



LABORATORY NAME:	
LABORATORY ADDRESS:	
DATE OF THIS PACKET:	
INSERT REVISION:	IN893000 Rev.6 10/2019

Clearview™ PBP2a SA Culture Colony Test REF 893-000 Laboratory Procedure

This procedure is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. **Any modifications to this document are the sole responsibility of the Facility.**

CLIA Complexity: Moderate

1. Intended Use

The **Clearview™ PBP2a SA Culture Colony Test** is a qualitative, *in vitro* immunochromatographic assay for the rapid detection of penicillin-binding protein 2a (PBP2a) in isolates identified as *Staphylococcus aureus* as an aid in identifying methicillin-resistant *Staphylococcus aureus* (MRSA).

2. Summary

Early detection of methicillin-resistant Staphylococcal infections is known to reduce health care costs, critical in efforts to decrease patient morbidity and mortality, reduce empiric use of vancomycin and permits cost-effective decisions for optimal patient management.¹

Conventional methods of antimicrobial susceptibility testing can take over 24 hours, and *mecA* gene identification is expensive and time-consuming to perform. However, penicillin-binding protein 2a (PBP2a) detection has the advantage over *mecA* in identifying strains that not only harbor the *mecA* gene but also produce the protein that confers resistance to methicillin.

The **Clearview™ PBP2a SA Culture Colony Test** provides a simple and rapid method to detect the PBP2a protein found in isolates of methicillin-resistant *Staphylococcus aureus*.

3. Test Principle

The **Clearview™ PBP2a SA Culture Colony Test** is a rapid immunochromatographic 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 a sample pad, a pink/purple conjugate pad, and an absorption pad to form a test strip.



Isolates are sampled directly from the culture plate and eluted into an assay tube containing Reagent 1. Reagent 2 is then added and the test strip is placed in the assay tube. Results are read visually at 5 minutes.



4. Specimen Collection/Treatment

A. Specimen:	<p>Specimens are bacterial isolates of <i>Staphylococcus aureus</i>. The use of fresh (<24 hours) cultures is recommended. The performance of the Clearview™ PBP2a SA Culture Colony Test has not been established for use with refrigerated specimens.</p> <p>Culture Media:</p> <p><i>S. aureus</i> colonies may be tested from any of the following culture media:</p> <ul style="list-style-type: none">• Tryptone Soy Agar (Tryptic Soy Agar) with 5% sheep blood (TSA blood)• Columbia Agar with 5% sheep blood• Mueller Hinton Agar
B. Handling Precautions:	<p>Patient samples, controls and tests should be handled as though they could transmit disease. Observe established precautions against microbial hazards.</p>

5. Reagents and Equipment

A. Reagents and Materials Provided

Materials Provided in the Clearview™ PBP2a SA Culture Colony Test Kit

Component	Content
Test Strips	A test strip covered with a plastic overlay with directional arrows to indicate testing position.
Reagent 1	A clear, blue alkaline solution. 
Reagent 2	A clear, slightly acidic solution containing sodium azide buffer and surfactants. 
Assay Tubes	
Test Racks	

B. Materials Required but Not Provided

Clock, timer or stopwatch, bacteriological loops, vortex mixer and external positive and negative control strains.

C. External Positive and Negative Controls

The recommended controls are listed below. Alternatively, laboratories may use resistant and sensitive *S. aureus* strains routinely used, providing control results are as expected.

Positive control: *Staphylococcus aureus*, ATCC # 43300

Negative control: *Staphylococcus aureus*, ATCC # 25923



6. Storage and Stability

Store kit components at room temperature or under refrigeration (2-30°C).

The **Clearview™ PBP2a SA Culture Colony Test** Kit and reagents are stable until the expiration dates marked on their outer packaging.

7. Quality Control

Daily Quality Control:

Clearview™ PBP2a SA Culture Colony Test has built-in positive and negative procedural controls. For quality control, Clearview suggests that you record these controls for each test run.

