VRE - This isolate exhibits a resistance to vancomycin and has been sent to referral laboratory for Van gene testing"** • Verify the result → set the Status to Final • Add test ?REFD and send to Prov. Lab for Van gene testing. • In order entry, copy report to Chief Medical Officer of Health (HPU1) and Infection Control (SOHS) if in-patient. • Freeze organism and record in patient isolate log

	If:	Then:
Vitek ID: *E.faecalis* and/or *E.faecium*	Vancomycin Etest MIC = ≥ 32 µg/mL	• Enter and suppress vancomycin E-test result • Verify the organism ID. List quantitation as "Isolated" • Report organism with isolate comment **&VRE** to state: **"***VRE – This isolate is resistant to Vancomycin***"** • Verify the result → set the Status to Final • In order entry, copy report to Chief Medical Officer of Health (HPU1) and Infection Control (SOHS) if in-patient. • In the patient demographics field click on ESO and add **"VRE Positive"** • Freeze organism and record in patient isolate log

LIMITATIONS:

- 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.
- 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.
- Strains of *E. faecalis* or *E. faecium* with intermediate resistance to vancomycin are infrequently encountered and may yield positive results.
- Some rare strains of *Lactobacilli* and *Pediococcus* can sometimes appear as pinpoint mauve colonies.
- Use of these plates may be difficult for individuals who have problems recognizing colors

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- 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, ASM Press, Washington, D.C.
- Dalynn Colorex VRE agar package insert, October 2014

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	26 Apr 2017	Initial Release	L. Steven
2.0	24 Jul 2017	Updated to reflect placement of inoculated VRE plates	L. Steven
3.0	25 Apr 2017	Change to reflect new Vitek 2 instrument	L. Steven