PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Rapid ESBL Procedure for Blood Cultures

Department of Microbiology

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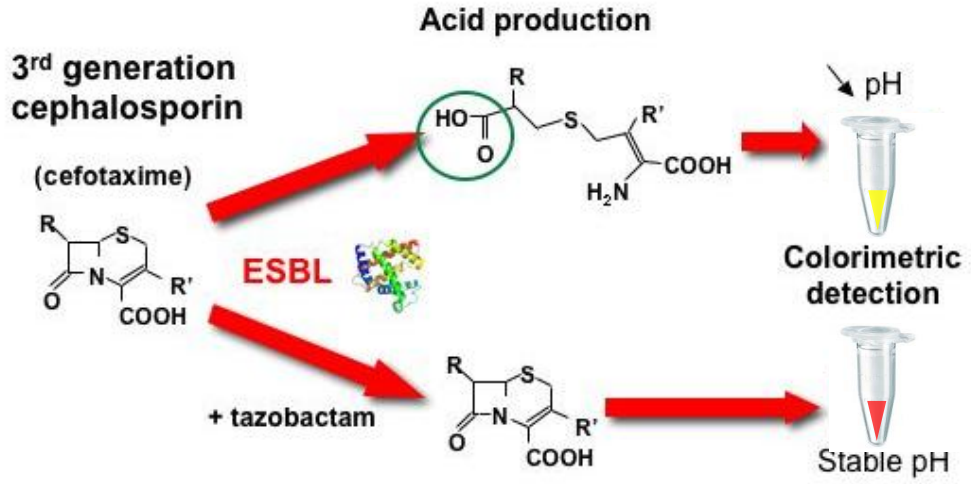
1.0 Clinical Significance

Extended-spectrum β-lactamases (ESBLs) confer resistance to the cephalosporins (i.e., cefotaxime, ceftriaxone, ceftazidime) and monobactams (i.e., aztreonam). A variety of ESBLs, mostly of the CTX-M, TEM, and SHV types, have been reported in *Enterobacteriaceae*. *E. coli* and *Klebsiella* are the main source of community- and hospital-acquired infections. Identification of ESBL-producing isolates is necessary for guiding appropriate antimicrobial therapy and improving infection control practices. Rapid identification of isolates causing serious infections, such as sepsis, is critical to reducing patient mortality.

2.0 Purpose or Principle

The ESBL NDP (Nordmann/Dortet/Poirel) test was developed for a rapid identification of ESBLs in *Enterobacteriaceae*. This biochemical test is based on the *in vitro* detection of a cephalosporin (cefotaxime) hydrolysis that is inhibited by tazobactam addition. ESBL activity is detected by a color change (red to yellow) of the phenol red pH indicator due to carboxyl-acid formation resulting from cefotaxime hydrolysis.



3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel trained to perform and interpret the assay.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

The reagents and/or chemicals that are used in this procedure may be hazardous to your health if handled incorrectly. More extensive information concerning the safe handling of the reagents used in this procedure, as well as other important safety information may be obtained by consulting the Material Safety Data Sheet (MSDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Airborne pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Gloves – must be worn when handling positive blood cultures.
- Laboratory Coat – must be worn when handling cultures and reagents.
- Biological Safety Cabinet – must be used when processing positive blood cultures.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- MSDS
- Chemical hygiene plan

5.0 Specimen Requirements

Testing should be performed on instrument-positive blood cultures that demonstrate Gram-negative bacilli on the smear. The rapid ESBL test may be performed concurrently with rapid identification testing, such as the BioFire FilmArray BCID assay. However, the rapid ESBL results should only be reported for those genera for which CLSI confirmatory testing is described (*E. coli, Klebsiella,* and *Proteus*).

6.0 Materials

6.1 Equipment

- Vortex
- Microcentrifuge
- Dry heating block for 1.5 mL tubes
- Timer

6.2 Consumables

- 15-mL sterile conical tubes
- 3 cc sterile syringes
- 20-gauge blunt transfer needles
- 2-mL screw-top microcentrifuge tubes
- 1.5-mL microcentrifuge tubes with snap cap
- Pipette (100-1000 µL) and filtered sterile tips
- Fine-tip transfer pipettes

6.3 Reagents

- Cefotaxime sodium salt (Fisher catalog no. BP29511)
- Tazobactam (Fisher catalog no. NC9359477)
- Phenol red solution, 0.5% (Fisher catalog no. NC0341766)
- Triton X-100 Surfact-Amps® Detergent, 10% (Fisher catalog no. P185111)
- B-PER® II, Bacterial Protein Extraction Reagent (Fisher catalog no. P178260)
- Sterile distilled water

6.4 Controls

- **Positive Control:** *E. coli* (ESBL) clinical isolate previously characterized by CLSI methods.
- **Negative Control:** *E. coli* ATCC 25922

7.0 Reagent Preparation & Storage

7.1 Reagent A

1. Mix 4 mL of phenol red solution in 33.2 mL of distilled water.
2. Adjust the pH of the solution to 7.8. The pH meter in Chemistry may be used to measure the pH of the solution. The pH can be lowered by adding small amounts (1 µL) of HCl (6 N). The pH can be raised by adding small amounts (< 10 µL) of NaOH (1N).