Procedural Controls:

- A. 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.
- B. In comparison to the color of the control line, the background color on the test strip should be white within 5 minutes.

External Positive and Negative Controls:

Good laboratory practice suggests the use of positive and negative controls to ensure that:

- test reagents are working, and
- the test is correctly performed.

For each new lot, external positive and negative controls should be tested. These will monitor the entire assay. The results of these control samples should be recorded. Please refer to the Sample Preparation Procedure section for further instructions.

Recommended Control Strains:

Positive control: *Staphylococcus aureus* ATCC # 43300

Negative control: *Staphylococcus aureus* ATCC # 25923



Other controls may be tested in order to conform with,

- local, state, and/or federal regulations,
- accrediting groups, and/or,
- your laboratory’s standard Quality Control procedures.

If the correct control results are not obtained, do not report results. Contact Technical Support (please refer to the Ordering and Contact Information section).

8. Precautions

1. For *in vitro* diagnostic use only.
2. If refrigerated, allow all kit components to equilibrate to room temperature (15-30°C) before use.
3. Leave test strip sealed in its foil pouch until just before use.
4. Avoid skin and eye contact with reagents and test strip.
5. Do not use kit past its expiration date.
6. Do not interchange or mix components from different kit lots.

7. As with all bacterial samples, controls and test strips may contain pathogenic organisms; handle with appropriate precautions and dispose of materials safely in biohazard waste receptacles.
8. The **Clearview™ PBP2a SA Culture Colony Test** should be performed only 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 and surfactants. **WARNING**: Causes serious eye irritation. 
11. Safety Data Sheets for this product are available upon request.
12. Follow your national, regional, and local ordinances accordingly for waste disposal regulations.

9. Control Procedure

1. Subculture the control strain onto a culture plate. Incubate the plate overnight at 33-35°C for 18-24 hrs.
2. Follow Test Procedure below.

10. Test Procedure

If refrigerated, allow reagents and test strips to equilibrate to room temperature (15-30°C) before testing.

The test can be performed from well-isolated colonies on the primary plate if there is sufficient growth, or from a subculture of the isolate.

1. Holding the dropper bottle vertically, add two drops of Reagent 1 to an assay tube.
2. Take one heaped 1 µL bacteriological loop (a heavy inoculum) of sample from well-isolated colonies on the culture plate, place into the tube and thoroughly mix.
3. Holding the dropper bottle vertically, add two drops of Reagent 2 to the tube.
4. 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).
5. Insert the test strip into the assay tube with arrows pointed downward.
6. At five (5) minutes, withdraw the test strip from the tube and read the assay result.

11. Interpretation of Test Results

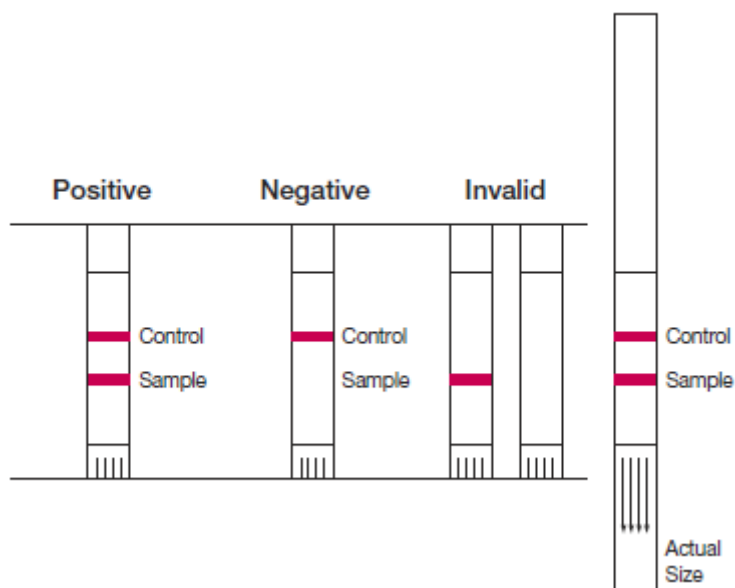
For help in reading the test strip, refer to the Result Interpretation Graphic.

