Laboratory Methods for Detection of Methicillin-Resistant *Staphylococcus Aureus* (MRSA)

In the U.S., most strains of *Staphylococcus aureus* are resistant to penicillin due to the production of the enzyme beta lactamase and, until the 1960s, were uniformly susceptible to beta-lactamase stable penicillins, such as methicillin and oxacillin.

Resistance to antistaphylococcal beta-lactamase stable penicillins has been historically referred to as methicillin-resistant *Staphylococcus aureus* or MRSA.

The acronym MRSA is still commonly used even though methicillin is no longer used for treatment. Antimicrobials like oxacillin and nafcillin now are used for treatment of *Staphylococcus aureus* infections. The acronym ORSA (oxacillin-resistant *Staphylococcus aureus*) may be used interchangeably with MRSA.

Accurate detection of MRSA can be difficult because susceptible and resistant populations may coexist in the same culture. This heteroresistance is a problem in the clinical laboratory because the resistant population may grow more slowly than the susceptible population.

The National Committee for Clinical Laboratory Standards (NCCLS) recommends the following for accurate detection of oxacillin/methicillin resistance.

**MIC Susceptibility Method**

1. **Direct Colony Inoculum**
   - Isolated colonies from an 18 to 24 hour nonselective agar plate are used to prepare a direct inoculum equivalent to a 0.5 McFarland Standard.
2. **Oxacillin is the preferred agent for detection of methicillin resistance.**
3. **Supplementation of Test Medium**
   - The addition of 2% NaCl to broth dilution is recommended to enhance detection of MRSA. Commercial susceptibility systems supplement with NaCl.
4. **Incubation Time and Temperature**
   - Tests must be incubated for a FULL 24 hours at 35° C.
   - Incubation temperature must not exceed 35° C.
5. **Interpretation of Results**

<table>
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<th><em>Staphylococcus aureus</em> MIC</th>
<th>Oxacillin-Susceptible</th>
<th>Oxacillin-Resistant</th>
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<td>≤2 ug/ml</td>
<td>≥ 4ug/ml</td>
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**IMPORTANT:** Oxacillin/methicillin resistance implies resistance to all penicillins, cephems, imipenem and beta lactam/beta lactamase inhibitor combinations such as ampicillin/sulbactam, amoxicillin/clavulanic acid, piperacillin/tazobactam, and ticarcillin/clavulanic acid regardless of the in vitro test results. This is because most cases of documented MRSA infections have responded poorly to beta lactam therapy or
because convincing clinical data have yet to be presented to document clinical efficacy. These antimicrobial agents **MUST** be reported as **RESISTANT**.

**DISK DIFFUSION SUSCEPTIBILITY METHOD**
1. Direct colony inoculum preparation
2. Mueller Hinton agar
3. 1 ug oxacillin disk
4. Incubate for a FULL 24 hrs at 35° C.
5. Interpretation of results
   - Resistant (MRSA): < 10 mm zone size of inhibition
   - Confirm with Oxacillin Screening Agar: 11-12 mm zone size of inhibition
   - Susceptible (No MRSA) ≥ 13 mm zone size of inhibition

**OXACILLIN AGAR SCREENING TEST**
When performed correctly, both disk diffusion and MIC tests accurately detect MRSA. The oxacillin screening plate can be used in addition to or as a backup method.

1. Mueller Hinton agar with 4% NaCl and 6 ug/ml of Oxacillin
2. Direct colony inoculum preparation
   - Dip swab into inoculum, express excess fluid and streak quadrant of the agar or spot inoculate 1 to 10 ul of the suspension to a quadrant of the plate.
3. Incubate full 24 hours at 35° C.
4. > 1 colony or light film of growth = oxacillin/methicillin-resistant

**INCORRECT IDENTIFICATION OF MRSA**
1. Mixed culture
   - Methicillin-resistant coagulase negative staphylococcus mixed with a methicillin-susceptible *Staphylococcus aureus*. It is advisable to perform a purity check of the inoculum suspension each time a susceptibility test is performed. This plate should be examined for the presence of a second organism. Isolate and retest if indicated.
2. Misidentification
   - Rarely, a coagulase negative staphylococcus may give a false positive reaction with commercially available slide agglutination methods. A test tube coagulase test may be indicated in select situations to confirm an identification of *Staphylococcus aureus*.

