PURPOSE:

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

SAMPLE INFORMATION:

Type	Swab • Amies with or without charcoal
Source	• VRE admission screen: rectum • VRE MRO screen: any site
Stability	If the sample is received in the laboratory and processed greater than 48 h from collection: • Add specimen quality comment **DELAY** to state: **"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, in the order entry screen, **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

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

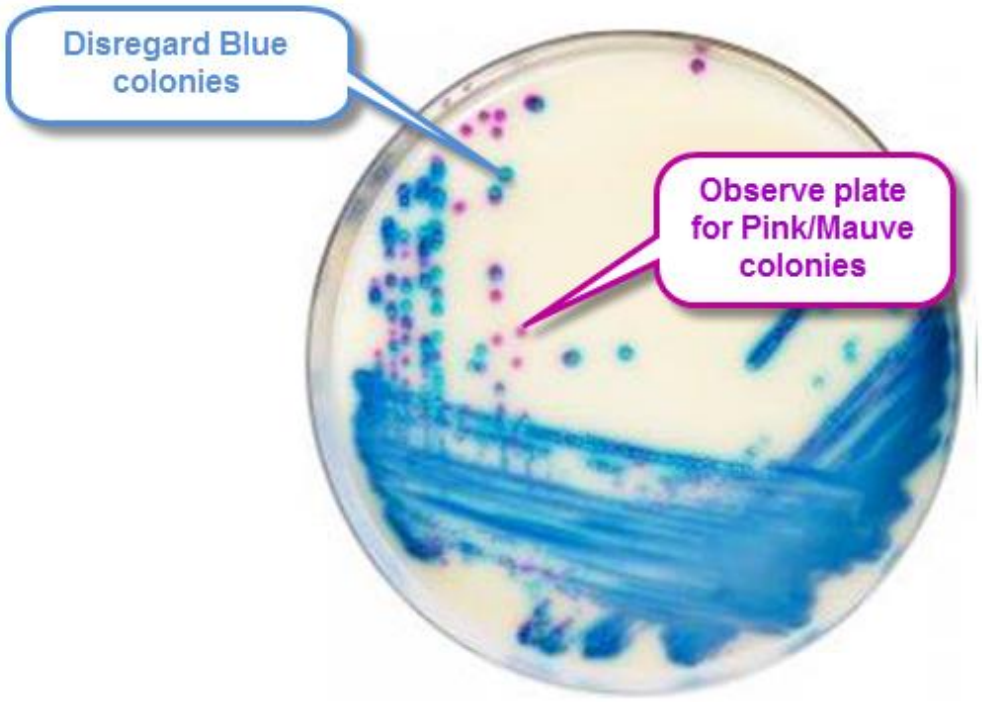
PROCEDURE INSTRUCTIONS:

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 with new urines and stools rack at 35° for 24 hours

INTERPRETATION OF RESULTS:

Action

- Remove cultures at 24 hours from O_2 incubator
- 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."**
- Observe plates for pink/mauve colonies
- 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



IF	THEN
No pink/mauve colonies seen at 24 hours	1. Log results into LIS 2. Re-incubate plates in O_2 incubator for additional 24 hours
No pink/mauve colonies seen at 48 hours	1. Log results into LIS 2. Report: **"No Vancomycin Resistant Enterococcus (VRE) isolated"**
Pink/mauve colonies seen at 24 or 48 hours	1. Subculture to BA plate 2. From BA plate, perform gram stain to confirm colonies are Gram-positive cocci. Perform Catalase (negative) and PYR (positive) 3. Set up GPI to identify species level of Enterococcus 4. Set up Vancomycin E-test to determine vancomycin MIC 5. Refer to table below for interpretation

Follow the steps in the table below to interpret the GPI and Vancomycin E-test MIC

IF	THEN
Vitek ID: *E.gallinarum* and/or *E.casseliflavus*	1. Verify the organisms ID. List quantitation as "Isolated" 2. Hide GPI results in the isolates tab: ➢ Change the Isolate # to a letter ➢ Verify the result even though it will be hidden from final report 3. Enter and verify vancomycin E-test result even though it will be hidden from final report **4.** Report: **"No Vancomycin Resistant Enterococcus (VRE) isolated"**

	IF	THEN
Vitek ID: *E.faecalis* and/or *E.faecium*	Vancomycin Etest MIC= < 4 µg/mL	1. Verify the organisms ID. List quantitation as "Isolated" 2. Hide GPI results in the isolates tab: ➢ Change the Isolate # to a letter ➢ Verify the result even though it will be hidden from final report 3. Enter and verify vancomycin E-test result even though it will be hidden from final report 4. Report: **"No Vancomycin Resistant Enterococcus (VRE) isolated"**
	Vancomycin Etest MIC= 4 µg/mL	Re-incubate vancomycin E-test for additional 24 hours. <u>If after 48 hours MIC is still 4 µg/mL:</u> 1. Verify the organisms ID. List quantitation as "Isolated" 2. Hide GPI results in the isolates tab: ➢ Change the isolate # to a letter ➢ Verify the result even though it will be hidden from final report 3. Enter and verify vancomycin E-test result even though it will be hidden from final report 4. Report: **"No Vancomycin Resistant Enterococcus (VRE) isolated"**
		<u>If after 48 hours MIC ≥ 8 µg/ML:</u> 1. Verify the organisms ID. List quantitation as "Isolated" 2. Enter and suppress vancomycin E-test result 3. 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."** 4. Verify the result → set the Status to Final 5. Add test ?REFD and send to DynaLIFE for Van gene testing as per Referral Specimens DynaLIFE and Provincial Laboratory (Category B) Microbiology procedure 6. Freeze organism in glycerol and record in patient isolate log

	IF	THEN
Vitek ID: *E.faecalis* and/or *E.faecium*	Vancomycin Etest MIC= 8-16 µg/mL	1. Check purity plate carefully 2. Repeat ID from Vanc E-test plate 3. Verify the organism ID 4. Enter and suppress vancomycin E-test result 5. 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"** 6. Verify the result → set the Status to Final 7. Add test ?REFD and send to DynaLIFE for Van gene testing as per Referral Specimens DynaLIFE and Provincial Laboratory (Category B) Microbiology procedure 8. Go to Order Entry; copy report to Chief Medical Officer of Health (HPU) and Infection Control Nurse (SOHS) if in-patient 9. Freeze organism in glycerol and record in patient isolate log
	Vancomycin Etest MIC = ≥ 32 µg/mL	1. Log results into LIS 2. Enter and suppress vancomycin Etest results **3.** Report organism with isolate comment **&VRE** to state: **"***VRE – This isolate is resistant to Vancomycin***"** 4. Verify the result → set the Status to Final 5. Go to Order Entry; copy report to Chief Medical Officer of Health (HPU) and Infection Control Nurse (SOHS) if in-patient 6. In the patient demographics field click on ESO and add **"VRE Positive"**

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