For a **Negative Sample**, a PINK/PURPLE Control Line appears in the top half of the test strip. No other line appears.

For a **Positive Sample**, the PINK/PURPLE Control Line appears AND a second PINK/PURPLE Sample Line appears below it in the bottom half of the test strip. Any Sample Line, even when very faint, is positive. A test is **Invalid** if the PINK/PURPLE Control Line does not appear, whether a Sample Line is present or not. Repeat invalid tests with a new test strip. Call Clearview™ Technical Support if the problem persists.

Reporting of Results

Result	Suggested Report
Positive	Positive for PBP2a
Negative	Negative for PBP2a
Invalid	Indeterminate; do not report. Re-test sample and contact Clearview™ Technical Service Support if problem persists.



12. Performance Characteristics

Clinical Performance

The clinical performance of the **Clearview™ PBP2a SA Culture Colony Test** was established in a multi-center clinical study conducted in 2013 at three (3) geographically diverse laboratories.

A total of 454 *S. aureus* samples were evaluated in the **Clearview™ PBP2a SA Culture Colony Test**, compared to results of 30 µg cefoxitin disk diffusion and interpreted according to CLSI standards. **Clearview™ PBP2a SA Culture Colony Test** performance versus cefoxitin disk diffusion, including 95% confidence intervals and stratified by plate type, is provided in Table 1.

All positive and negative daily controls generated the expected results.

Table 1: Clearview™ PBP2a SA Culture Colony Test Performance vs. Cefoxitin (30 µg) Disk Diffusion in *S. aureus* Isolates: Results by Plate Type

Plate Type	Sensitivity	95% C.I.	Specificity	95% C.I.
Primary Plate¹	100.0% (129/129)	(97.1, 100.0)	98.5% (134/136)	(94.8, 99.6)
Tryptic Soy Agar with 5% sheep blood	99.1% (213/215)	(96.7, 99.8)	99.2% (237/239)	(97.0, 99.8)
Columbia Agar with 5% sheep	98.6% (212/215)	(96.0, 99.5)	99.2% (237/239)	(97.0, 99.8)

blood				
Mueller Hinton with 30 µg cefoxitin induction	99.1% (213/215)	(96.7, 99.8)	99.6% (238/239)	(97.7, 99.9)

1: **Clearview™ PBP2a SA Culture Colony Test** was performed from primary plates at 2 out of 3 clinical sites. Primary plates were either Tryptic Soy Agar or Columbia Agar, with the exception of two samples of unknown plate type.

Analytical Performance

Analytical Reactivity and Specificity

162 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and 112 strains of methicillin-sensitive *Staphylococcus aureus* (MSSA) were tested with **Clearview™ PBP2a SA Culture Colony Test** with expected results. These bacterial strains were obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA), American Type Culture Collection (ATCC) and a collection of strains from the Department of Infectious Disease Epidemiology of the Imperial College in London, England. Please note that isolates tested were not cultured using Columbia agar plates. All positive and negative daily controls generated the expected results.

Reproducibility Study

A study of **Clearview™ PBP2a SA Culture Colony Test** was conducted at 3 separate sites using panels of blind coded specimens containing negative and positive samples in duplicate for each panel. Participants each tested one panel on 5 different days. There was 100.0% (600/600) agreement with expected test results. There were no significant differences within run (replicates tested by one operator), between run (5 different days), between sites (3 sites), or between operators (6 operators). Please note that isolates tested were not cultured using Columbia agar plates. All positive and negative daily controls generated the expected results.

13. References

1. Lodise 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.

Ordering and Contact Information

893-000 Clearview™ PBP2a SA Culture Colony Test

US: 1 877 441 7440

OUS: +1 321 441 7200

Technical Support

Advice Line

Further information can be obtained from your distributor, or by contacting Clearview™ Technical Support on:

US

1-877-866-9341 TS.SCR@abbott.com

Africa, Russia, CIS

+44 161 483 9032 EMEprouductsupport@abbott.com

Asia Pacific

+61 7 3363 7711 APprouductsupport@abbott.com

Canada



+57 (1) 482 4033 CANproductsupport@abbott.com

Europe & Middle East

+44 161 483 9032 EMEprouductsupport@abbott.com













Latin America

+57 (1) 4824033 LAproductsupport@abbott.com

REF

893-000

Symbols

 <p>Consult instructions for use</p>	 <p>Authorized Representative in the European Community</p>	 <p><i>In vitro</i> diagnostic medical device</p>
 <p>Catalog number</p>	 <p>CE Mark</p>	 <p>Manufacturer</p>
 <p>Temperature limitation</p>	 <p>Hazard Pictogram. See precautions.</p>	 <p>Kit contains sufficient materials for 25 tests</p>
 <p>Do not reuse</p>	 <p>Batch code</p>	 <p>Use by</p>



Test Procedure Approval and Review Sheet

PREPARED BY:	
DATE:	
SUPERVISOR REVIEW:	
DATE:	
LABORATORY DIRECTOR OR DESIGNEE APPROVAL:	
IMPLEMENTATION DATE:	
SUPERSEDES PROCEDURE DATED:	
DATE PROCEDURE RETIRED:	

LABORATORY DIRECTOR OR DESIGNEE	DATE REVIEWED	LABORATORY DIRECTOR OR DESIGNEE	DATE REVIEWED



Clearview™ PBP2a SA Culture Colony Test Verification Form

ACCOUNT NAME: _____

ADDRESS: _____

TELEPHONE: _____

**CLEARVIEW™ PBP2A SA CULTURE
COLONY TEST**

LOT #/EXP: _____

DATE: _____

SUPERVISOR SIGNATURE: _____

Record the results from reference samples below.

Record the Sample #, the **Clearview™ PBP2a SA Culture Colony Test** results, Tester's Initials, and any comments. After the **Clearview™ PBP2a SA Culture Colony Test** results have been recorded (positive or negative) then record the Expected Results (positive or negative).

SAMPLE #	EXPECTED RESULTS	CLEARVIEW™ PBP2A SA CULTURE COLONY TEST RESULTS	TESTER'S INITIALS	COMMENTS



Clearview™ PBP2a SA Culture Colony Test Verification Form (continued)

SAMPLE #	EXPECTED RESULTS	CLEARVIEW™ PBP2A SA CULTURE COLONY TEST RESULTS	TESTER'S INITIALS	COMMENTS

REVIEW: _____ DATE: _____

LABORATORY DIRECTOR REVIEW AND APPROVAL FOR CLINICAL USE: _____

DATE: _____



Clearview™ PBP2a SA Culture Colony Test External Quality Control

There are two options for complying with CLIA's daily QC requirements for non-waived test systems under Section 493.1256 of the regulations:

- Run two levels of external controls daily before patient testing OR
- Laboratories may develop and implement an IQCP for each non-waived test system.

Clearview IQCP Support Documents may be found at <http://www.alere.com/IQCP>. The following listed conditions are also required as a minimum requirement:

External QC testing is recommended:

- To be performed and recorded for each new lot
- Additional tests can be performed with the controls to meet the requirements of local, state, and/or federal regulation and/or accrediting groups, and/or your laboratory's standard QC Procedures

DATE	CLEARVIEW™ PBP2A SA CULTURE COLONY TEST KIT LOT/EXP	POSITIVE CTRL LOT/EXP	NEGATIVE CTRL LOT/EXP	POSITIVE CONTROL RESULT	NEGATIVE CONTROL RESULT	TESTER'S INITIALS	COMMENTS

REVIEWED BY: _____

DATE: _____



Clearview™ PBP2a SA Culture Colony Test Internal Controls and Patient Record

LOT NUMBER _____ EXP. DATE _____

Record the Date, Patient's Name, Patient Test Result, Internal Control Results and the Tester's initials.