**ADDITIONAL TESTS TO DETECT MRSA**
1. Amplification tests, like those based on polymerase chain reaction (PCR), are available to detect the mecA gene.
2. Latex agglutination methods are available for detection of the penicillin-binding protein 2a (PBP2a). High level methicillin resistance in *Staphylococcus aureus* is dependent upon the acquisition of the mecA gene encoding PBP2a. This protein is involved in the assembly of the cell wall in bacteria and is poorly inactivated by beta-lactam antibiotics.
**Vancomycin-Intermediate Staphylococcus Aureus (VISA)**

The first occurrence of *Staphylococcus aureus* strains with reduced susceptibility to vancomycin (MIC 4-8 ug/ml) was reported from Japan in 1997, followed by reports from the U.S. and other countries. All strains of *Staphylococcus aureus* with vancomycin MIC of $\geq$ to 4ug/ml should be considered candidate strains for VISA.

1. Disk diffusion is not an acceptable method for vancomycin susceptibility testing of *Staphylococcus aureus*. CDC indicates none of the known strains of *Staphylococcus aureus* with reduced susceptibility to vancomycin have been detected by this method.
2. An MIC method or Epsilometer test (E test) (AB Biodisk, Solna, Sweden) should be used.
3. Test must be incubated for a full 24 hours before reading. The vancomycin agar screen test used for enterococci may also be used to detect VISA.
4. The laboratory should ensure the strain is a pure culture. Repeat the test for confirmation. Immediately contact the infection control department and contact the state health department.

**Vancomycin Resistant Staph Aureus (VRSA)**

The first occurrence of VRSA in the U.S. was isolated in Michigan in July 2002. Refer to the guidelines for VISA.

**Emerging Resistance To Staphylococcus**

For guidelines for emerging resistance to *Staphylococcus* and other microorganisms, consult the current NCCLS M100 document issued in January of each year.

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References:

4. Draft: Health System Response to Control the First Sentinel Case of VRSA.
Laboratory Methods For Detection of Vancomycin Resistant Enterococci (VRE)

Recommendations of the Hospital Infection Control Practices Advisory Committee

Role of the microbiology laboratory in the detection, reporting and control of VRE

The microbiology laboratory is the first line of defense against the spread of VRE in the hospital. The laboratory’s ability to identify enterococci and detect vancomycin resistance promptly and accurately is essential in recognizing VRE colonization and infection and avoiding complex, costly containment efforts that are required when recognition of the problem is delayed. In addition, cooperation and communication between the laboratory and the infection control program will facilitate control efforts substantially.

Identification of Enterococci
Presumptively identify colonies on primary isolation plates as enterococci by using the colonial morphology, Gram stain, and pyrrolidonyl arylamidase (PYR) test. Although identifying enterococci to the species level can help predict certain resistance patterns (e.g., *Enterococcus faecium* is more resistant to penicillin than is *Enterococcus faecalis*) and may help determine the epidemiologic relatedness of enterococcal isolates, such identification is not routinely necessary if antimicrobial susceptibility testing is performed. However, under special circumstances or as laboratory resources permit, biochemical tests can be used to differentiate between various enterococcal species. Most commercially available identification systems adequately differentiate *Enterococcus faecalis* from other species of enterococci. However, additional tests for motility and pigment production are required to distinguish *Enterococcus gallinarum* (motile and nonpigmented) and *Enterococcus casseliflavus* (motile and pigmented) and *Enterococcus faecium* (nonmotile and nonpigmented).

Tests for Antimicrobial Susceptibility
All enterococcal isolates should be tested for susceptibility to penicillin, ampicillin and vancomycin and other drugs as clinically indicated. Determination of actual MIC for blood and CSF should be considered.

