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

SAMPLE INFORMATION:

Type	Swab • Amies Stool specimen
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 and follow up action	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. Dry swab. 5. Nasal and axilla swabs will not be processed for VRE. 6. For swabs not visibly soiled with fecal matter, add specimen quality comment **VRE** to state: **"No fecal matter visible on swab"**

REAGENTS and/or MEDIA:

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

SUPPLIES:

- Disposable inoculation needles
- Biosafety cabinet
- 35° ambient air incubator
- Microscope slides
- 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

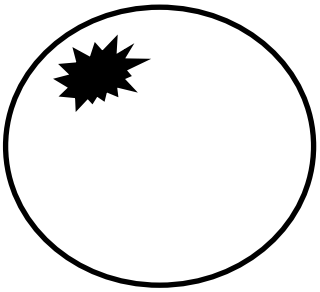
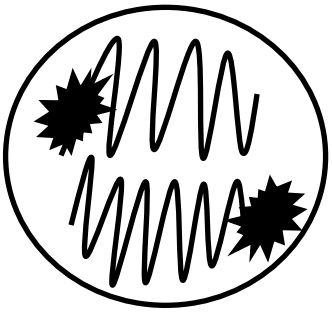
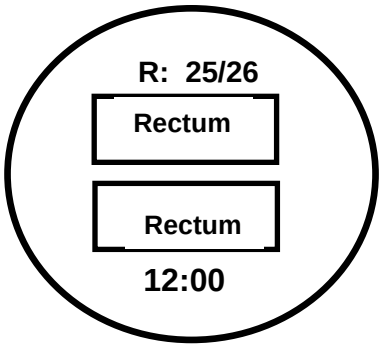
QUALITY CONTROL:

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

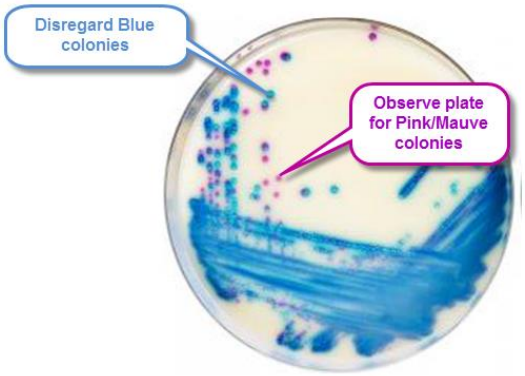
PROCEDURE INSTRUCTIONS:

NOTE: Monday to Friday: VRE swabs are processed at noon and 17:00.

Saturday and Sunday: VRE swabs are processed before 15:00.

Step	Action
Processing swabs for VRE screening	
1	In the biosafety cabinet, inoculate the top-left corner of VRE agar from the swab, ensuring all surfaces of swab make contact with the agar: 
2	Streak for confluent growth using a disposable inoculation needle:  Streak out to cover half the plate.
3	Mark on VRE plate: • **R** (for read) followed by the read dates (24 hours and 48 hours from day of planting) • Time of planting (12:00 or 17:00) **Reason:** Plates are read at 24 and 48 hours after incubation. 
4	Incubate plate in O_2 incubator at 35° for 24 hours in appropriate rack, depending on time of incubation.

INTERPRETATION OF RESULTS:

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td>1</td><td colspan="2">Remove culture plate after 24 hours incubation.</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, 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>3</td><td colspan="2">Observe plate for pink/mauve colonies, disregard blue colonies:
 
</td></tr>
<tr><td rowspan="4">4</td><td>IF</td><td>THEN</td></tr>
<tr><td>No pink/mauve colonies seen at 24 hours</td><td>• 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>• Record observations in the LIS.
• Workup complete. VRE not isolated.</td></tr>
<tr><td>Pink/mauve colonies seen at 24 or 38 hours</td><td>• Record observations in the LIS.
• Subculture to BA plate.
• From BA plate, perform:
1. Gram stain (Gram-positive cocci)
2. Catalase (negative)
3. PYR (positive).
• Set up GPI to identify species level of Enterococcus.
• Set up vancomycin E-test to determine MIC.</td></tr>
</table>

REPORTING RESULTS:

<table>
<tr><th>IF</th><th>THEN</th></tr>
<tr><td>VRE not isolated</td><td>• Report:
“No Vancomycin Resistant Enterococcus (VRE) isolated”</td></tr>
<tr><td>Vitek ID:
E.gallinarum
and/or
E.casseliflavus</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.
• Leave the vancomycin E-test result suppressed to prevent it from printing 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
• Enter and verify vancomycin E-test result.
• Leave the vancomycin E-test result suppressed to prevent it from printing 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
• Enter and verify vancomycin E-test result.
• Leave the vancomycin E-test result suppressed to prevent it from printing on the final report.
• Report: “No Vancomycin Resistant Enterococcus (VRE) isolated”</td></tr>
</table>

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 verify vancomycin E-test result. • Leave the vancomycin E-test result suppressed to prevent it from printing on the final report. • 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"** • Add test ?REFD and finalize with "." • In order entry, copy report to Chief Medical Officer of Health (HPU1) and if in-patient, Infection Control (SOHS). • In order entry add ESO code "VRE". • Freeze organism and record in patient isolate log. • Send to DynaLIFE for vancomycin gene testing.
	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. • Leave the vancomycin E-test result suppressed to prevent it from printing on the final report. • 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"** • Add test ?REFD and finalize with "." • In order entry, copy report to Chief Medical Officer of Health (HPU1) and if in-patient, Infection Control (SOHS). • In order entry add ESO code "VRE". • Freeze organism and record in patient isolate log. • Send to DynaLIFE for vancomycin gene testing.

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. • Leave the vancomycin E-test result suppressed to prevent it from printing on the final report. • Report organism with isolate comment **&VRE** to state: **"***VRE – This isolate is resistant to Vancomycin***"** • In order entry, copy report to Chief Medical Officer of Health (HPU1) and Infection Control (SOHS) if in-patient. • In order entry add ESO code "VRE". • Freeze organism and record in patient isolate log.

NOTE: Refer to MIC10510 – Referral of Category B Specimens to DynaLIFE and Prov. Lab. for sending isolates to DynaLIFE.

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.

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	30 Nov 2018	Updated to reflect new Vitek 2 instrument and two specimens per plate.	L. Steven