Positive Internal Control = The appearance of a pink/purple line at the "control line" position can be considered an internal positive procedural control.

Negative Internal Control = In comparison to the color of the control line, the background color on the test strip should be white within 5 minutes.

DATE	PATIENT NAME	PATIENT ID NUMBER	PATIENT RESULTS	INTERNAL CONTROL RESULT		INTERNAL CONTROLS		COMMENTS	TESTER'S INITIALS
				INVALID	VALID	+	-		

REVIEWED BY: _____

DATE: _____



Clearview™ PBP2a SA Culture Colony Test Lot to Lot Comparisons

NAME OF FACILITY: _____

External Quality Controls are required to test a new lot of reagents.

- For each new lot
- When required by local, state, and/or federal regulations, accrediting groups, or your lab's Quality Control procedures

	CURRENT CLEARVIEW™ PBP2a SA CULTURE COLONY TEST IN-USE KIT					NEW CLEARVIEW™ PBP2a SA CULTURE COLONY TEST KIT					
DATE	CLEARVIEW™ PBP2a SA CULTURE COLONY TEST KIT LOT/EXP	POSITIVE CONTROL LOT/EXP	NEGATIVE CONTROL LOT/EXP	POSITIVE CONTROL RESULT	NEGATIVE CONTROL RESULT	CLEARVIEW™ PBP2a SA CULTURE COLONY TEST KIT LOT/EXP	POSITIVE CONTROL LOT/EXP	NEGATIVE CONTROL LOT/EXP	POSITIVE CONTROL RESULT	NEGATIVE CONTROL RESULT	TECH'S INITIALS

REVIEWED BY: _____

DATE: _____



Quality Assessment Review Form and Checklist

These forms are used for periodical review of the patient testing process. These should be filed with the quality assessment records.

QUALITY ASSESSMENT ACTIVITY	COMMENTS	DATE	INITIALS
Patient Test Management: Evaluate criteria for specimen submission, handling, and rejection; test results requisitions and reporting, accuracy and reliability of reports.			
Quality Control: Assess control data, errors in reporting results, and corrective actions taken with appropriate documentation records.			
Proficiency Testing: Review the effectiveness of corrective actions taken for unsatisfactory performance or failures.			
Comparison of Test Results: Review at least semi-annually comparative results for multiple methods, instruments, or site correlations when more than one procedure exists.			
Relationship of Patient Test Information to Test Results: Evaluate patient test reports for accuracy of patient information, test results, and normal ranges. Identify and evaluate results inconsistent with Patient's age, sex, diagnosis, and other test parameters.			
Personnel: Evaluate the effectiveness of policies and procedures for assuring employees' competence of testing and reporting test results.			
Communications: Evaluate documented problems and corrective actions that occur between the laboratory and the authorized individual who orders or receives the test result.			
Complaint Investigation: Evaluate documented complaints and corrective actions.			
Quality Assessment Reviews with Staff: Document discussion with Staff regarding identified problems and corrective actions during the QA review.			



Corrective Action Form

PROBLEM/ERROR	CORRECTIVE ACTION

TECHNOLOGIST: _____

DATE: _____

SUPERVISOR: _____

DATE: _____

LABORATORY DIRECTOR: _____

DATE: _____



Tips for Successful Proficiency Testing (PT) Performance

- Strictly follow the PT provider's storage or handling requirement ***before testing PT specimens***.
- Analyze PT specimens ***within the time frame*** provided by the PT provider.
- Contact the PT provider ***promptly*** when specimens are received damaged. You may be able to receive a replacement immediately.
- Avoid clerical error when filling out PT answer sheets. Be sure to ***enter the correct result next to the correct analyte*** on the answer form.
- Remember to identify the instrument or method you are using to perform your PT so you are ***graded among your peer group***.
- Make copies of all answer forms ***before submitting them*** to your PT provider.
- Please contact Technical Support at 1-877-866-9341 or ts.scr@abbott.com for further information on proficiency providers.



