PURPOSE: The cefoxitin screen is used to detect *mec*A-mediated resistance to oxacillin and other penicillinase-stable penicillins. Cefoxitin is used as a surrogate for oxacillin resistance and oxacillin is reported based on the cefoxitin result.

SAMPLE INFORMATION:

Type	Few, well isolated colonies that are 18 to 24 hours old

REAGENTS and/or MEDIA:

Type	Oxoid 30 µg Cefoxitin Disk
Stability and Storage Requirements	• Unopened cartridges must be stored at 2°C to 8°C. • Unopened cartridges should be allowed to come to room temperature before removing them from the packaging to minimize condensation. • Opened cartridges need to be stored at 2°C to 8°C, in an opaque, air tight container with a charged desiccant to protect the disks from moisture. • Once a cartridge is opened, it should be stored for no longer than a month.

SUPPLIES:

- Plastic Vitek tubes and caps
- 0.9% sterile saline
- Sterile swabs
- DensiCHEK Plus
- Mueller Hinton agar
- Forceps
- 35° ambient air incubator
- Small, metric ruler

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 when 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:

- Quality control is performed weekly:
  - Resistant: *Staphylococcus aureus* ATCC 43300, Zone size = ≤ 21 mm
  - Sensitive: *Staphylococcus aureus* ATCC 25923, Zone size = 23 – 29 mm
- A TQC order is automatically generated on Wednesdays to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing the cefoxitin screen	
1	Remove the antibiotic disks from the refrigerator for 1 hour and bring to room temperature.
2	Remove Mueller Hinton agar from the refrigerator and bring to room temperature.
3	Dispense 3 mL of 0.9% sterile saline into a labelled plastic test tube. Pick several colonies from a fresh agar plate and prepare a suspension equivalent to a 0.5 McFarland standard.
4	Within 15 minutes of adjusting turbidity, dip a sterile cotton swab into the inoculum and rotate against the wall of the tube above the liquid to remove excess inoculum.
5	Swab the entire surface of the agar three times, rotating plate approximately 60° between streaking to ensure even distribution. To minimize aerosols, avoid hitting the sides of the plate. Finally, run swab around the edge of the agar to remove any excess moisture. Allow inoculated plate to stand for 3 to 15 minutes before applying disks.
6	Apply the cefoxitin disk to the agar surface with forceps. Apply gentle pressure to ensure complete contact of disk with agar.
7	Invert the plate and incubate within 15 minutes of the disk application: • *S.aureus* and *S.lugdunensis* in the O_2 incubator for 16 to 18 hours. • Coagulase-negative *Staphylococcus* (except *S.lugdunensis*) in the O_2 incubator for 24 hours.
8	After incubation, read plates only if lawn of growth is confluent.
9	Use a ruler held on the back of the plate to measure the diameter of inhibition zone to the nearest millimeter, including the disk for diameter.

INTERPRETATION OF RESULTS:

IF	THEN
Zone of ≤ 21mm for *S.aureus* and *S.lugdunensis* Zone of ≤ 24mm for other Coagulase neg. *Staphylococcus*	**Cefoxitin screen = Positive** ***mec*A positive**
Zone of ≥ 22mm for *S.aureus* and *S.lugdunensis* Zone of ≥ 25mm for other Coagulase neg. *Staphylococcus*	**Cefoxitin screen = Negative** ***mec*A negative**

LIMITATIONS/PRECAUTIONS:

1. If cefoxitin is used as a surrogate, the isolate should be reported as oxacillin susceptible or oxacillin resistant based on the cefoxitin result.
2. Because of the rare occurrence of resistance mechanisms other than *mec*A in *S.aureus*, isolates that are negative for the *mec*A gene or that do not produce PBP2a but for which oxacillin MICs are ≥ 4 µg/mL should be reported as oxacillin resistant.
3. Oxacillin-resistant *Staphylococci* are considered resistant to all penicillins, cephems (except for cephalosporins with anti-MRSA activity), β-lactam/β-lactamase inhibitors and carbapenems. This recommendation is based on the fact that most cases of documented MRS infections have responded poorly to β-lactam therapy or because clinical data that demonstrate efficacy for these agents in MRS infections are not available.
4. The zone diameter needs to be read with reflected, not transmitted light (plate held up to the light).

REFERENCES:

- Oxoid, Antimicrobial Susceptibility Test Disks product insert, 2018-07
- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	4 APR 19	Initial Release	L. Steven