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Test Information

Acuitas™ MDRO Gene Test

Detect Antibiotic Resistance Genes (KPC, NDM, VIM, IMP, OXA, CTX-M and VanA) from Peri-Anal Swabs

Indication:

The Acuitas MDRO Gene Test provides rapid detection for 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). The test is performed directly on peri-anal swabs from infected patients or subjects at high risk for colonization to aid infection prevention and control and in the selection of empiric antibiotic therapy. The test is also validated for the identification of MDRO genes in clinical isolates.

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.

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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 from 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 $bla_{CTX\text{-}M}$ 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 Acuitas MDRO Gene Test is performed by OpGen's Clinical Services Laboratory in Gaithersburg, Maryland under CLIA certification. Genomic DNA is extracted from 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 without microbiology culture.

The Acuitas MDRO Gene Test detects more than 200 gene subtypes across the beta-lactamase gene family targets

Specimen Type & Storage:

- **Peri-anal swabs:** specimens should be collected with either a Copan Eswab™ White Cap (#20245) or a BD Liquid Amies Elution Swab (ESwab) