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OpGen®

Test Information

Acuitas™ CR Elite Test with ID/AST Reflex (C0301)
Acuitas™ CR Elite Test (C0302)

The test provides direct detection of antibiotic resistance genes (KPC, NDM, VIM, IMP, OXA, CTX-M and VanA) from a peri-anal swab specimen plus culture isolation of carbapenem-resistant Enterobacteriaceae (CRE) from the same peri-anal swab specimen followed by optional species identification and antibiotic susceptibility testing of isolated CREs.

Indication:

The Acuitas CR Elite Test and CR Elite Test with ID/AST Reflex use peri-anal swab specimens for rapid and direct detection of seven gene families of antibiotic resistance (KPC, NDM, VIM, IMP, OXA, CTX-M and VanA) associated with multidrug-resistant organisms (MDROs) including carbapenem-resistant Gram-negative bacteria and extended-spectrum beta-lactamase (ESBL) producers along with vancomycin-resistant *Enterococci* (VRE). Additionally, the peri-anal swab specimens are screened for CREs through selective culture followed by species identification and antibiotic susceptibility testing of isolated CREs. The test is designed for infected patients or subjects at high risk for colonization with MDROs as an aid to infection prevention and control and antibiotic stewardship.

Clinical and Biological Background:

KPC Gene. The *bla*$_{KPC}$ gene encodes the *Klebsiella pneumoniae* carbapenemase (KPC), an Ambler class A beta-lactamase encoded on plasmids or transposons with inhibition against penicillins, cephalosporins, aztreonams and carbapenems. KPC has been described in numerous Gram-negative bacteria including *Klebsiella* spp., *Escherichia coli, Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa, Citrobacter freundii, Serratia marcescens, Salmonella enterica, Raoultella* spp. and *Proteus mirabilis*. KPC is the most common carbapenemase among Enterobacteriaceae in the United States. The rapid dissemination and rising prevalence of KPC in Gram-negative bacteria highlight the transmissible nature of plasmid-encoded resistance in response to antibiotic selective pressure [1, 2].

NDM Gene. The *bla*$_{NDM}$ gene encodes the New Delhi metallo (NDM) beta-lactamase, an Ambler Class B enzyme encoded on plasmids or transposons with inhibition against penicillins, cephalosporins and carbapenems [3]. NDM has been described in numerous Gram-negative bacteria including *Klebsiella* spp., *E. coli, Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas aeroginosa* and *Citrobacter freundii.* Clusters of carbapenem-resistant Enterobacteriaceae (CREs) with NDM have been recovered from patients in the United States, some with ties to South Asian endemic areas [4]. The Centers for Disease Control (CDC) recently reported a large hospital outbreak of NDM CREs in Denver, Colorado, where unidentified asymptomatic carriers likely contributed to the outbreak and underscore the importance of active surveillance for infection control [5].

VIM Gene. The *bla*$_{VIM}$ gene encodes the Verona integrin-encoded metallo (VIM) beta-lactamase, an Ambler Class B enzyme encoded on plasmids or transposons with inhibition against penicillins, cephalosporins and carbapenems [3]. VIM carbapenemases have been described in numerous Gram-negative bacteria including *Pseudomonas aeruginosa, Klebsiella* spp., *E. coli, Enterobacter* spp., *C. freundii, Acinetobacter baumannii, Morganella morganii* and *Providencia stuartii*. VIM isolates have been reported throughout the world with higher prevalence in Southern Europe. The transmissible nature of transposon-encoded carbapenemases and increased international travel provide opportunity for introduction across healthcare settings.

IMP Gene. The *bla*$_{IMP}$ gene encodes an Ambler Class B metallo beta-lactamase encoded on plasmids or transposons with inhibition against penicillins, cephalosporins and carbapenems as initially recognized with imipenem [3]. IMP carbapenemases have been described in numerous Gram-negative bacteria including *Klebsiella* spp., *Escherichia coli, Enterobacter* spp., *Acinetobacter* spp., *P. aeruginosa, C. freundii* and *S. marcescens.* Highly transmissible Gram-negative bacteria with IMP have been reported in the United States and throughout the world [6].

