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| PBP2a SA Culture Colony Test |
| **Purpose** | This procedure provides instructions for PBP2a SA Culture Colony Test. It is a qualitative, *in vitro* immunochromatographic assay for the rapid detection of penicillin-binding protein 2a (PBP2a) in isolates identified as *Staphylococcus aureus* as anaid in identifying methicillin-resistant *Staphylococcus aureus*.  |
| **Policy Statements** | This procedure applies to microbiologists who perform microorganism identification.  |
| **Principle and Clinical Significance** | The Alere™ PBP2a SA Culture Colony Test is a rapidimmunochromatographic membrane assay that uses highly sensitive recombinant monoclonal antibody fragments (rFabs) to detect the PBP2a protein directly from bacterial isolates. The rFab and a control protein are immobilized onto a nitrocellulose membrane as two distinct lines and combined with the sample pad, a pink/purple conjugate pad, and an absorption pad to form a test strip. Early detection of MRSA is critical in efforts to decrease morbidity and mortality, reduces empiric use of vancomycin and reduces health care costs. The PBP2a has the advantage over *mec*A in identifying strains that not only harbor the *mec*A gene but also produce the protein that confers resistance to methicillin. The PBP2a allows for a short turnaround time compared to conventional and molecular methods.  |
| **Test Code** | PBP2 |
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| **Materials** | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * Test Strips
* Reagent 1-clear blue alkaline solution
* Reagent 2-clear acidic solution
 | * Assay tubes
* Test Racks
 | * Clock
* Timer
* Bacterial loops
* Vortex
 | * Columbia Agar with 5% sheep blood
* Mueller Hinton Agar
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| **Sample** | * Fresh bacterial isolate (<24 hours) of *Staphylococcus aureus* from Columbia Agar with 5% sheep blood or Mueller Hinton agar.
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| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**1. [*Biohazard Containment*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.1%20Biohazard%20Containment.docx)
2. [*Safety in the Microbiology/Virology Laboratory*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx)
* [*Biohazardous Spills*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.4%20Biohazardous%20Spills.docx)
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| **Storage** **and stability**  | Store kit components at room temperature or in refrigeration (2-30oC). The Alere™ PBP2a test kit and reagents are stable until the expiration date marked on the outer packaging.  |
| **Quality Control** | Perform External Quality Control, Positive and Negative with each new lot, new shipment to ensure the test reagents are working and the test is correctly performed. * *Staphylococcus aureus* ATCC # 43300 for the positive control.
* *Staphylococcus aureus* ATCC # 25923 for the negative control.
* Record in QC binder.

Record Daily Quality Control from the built in positive and negative procedural controls with each run in Workups in Sunquest MRE.* Procedural Controls: The appearance of a pink/purple line at the “control line” position can be considered an internal positive procedural control. If capillary flow has occurred, this line will always appear.
* In comparison to the color of the control line, the background color on the test strip should be white within 5 minutes.
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| **Procedure****Interpretation of Test** | 1. Perform test on all *Staphylococcus aureus* isolates to distinguish between MSSA or MRSA.
2. Wearing gloves hold the dropper bottle vertically; add two drops of Reagent 1 to an assay tube.
3. Take one heaped 1ul loop (a heavy inoculum) of sample from well-isolated colonies on the culture plate, place into the tube and thoroughly mix.
4. Holding the dropper bottle vertically, add two drops of Reagent 2 to the tube.
5. Vortex briefly. The blue solution must turn a clear color (if the color does not change, add one more drop of Reagent 2 and mix until the sample turns clear).
6. Insert the test strip into the assay tube with arrows pointed downward.
7. At five minutes, withdraw the test strip from the tube and read the assay result.
* **Negative:** Pink/Purple Control Line appears in the top half of the test strip. No other lines appear.
* **Positive:** Pink/Purple Control Line appears and a second Pink/Purple Sample Line appear below it in the bottom half of the test strip. Any Sample Line, even when very faint, is positive.
* **Invalid:** If the Pink/Purple control Line does not appear, whether a Sample Line is present or not, the test is Invalid. Repeat invalid tests with a new strip. Call Alere™ Technical Support if the problem persists.
* The following figure displays how results are to be interpreted:

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| **SAUR-SCV cefoxitin susceptible testing** | 1. For testing SAUR-SCV strains, use cefoxitin induced growth from MHSB.
2. Prepare a 0.5 McFarland with a fresh isolate.
3. Inoculate the MHSB plate. First streak of swab should go down the middle of the plate. Swab across the entire agar surface at a 90º angle.
4. Repeat this procedure 3 times, rotating the plate approximately 60º between streaking to ensure even distribution. Avoid hitting the sides of the plate to prevent aerosols.
5. Allow plate to stand 3-5 minutes before applying the disk.
6. Apply FOX disc, invert plates and incubate at 35ºC in CO2 incubator for 24 hours.
7. When the cefoxitin zone is susceptible, perform PBP2a using the induced growth, the growth taken from the zone margin surrounding the cefoxitin disc.
8. When the cefoxitin zone is resistant, PBP2a testing does not need to be performed.