Vancomycin Resistance
Although different test methods can be used, it is critical that all laboratories use the same interpretive standards for determining vancomycin resistance. Since breakpoints for disk diffusion or MIC susceptibility tests are periodically changed by the National Committee for Clinical Laboratory Standards (NCCLS), these documents should be reviewed annually. The 2004 NCCLS breakpoints for determining vancomycin resistance by disk diffusion (30 μg) are: susceptible (≥ 17 mm), intermediate (15-16 mm) and resistant (≥ 14 mm). For dilution testing, the 2003 breakpoints are susceptible (≤ 4 μg/mL), intermediate (8-16 μg/mL), and resistant (≥ 32 μg/mL). Accurate detection of vancomycin-resistant enterococci by the agar or broth dilution test requires incubation for a full 24 hours (rather than 16 to 20 hours). The plates, tubes, or
wells must be examined carefully for evidence of faint growth. A vancomycin screen agar, containing 6 µg/mL of vancomycin, also may be used. However, it cannot differentiate between intermediate or high level resistance. The MIC test is recommended for confirmation of vancomycin resistance isolates. Speciation of enterococcus should be done whenever possible.

- **Disk Diffusion**
  Although this method has been shown to be unreliable for detecting resistance in strains with intermediate or low-level resistance to vancomycin, it remains an acceptable alternative for those laboratories not routinely performing MIC tests. A disk content of 30 µg should be used. Plates should be read using transmitted light rather than reflected light, since some vancomycin-resistant strains produce hazy growth with a larger zone of inhibition. In addition, disk diffusion plates should be incubated for a full 24 hours. MIC tests should be performed for strains demonstrating intermediate zones if vancomycin is being considered for treatment.

- **Minimum Inhibitory Concentrations (MICs)**
  MICs can be determined by agar dilution, agar gradient dilution, broth macrodilution, or broth microdilution. These test systems should be incubated for 24 hours.

- **Vancomycin Agar Screening Plate**
  Commercially prepared agar screening plates are an acceptable method of determining vancomycin susceptibility in the absence of a reliable MIC method; however, as mentioned previously, isolates such as *Enterococcus gallinarum* and *Enterococcus casseliflavus* which have intermediate levels of resistance to vancomycin will grow on screening plates containing 6 µg/mL of vancomycin.

**Procedure**
1. BHI agar with 6µg/ml of vancomycin
2. Direct colony inoculum preparation
3. Spot inoculate 1-10ul of preparation onto agar surface.
4. Incubate full 24 hours at 35°C.
5. >1 colony = presumptive resistance

If the screening plate demonstrates presumptive resistance (i.e., >1 colony), an MIC for vancomycin and tests for motility and pigment production should be performed to distinguish species with acquired resistance from those with intrinsic, intermediate-level resistance to vancomycin such as *Enterococcus gallinarum* or *Enterococcus casseliflavus*. These species often grow on the vancomycin screen plate. In contrast to other enterococci, *Enterococcus casseliflavus* and *Enterococcus gallinarum* with vancomycin MICs of
8-16 µg/mL (intermediate) differ from *vancomycin-resistant Enterococcus* for infection control purposes.

Procedure to follow when VRE are isolated from a clinical specimen:

Confirm vancomycin resistance by repeat testing. Assure culture purity by performing a Gram stain of the growth from the vancomycin susceptibility test. Common causes of false positive vancomycin resistance are mixed culture (Gram negative rods or yeast mixed with enterococci) or misidentification.

**Recommendations of the Hospital Infection Control Practices Advisory Committee**

Immediately while performing confirmatory susceptibility tests, notify the patient’s primary caregiver, patient-care personnel on the ward on which the patient is hospitalized, and infection control personnel regarding the presumptive identification of VRE so that the patient can be placed on appropriate isolation precautions promptly. Follow this preliminary report with the (final) result of the confirmatory test. Additionally, highlight the report regarding the isolate to alert staff that isolation precautions are indicated.

References:
2. *Performance Standards for Antimicrobial Susceptibility Testing; NCCLS 2004; M100 S14; Vol. 24 No. 1.*