Laboratory Procedure Manual

Analyte: **Methicillin-Resistant *Staphylococcus aureus***

Matrix: **Swab**

Method: ***S. aureus* Isolate Screening for Methicillin Resistance**

Method No.:

Revised: **October 2004**

as performed by: *Epidemiology and Laboratory Branch*
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National Center for Infectious Diseases

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Important Information for Users

The National Center for Infectious Diseases periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2003–2004 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
l35_c	LBXMS1	*S. aureus* present 1
	LBXM1	MRSA 1
	LBXMT1	tetracycline 1
	LBXMZ1	trimethoprim/sulfamethoxazole 1
	LBXMC1	clindamycin 1
	LBXME1	erythromycin 1
	LBXMP1	penicillin 1
	LBXMI1	imipenem 1
	LBXMV1	vancomycin 1
	LBXMF1	cefazolin 1
	LBXMO1	oxacillin 1
	LBXMG1	gentamicin 1
	LBXMD1	ciprofloxacin 1
	LBXML1	levofloxacin 1
	LBXMR1	rifampin 1
	LBXMY1	amoxicillin/k clavulanate 1
	LBAMMT1	molecular type 1
	LBXMS2	s. aureus present 2
	LBXMT2	tetracyline 2
	LBXMZ2	trimethoprim/sulfamethoxazole 2
	LBXMC2	clindamycin 2
	LBXME2	erythromycin 2
	LBXMP2	penicillin 2

l35_c	LBXMI2	imipenem 2
	LBXMV2	vancomycin 2
	LBXMF2	cefazolin 2
	LBXMO2	oxacillin 2
	LBXMG2	gentamicin 2
	LBXMD2	ciprofloxacin 2
	LBXML2	levofloxacin 2
	LBXMR2	rifampin 2
	LBXMY2	amoxicillin/k clavulanate 2
	LBAMMT2	molecular type 2
	LBXETA	enterotoxin A
	LBXETB	enterotoxin B
	LBXETC	enterotoxin C
	LBXETD	enterotoxin D
	LBXETE	enterotoxin E
	LBXETH	enterotoxin H
	LBXTSS	toxic shock syndrome toxin 1
	LBXPVL	Panton Valentine leukocidin
	LBXSCC	SCCmec Type

Methicillin-Resistant *Staphylococcus aureus* Screening

Nasal cultures are collected from both anterior nares using a culturette swab (Becton Dickinson Microbiology Systems, Cockeysville, MD) and refrigerated until shipped overnight on cold packs to CDC. Swabs are first examined for proper labeling and integrity and then plated on mannitol salt agar (MSA; Becton Dickinson Microbiology Systems), a selective media for the isolation of *S. aureus.* MSA plates are incubated at 37°C for 48 hours. Mannitol fermenting colonies (yellow or gold) are selected from the MSA plates and subcultured to trypticase soy agar + 5% sheep blood plates (BAP; Becton Dickinson Microbiology Systems) and incubated at 37°C overnight. MSA plates with little or no growth are re-incubated at 37°C overnight, and plates with non-mannitol fermenting growth are held at room temperature. These plates are reexamined the next day, and any yellow or gold colonies are subcultured to BAP.

Overnight cultures on BAP are first screened using Staphaurex, a rapid latex kit for the identification of *S. aureus* (Remel, Lenexa, KS). A tube coagulase test using rabbit plasma with (ethylenedinitrilo)tetraacetic acid (EDTA; Becton Dickinson Microbiology Systems) is then performed on Staphaurex-negative isolates from BAP with morphology consistent with *S. aureus* and Staphaurex-positive isolates with morphology inconsistent with *S. aureus* (non-hemolytic). Staphaurex-positive isolates and Staphaurex-negative tube coagulase-positive isolates are identified as *S. aureus* and saved for further testing. Staphaurex-positive, tube coagulase-negative isolates are discarded.

S. aureus isolates are screened for methicillin resistance following the National Clinical and Laboratory Standards Institute (NCCLS) disk diffusion method. Overnight cultures from BAP are plated on Mueller-Hinton (MH) agar, and a 1-µg oxacillin (OX) disk is placed on the inoculated plate. Zone diameters are measured and recorded after 24-h incubation at 37°C (sensitive, ≥ 13 mm; intermediate, 11–12 mm; resistant, ≤ 10 mm).

Isolates resistant to OX (MRSA), intermediate to OX, and every 10th isolate sensitive to OX (MSSA) by disk diffusion are saved for additional testing of organism characteristics. These tests include antibiotic susceptibility testing (MIC) by using broth microdilution using NCCLS reference methods, MicroScan Pos combo 10 panels (Dade MicroScan, West Sacramento, CA); strain typing by pulsed-field gel electrophoresis (PFGE) using *SmaI* enzyme; singleplex polymerase chain reaction (PCR) for detection of enterotoxins, toxic shock syndrome toxin-1, and Panton-Valentine Leukocidin toxin; and SCC-mec cassette typing by PCR.