Certification of Training

This is to verify that personnel responsible for running the **Clearview™ PBP2a SA Culture Colony Test** at _____ have been thoroughly in-serviced on the test and the test procedure. This has included:

- **Review of the package insert**
- **Demonstration of the product assay**
- **Successful performance of the Clearview™ PBP2a SA Culture Colony Test and interpretation of results**

Names of the personnel who have been trained with the **Clearview™ PBP2a SA Culture Colony Test** and are responsible for reporting patient results:

PRINT NAME	SIGNATURE	DATE

Signature of Laboratory Director(s) responsible for personnel and testing:

SIGNATURE

DATE

SIGNATURE

DATE

TRAINER

DATE



Testing Personnel Training Assessment

Test Method: Clearview™ PBP2a SA Culture Colony Test

PROCEDURE	SATISFACTORY	UNSATISFACTORY	NOT APPLICABLE	COMMENTS / CORRECTIVE ACTIONS
<i>Observation of Test Performance:</i>				
Patient Sample Preparation (if applicable)				
Specimen Handling/Processing				
Testing				
Recording/Reporting Results				
<i>Assessment of Test Performance Using Known Samples</i>				
<i>Review of Records:</i>				
Patient/Quality Control Log Sheet Records				
Proficiency Testing Records				
<i>Assessment of Problem Solving Skills</i>				

(Attach all supporting documents)

EVALUATOR: _____

DATE: _____

EMPLOYEE: _____



Clearview™ PBP2a SA Culture Colony Test Quiz

Name: _____

Date: _____ Score: _____

Circle T (True) or F (False) for each Question:

- | | | |
|--|---|---|
| 1. The Clearview™ PBP2a SA Culture Colony Test kit components may be stored at room temperature or refrigerated. | T | F |
| 2. If refrigerated, allow all kit components to equilibrate to room temperature before use. | T | F |
| 3. External positive and negative control stains are included in the Clearview™ PBP2a SA Culture Colony Test kit. | T | F |
| 4. Read the test result at 8 minutes. | T | F |
| 5. The test can be performed from well-isolated colonies on the primary plate if there is sufficient growth, or from a subculture of the isolate. | T | F |
| 6. After adding Reagent 2, the solution should be blue when adding the test strip. | T | F |
| 7. The use of fresh (<24 hours) cultures is recommended. | T | F |
| 8. One pink-purple line that appears in the top half of the test strip at the Control position and no other visible line is interpreted as a positive test result. | T | F |
| 9. Insert the test strip into the assay tube with arrows pointed downward. | T | F |
| 10. The Clearview™ PBP2a SA Culture Colony Test is a quantitative test based on the intensity of color. | T | F |



Clearview™ PBP2a SA Culture Colony Test Quiz Answer Key

ANSWER KEY		EXPLANATION
1.	T	Store kit components at room temperature or under refrigeration (2-30°C).
2.	T	If refrigerated, allow all kit components to equilibrate to room temperature (15-30°C) before use.
3.	F	External positive and negative control stains are not included in the kit. External controls are required. Recommended controls can be found in the insert.
4.	F	At five (5) minutes, withdraw the test strip from the tube and read the assay result.
5.	T	The test can be performed from well-isolated colonies on the primary plate if there is sufficient growth, or from a subculture of the isolate.
6.	F	After adding Reagent 2, vortex briefly. Then 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).
7.	T	The use of fresh (<24 hours) cultures is recommended. The performance of the Clearview™ PBP2a SA Culture Colony Test has not been established for use with refrigerated specimens.
8.	F	For a Negative Sample, a PINK/PURPLE Control Line appears in the top half of the test strip. No other line appears.
9.	T	Insert the test strip into the assay tube with arrows pointed downward.
10.	F	The test is qualitative.