7.2 Reagent B

1. Weigh 0.06 g of cefotaxime powder in a 15-mL conical tube.
2. Add 10 mL of Reagent A and dissolve cefotaxime.

7.3 Reagent C

1. Prepare another tube of Reagent B.
2. Weigh 0.04 g of tazobactam powder in a separate15-mL conical tube.
3. Add 1 mL of sterile distilled water and dissolve tazobactam.
4. Combine 10 mL of Reagent B with the 1 mL of tazobactam solution and mix.

7.4 Storage

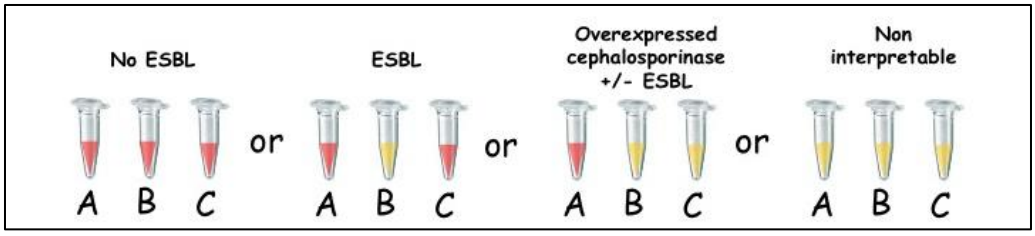
Reagent A, B, and C may be stored in 100 µL aliquots in 1.5-mL microcentrifuge tubes at -70°C for up to 1 year. Label the tops of each tube with the respective A, B, or C. Label the storage container with the reagent preparation and expiration dates.

8.0 Test Protocol

1. Gram stain positive blood culture to determine if Gram-negative rods are present.
2. Retrieve a tube of Reagent A, Reagent B, and Reagent C from the -70°C freezer. If QC has not been performed for the day, retrieve two more sets of reagents. Set the reagents aside to thaw at room temperature.
3. Under a biosafety hood, transfer 1.5 mL of a positive blood culture to a 2-mL screw-cap microcentrifuge tube using a 3-cc syringe with a 20-gauge blunt transfer needle.
4. Add 150 µL of Triton X-100 Surfact-Amps® Detergent.
5. Cap the tube, and vortex for 30 s.
6. Let tube sit for 5 min.
7. Centrifuge at 13,000 x *g* for 2 min.
8. Under a biosafety hood, use a fine-tip transfer pipette to remove and discard the supernatant in the biohazardous waste.
9. Suspend the pellet in 1,000 µL of distilled water by mixing up and down with the pipette.
10. Centrifuge at 13,000 x *g* for 2 min.
11. Under a biosafety hood, use a fine-tip transfer pipette to remove and discard the supernatant in the biohazardous waste.
12. Suspend the pellet in 310 µL of B-PER® II, Bacterial Protein Extraction Reagent by mixing up and down with the pipette.
13. Transfer 100 µL of the bacterial extraction to each tube of Reagent A, B, and C.
14. Incubate the tubes in a heat block at 37°C for 30 min.
15. Examine the color of the reagents in each tube.

9.0 Interpretation, Reporting, and Confirmation

Interpretation Guide



Tube A: no antibiotic

Tube B: cefotaxime

Tube C: cefotaxime + tazobactam

9.1 Negative for ESBL

- **Interpretation:** all tubes will all remain red.
- **Report:** "Negative extended spectrum beta-lactamase (ESBL) screen. Confirmatory testing is pending."
- **Confirmation:** Negative results should be confirmed by Phoenix NMIC testing. If the Phoenix flags the isolate for ESBL, CLSI disk confirmation must be performed.

9.2 Positive for ESBL

- **Interpretation:** tube B will be orange/yellow and tube A & C will remain red.
- **Report:** "Positive extended spectrum beta-lactamase (ESBL) screen. Isolate may be resistant to penicillins, cephalosporins and aztreonam. Confirmatory testing is pending."
- **Confirmation**: Positive results should be confirmed by the CLSI disk method.

9.3 Uninterpretable Results

- **Interpretation:** If tube B is yellow/orange, and either tube A or tube C are also yellow/orange, the result is uninterpretable.
- **Report:** "Uninterpretable extended spectrum beta-lactamase (ESBL) screen. Confirmatory testing is pending."
- **Confirmation**: If the rapid ESBL screen is uninterpretable, proceed with Phoenix NMIC testing. If the Phoenix flags the isolates for ESBL, CLSI disk confirmation must be performed.

10.0 Quality Control & Quality Assurance

Quality control should be performed each day when patient samples are tested. Controls should also be performed by each new user. Control material consists of blood cultures spiked with the control strains. New control cultures should be inoculated on Monday of each week.

- **Positive Control:** *E. coli* (ESBL) clinical isolate previously characterized by CLSI ESBL disk confirmation test. Do not use *K. pneumoniae* ATCC 700603. This strain contains an uncommon ESBL phenotype that reacts weakly with the rapid ESBL assay.
- **Negative Control:** *E. coli* ATCC 25922

11.0 Limitations

1. This test was validated using BacT/Alert® SA and SN blood culture bottles. Bottles containing activated charcoal have not been evaluated. Blood culture bottles from other manufacturers have not been evaluated.
2. A variety of phenotypes are associated with β-lactam resistance. While this assay has performed well with the common types currently encountered, there may be variant phenotypes that the assay may not detect. Negative results should be confirmed by Phoenix NMIC testing. Positive results should be confirmed by the CLSI disk method.
3. The red-to-yellow color change seen with many ESBL-producing isolates occurs within the first 1 to 5 min. However, some isolates may react weakly and require up to 20 to 30 min to produce a color change.
4. Although the assay produced correct results for 3 mixed patient cultures in the validation (1 positive and 2 negative), it is uncertain how mixed cultures might affect the assay results.

12.0 Validation Information

The rapid ESBL assay was evaluated with a total of 206 positive blood cultures. This included 54 positive patient blood cultures and 152 seeded blood cultures. The seeded blood cultures were created using clinical isolates. These isolates were grown overnight on blood agar plates and then used to create bacterial suspensions equivalent to a 0.5 McFarland. The suspensions were serially diluted to an approximate concentration on 1×10^4 CFU/mL. Instrument-negative patient blood cultures were inoculated with 100 µL of the isolate suspensions to achieve a final inoculum of 10^3 CFU/bottle. The bottles were incubated at 35 ± 2°C for 12 to 24 h and tested with the rapid ESBL assay.

The ESBL status for all of the isolates was confirmed by the CLSI disk method. Of the 206 cultures tested, 104 (50%) of the isolates were ESBL-positive and 102 (50%) were ESBL-negative. Three of the ESBL-producing isolates were recovered from patient blood cultures and the remaining 101 were in seeded cultures. The isolates included 173 *E. coli,* 29 *Klebsiella pneumoniae,* 3 *Klebsiella oxytoca,* and 1 *Proteus mirabilis.* Three of the patient blood cultures were mixed cultures. One EBSL-negative cultures grew *K. pneumoniae, E. cloacae,* and, *S. maltophilia.* Another ESBL-negative blood culture grew *E. cloacae* and *K. pneumoniae.* One ESBL-positive patient blood culture grew *E. coli* (ESBL+) and *K. pneumoniae* (ESBL-).

Analytical Sensitivity

The analytical sensitivity of the rapid ESBL assay in this validation was 100%. All 104 of the isolates were detected. Most of the positive results were evident within the first 15 min of test incubation. Six (6%) produced weak reactions that were easier to read at 30 min incubation.

Analytical specificity

The analytical specificity of the assay in this validation was 99%. One false-positive result was obtained from a seeded blood culture containing a *K. oxytoca* isolate. The isolate tested negative for ESBL by the CLSI disk diffusion method.

Statistical Summary

	Negative CLSI Disk	Positive CLSI Disk	Total
Negative Rapid ESBL	101	0	101
Positive Rapid ESBL	1	104	105
Total	102	104	206

Statistical Analysis

Sensitivity	100% (96.4 to 100%)
Specificity	99% (94.7 to 99.8%)
Predictive Value Positive	99%
Predictive Value Negative	100%

Conclusion

This assay has proven to be highly sensitive and specific for the rapid detection of ESBL in positive blood cultures. When used in conjunction with a rapid identification test, such as the BioFire FilmArray BCID assay, the results should provide information to help guide therapy in patients with sepsis associated with *E. coli, Klebsiella* species, and *Proteus* species. Data will be gathered prospectively with the aid of the pharmacy antimicrobial stewardship program to determine the impact of implementing the assay.

13.0 References

1. Protocol obtained through personal communication: “Rapid detection of ESBL activity in *Enterobacteriaceae* – ESBL NDP test directly with blood cultures.” P. Nordmann, May 2013.

2. Nordmann P, Dortet L, Poirel L; European Network on Carbapenemases. 2012. Rapid detection of extended-spectrum-beta-lactamase-producing *Enterobacteriaceae*. J. Clin. Microbiol. 50:3016–3022.
3. Clinical and Laboratory Standards Institute; 2014. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement.* M100-S24.

14.0 Document Control History

Microbiology Director Approval: Dr. Ann Robinson 11/03/2014

Microbiology Supervisor Reviews: Jerry Claridge 11/03/2014