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| **Limitations** | 1. The use of fresh (<24 hours) culture is recommended.
2. The performance of the Alere™ PBP2a SA Culture Colony Test has not been established for use on refrigerated specimens.
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| **Method Performance Specifications** |  1. For *in vitro* diagnostic use only.
2. If refrigerated, allow all kit components to equilibrate to room temperature before use.
3. Leave test strip sealed in pouch until just before use.
4. Avoid skin and eye contact with reagents and test strip.
5. Do not use past expiration date.
6. Do not interchange or mix components for different kit lots.
7. Controls and test strips may contain pathogenic organisms; handle with appropriate precautions and dispose of materials in biohazard waste receptacles.
8. The Alere™ PBP2a SA Culture Colony test should only be performed on isolates of *Staphylococcus aureus*.
9. **Reagent 1 contains sodium hydroxide. Danger: Causes severe skin burns and eye damage.**
10. **Reagent 2 contains sodium azide.**
11. Follow all ordinances for waste disposal regulations.
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| **Result Reporting** | 1. Record results in Sunquest MRE in the Culture Entry tab. Click on the Workups button. Enter results of POS or NEG at PBP2 prompt in the result box. Enter POS NEG at the QPBP prompt to document the internal Positive and Negative QC.
2. **Positive test:** Result as MRSA and add comment code PPBP for POSITIVE for PBP2a after MRSA code.
3. **Negative test**: Result as SUMP-MSSA and add comment code NPBP for NEGATIVE for PBP2a after MSSA code. Add comment PBPC. The SUMP code will be removed after susceptibilities have been confirmed by either the Vitek susceptibility card or the FOXS.
4. If susceptibility results are not needed, results do not need to be confirmed. Do not add SUMP before MSSA with negative PBP2a results.
5. Add bill code PB2A in the billing tab. See [MCVI 5.3 Billing Add-On Charges](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%205%20Computer%5CMCVI%205.3%20Billing%20Add-on%20Charges.docx) for additional codes.
6. MRSA isolation requires a “Called to” if not from E.D. or a repeat isolate. Freeze isolates for future reference.

Observations: 1. 2+ METHICILLIN-RESISTANT STAPH AUREUS \*\*\*MDRO\*\*\* POSITIVE for PBP2aWorkups: Workup # 1 Workup Components Med : SB CAT : POS Desc : BH QPBP : POS NEG ID : SAUR PBP2 : POS SLC : POSObservations: 1. 2+ PRESUMPTIVE STAPH AUREUS, METHICILLIN SENSITIVE NEGATIVE for PBP2a 2. This test detects mecA derived from PBP2a which confers methicillin resistance in S.aureus. It is not designed to detect rare mechanisms of resistance such as mecC. |
| **Interpretation /Discrepant Results** |

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| PBP2a | Cefoxitin (Vitek) | Oxacillin (Vitek) | Cefoxitin Disk | Final Interpretation |
| Negative\* | Negative | Susceptible | Not done | MSSA |
| Positive | Positive | Resistant | Not done | MRSA |
| Positive | Negative | Susceptible | Resistant | MRSA |
| Positive  | Negative | Susceptible | Susceptible | MSSA (repeat PBP2a, if positive contact Lab Director/Supervisor) |
| Negative\* | Negative | Resistant | Resistant | MRSA (confirm FOX result before reporting) |
| Negative\* | Negative | Resistant | Susceptible | MSSA (confirm FOX result before reporting) |
| Negative\* | Positive | Resistant | Resistant | MRSA (repeat PBP2a) |
| Negative\* | Positive | Resistant | Susceptible | MSSA (repeat PBP2a) |
| Negative (no susceptibility needed) |  |  |  | MSSA |
| Positive (no susceptibility needed) |  |  |  | MRSA  |

\*Initial result would be presumptive |
| **References** | Alere™ PBP2a SA Culture Colony Test Product Insert. Alere™ Scarborough, Inc Scarborough, ME 2017/03Lodise TP, McKinnon PS. Clinical and economic impact of methicillin resistance in patients with Staphylococcus aureus bacteremia. Diagn Microbiol Infect Dis. 2005 Jun: 52(2):113-22.CLSI M100 S28 Clinical Standards for Antimicrobial Susceptibility Testing Table 2C Staphylococcus spp. Page 57. |
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | * 1. Direct observation.
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Susan DeMeyere | 3/14/2018 | Initial Version |
| 2 | Susan DeMeyere | 4/11/2018 | Changed testing to all SAUR isolates. Added instruction for SAUR SCV testing. Added table for interpretation and discrepant results. Added instruction for negative results.  |
| 3 | Susan DeMeyere | 5/30/2019 | Added to and updated table. |
| **Archived by:** |  | **Archived Date:** |  |