

Carbapenem Resistant Organism (CRO) Testing Instructions

I. CURRENT DCLS LABORATORY TESTING

Test Type	Method
Identification Confirmation	MALDI-TOF (Bruker Biotyper)
Antimicrobial Susceptibility Testing (AST)*	Broth Microdilution/Sensititre
Phenotypic testing - Carbapenemase Production	Modified Carbapenem Inactivation Method (mCIM)
Resistance Mechanism Identification	Real-Time PCR

* Submitting laboratories are **REQUIRED** to provide AST results when submitting suspected CRO isolates to DCLS for testing. These AST results are assessed upon receipt to determine suitability for testing and potential pan-resistance.

TESTING METHODS

- **mCIM** – Phenotypic testing for carbapenemase production will be performed for all Carbapenem Resistant Enterobacteriaceae (CRE) and Carbapenem Resistant *Pseudomonas aeruginosa* (CRPA) isolates.
- **AST** – The following drug Minimum Inhibitory Concentration (MIC) values will be determined only for mCIM positive CRE and CRPA isolates:
 1. Amikacin
 2. Aztreonam*
 3. Cefepime*
 4. Cefotaxime* (CRE only)
 5. Ceftazidime*
 6. Ciprofloxacin
 7. Colistin
 8. Ertapenem (CRE only)
 9. Imipenem*
 10. Meropenem*
 11. Piperacillin/Tazobactam**
 12. Polymyxin B
 13. Tobramycin
 14. Trimethoprim/Sulfamethoxazole (CRE only)

* Testing required by the CDC's Antibiotic Resistance Laboratory Network (ARLN) for CRE and CRPA isolates.

** Testing required by the CDC's ARLN for *Pseudomonas aeruginosa* isolates.

- **Real-time PCR** - Analyses will be performed on the following resistance marker genes for Carbapenem Resistant *Acinetobacter baumannii* (CRAB) and mCIM positive CRE and CRPA isolates:
 1. KPC
 2. NDM
 3. OXA-48-like*
 4. VIM
 5. IMP

*OXA-48 like PCR testing will only be performed on CRE isolates.

II. SPECIMEN TYPES

- Pure suspect CRE isolates on slant or plate media.
 - 1) Member of the Enterobacteriaceae family
 - 2) Resistance to at least 1 carbapenem:
 - imipenem, meropenem or doripenem = MIC of $\geq 4 \mu\text{g/mL}$
 - ertapenem = MIC of $\geq 2 \mu\text{g/mL}$

Note: *Morganella spp.*, *Proteus spp.*, and *Providencia spp.* have intrinsic elevated MICs to imipenem. MIC results for meropenem, doripenem, and/or ertapenem must be used to determine if these organisms should be forwarded to DCLS for testing.

- Pure suspect CRPA isolates on slant or plate media.
 - 1) Isolate identified as *Pseudomonas aeruginosa*
 - 2) Resistance to at least 1 carbapenem:
 - imipenem, meropenem or doripenem = MIC of $\geq 8 \mu\text{g/mL}$

- 3) Mucoid *Pseudomonas aeruginosa* isolates will NOT be tested
- Pure suspect CRAB isolates on slant or plate media.
 - 1) Isolate identified as *Acinetobacter baumannii* complex
 - 2) Resistant to at least 1 carbapenem:
 - imipenem, meropenem or doripenem = MIC of ≥ 8 $\mu\text{g/mL}$

III. SPECIMEN TRANSPORT

- Ship isolates at room temperature.
- Submit a completed DCLS Clinical Microbiology/Virology Test Request form with isolate, to include the following:
 - 1) Complete patient information (including address)
 - 2) Complete submitter information
 - 3) Date of specimen collection
 - 4) Specimen source
 - 5) Organism identification, denoting “suspected carbapenem resistance”
 - 6) Outbreak ID number (if applicable)
- Include copy of laboratory’s AST results in specimen package
- **Shipping Address:**
Division of Consolidated Laboratory Services
Attn: CRE Team
600 North 5th Street
Richmond, Virginia 23219

IV. RESULTS REPORTING

- Alert value results (**results possibly requiring additional reference laboratory testing**) will be verbally reported within 1 working days of results. An alert email notification will be sent to the CDC as well.
- Non-alert-value, positive results (**results NOT requiring additional reference laboratory testing**) will be verbally reported to the submitting laboratory within 2 working days of results.
- Negative results will not be verbally reported.
- A hard copy report of final test results will be provided to the submitter by mail.
 - CRAB isolates will include: identification and molecular results only
 - mCIM positive isolates will include: identification, AST, phenotypic, and molecular results
 - mCIM negative isolates will include: identification and phenotypic result only
- VDH will receive electronic reporting of results.
- Reference laboratory results for additional characterization testing will be provided to the submitting laboratory and VDH within 2 working days of receipt from the reference laboratory.

V. CONTACT INFORMATION

- **For questions regarding testing, specimen collection or shipment, please contact one of the following:**
 - Dr. LaToya Griffin-Thomas (804-648-4480, ext. 281)
 - Microbial Reference Laboratory (804-648-4480, ext. 345 or 251)
 - Molecular Detection and Characterization Laboratory (804-648-4480, ext. 298)
 - DCLS 24/7 Emergency Mobile Number (804-335-4617)

VI. RESOURCES

Updated information on CRE/CRPA testing can be found on the DCLS website, under the “Hot Topics” link:
<https://dgs.virginia.gov/division-of-consolidated-laboratory-services/updates/hot-topics/>

Link to DCLS Clinical Microbiology/Virology Test Request Form:
https://dgs.virginia.gov/globalassets/business-units/dcls/documents/sample-kits/dcls-micro_viro-form_fillable_rw.pdf