OXA Gene. The *bla*$_{OXA}$ family of genes encodes a large and diverse group of Ambler Class D beta-lactamases with a wide range of inhibition against penicillins, cephalosporins and carbapenems as initially recognized with oxacillin. OXA gene subtypes can share less than 20% sequence homology. The OXA-48 family represents plasmid- or transposon-encoded carbapenemases found primarily in *Klebsiella pneumoniae* along with *Escherichia coli* (CREs). OXA-48 carbapenemases can be enhanced by accompanying resistance mechanisms such as cell permeability defects. OXA-48 CREs were first reported in the Middle East and North Africa with more recent expansion into Europe, Asia and the Americas. United States cases of OXA-48 CREs in 2013 involved patients arriving Saudi Arabia and India [7]. The OXA-23 family includes plasmid- or transposon-encoded ESBLs and carbapenemases found primarily in *A. baumannii*. The OXA-51 family represents beta lactamases very common to *A. baumannii* with little carbapenemase activity unless activated by an upstream transposon-encoded gene promoter [8].

CTX-M. The *bla*$_{CTX-M}$ gene encodes Class A extended-spectrum beta-lactamases (ESBLs) against third-generation cephalosporins and aztreonams. CTX-M enzymes are blocked by beta-lactamase inhibitors such as clavulanic acid. CTX-M has become the most common ESBL among Enterobacteriaciae worldwide, surpassing prevalence of ESBLs encoded by TEM and SHV genes. The United States recently experienced a rapid emergence and spread of *Klebsiella pneumoniae* and *E. coli* expressing CTX-M [9].

VanA Gene. The VanA operon encodes resistance to vancomycin and teicoplanin in Enterococci (vancomycin-resistant Enterococci, VRE). The VanA operon is transferrable on plasmids between *Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus durans, Enterococcus raffinosus* and *Enterococcus avium* [10]. VREs commonly cause nosocomial outbreaks with detection of asymptomic carriers being an important step toward infection control.

Methodology:

The tests are performed by OpGen's Clinical Services Laboratory in Gaithersburg, Maryland under CLIA certification. Genomic DNA is extracted from a portion of peri-anal swab specimens followed by analysis in a micro fluidic PCR array for direct detection of antibiotic resistance genes with high sensitivity and specificity. The test detects more than 200 gene subtypes across seven gene families of antibiotic resistance (KPC, NDM, VIM, IMP, OXA, CTX-M and VanA).

The peri-anal swab specimen is also screened for CREs by selective plate culture followed by species identification and antibiotic susceptibility testing of positive CRE isolates. Minimal inhibitory concentrations and AST interpretations using CLSI 2012 (M100-S22) or FDA breakpoints are provided for the following antibiotics: Amikacin, Ampicillin/Sublactam, Aztreonam, Cefazolin, Cefepime, Ceftazidime, Ceftriaxone, Ciprofloxacin, Ertapenem, Gentamicin, Imipenem, Levofloxacin, Meropenem, Piperacillin/Tazobactam, Tigecycline, Tobramycin and Trimethoprim/Sulfamethoxazole.

Specimen Type & Storage:

- **Peri-anal swabs:** Peri-anal specimens should be collected with either a Copan Eswab™ White Cap (#480C) or a BD Liquid Amies Elution Swab (ESwab) Collection and Transport System (#220245). Ship Eswabs at 15-25 °C within 24 hours of collection. Eswab specimens are stable for 48 hours at 4-25 °C.
- **Other specimen types:** Contact OpGen for specific information.

Results:

Test results for the presence of antibiotic resistance genes (KPC, NDM, VIM, IMP, OXA, CTX-M and VanA) are electronically reported next day after receipt of the swab specimen. Positive detection of a CRE culture isolate is reported wihin 48 hours after receipt of the swab specimen with corresponding identification and antibiotic susceptibility results reported approximately 72 hours after receipt of the swab specimen.

Test performance for direct detection of antibiotic resistance genes (KPC, NDM, VIM, IMP, OXA, CTX-M and VanA) from peri-anal swabs

Limit of detection (LOD) was determined in serial dilution studies with negative peri-anal swabs spiked with an organism containing the target. The LOD range was determined to be between 13 and 250 CFU/mL.

Gene	Organism	LOD (CFU/mL)
KPC	*E. cloacae*	84
NDM	*K. pneumoniae*	93
VIM	*S. marcescens, P. aeruginosa, E. cloacae*	37-154
IMP	*K. pneumoniae*	13-66
OXA-48	*K. pneumoniae*	79
OXA-23	*A. baumannii*	109
OXA-51	*A. baumannii*	125
CTX-M	*K. pneumoniae*	79-151
VanA	*E. faecium*	250

Analytical Specificity was determined against 243 clinical isolates with known results for the MDRO genes (KPC, NDM, VIM, IMP, OXA, CTX-M and VanA). The test correctly detected the MDRO genes with perfect specificity. No cross-reactivity was observed.

Acuitas	Confirmed MDRO Gene Presence	Confirmed MDRO Gene Absence
+	100	0
-	0	143

Accuracy was determined by testing 118 spiked-perianal swabs in a blinded manner with multiple operators. One-hundred – eight (108) swabs contained clinical isolates with 42 known genes covering all the MDRO genes (KPC, NDM, VIM, IMP, OXA, CTX-M and VanA) with multiple representative genotypes. The positive peri-anal swabs were prepared in a manner such that at least one-third of the swabs contained MDROs close to the Limit-of-Detection. Ten (10) swabs contained clinical isolates without drug-resistance genes detected by the Acuitas MDRO Gene Test.

Of 108 positive swabs, the Acuitas MDRO Gene Test identified all 108 positive MDRO samples and accurately detected the correct gene in each case except for one swab in which two VIM reactions were falsely positive. The accuracy data are presented below to demonstrate reaction level or gene detection accuracy.

Reaction Level (Gene Detection) Accuracy of 108 positive swabs		
	Targeted MDRO Gene Positive	Targeted MDRO Gene Negative
Acuitas Positive	42	2
Acuitas Negative	0	1596
Sensitivity		100%
Specificity		99.87%
Positive Predictive Value		95%
Negative Predictive Value		100%

Gene Test Limitations:

The test may detect the presence of antibiotic resistant genes in non-viable organisms present in clinical specimens. A negative test result does not exclude the presence of MDROs because not all forms of antibiotic resistance are detected.

Test performance for culture screening of CREs from peri-anal swabs

Limit of detection (LOD) was determined through serial dilution studies with negative peri-anal swabs spiked with two clinical isolates of *Klebsiella pneumoniae* harboring the KPC gene. The LOD was 16 to 29 colony forming units per peri-anal swab.

Sensitivity and specificity were determined against 69 clinical Enterobacteriaceae with reported antibiotic susceptibility profiles and CRE genotypes based on detection of the carbapenemase genes KPC, NDM, VIM, IMP and OXA. The CRE culture screen is sensitive and specific in comparison with either the reported antibiotic susceptibility or CRE genotype as shown below. The CRE culture screen detects some Enterobacteriaceae that are susceptible to carbapenems but non-susceptible to third and fourth generation cephalosporins. These cephalosporin-resistant isolates were correctly identified as such through subsequent species identification and antibiotic susceptibility testing following the normal protocol for the Acuitas CR Elite Test with ID/AST Reflex.

Detection of Confirmed CRE† (10 pos/59 neg)			
Acuitas CRE Culture		Acuitas MDRO Gene Test	
		Positive	Negative
	Positive	9	4**
	Negative	1*	55

* *CRE K. pneumoniae* with IMP-8 & SHV-27(e) detected by MDRO Gene Test but not by CRE culture

** The following false culture positives were ruled out by MDRO Gene Test

- ESBL *E. cloacae* with CTX-M-9 and ACT/MIR
- ESBL *E. coli* with CTX-M-9
- ESBL *K. pneumoniae* with CTX-M-2
- Cephalosporin resistant *E. coli* with CMY-2 and TEM-20

Test Performance for the detection of CRE†	
Acuitas MDRO Gene Test	
Sensitivity	100%
Specificity	100%
Acuitas CRE Culture	
Sensitivity	90%
Specificity	93%

†CRE defined as non-susceptible to Imipenem, Meropenem or Doripenem by standard susceptibility testing methods [11].

Culture Limitations:

The CRE culture screen does not detect non-CREs regardless of carbapenemase activity. The CRE culture screen may be negative for CREs with low levels of carbapenemase activity. The CRE culture screen may not detect colonization with CREs due to test sensitivity or variables in peri-anal swab collection and transport.

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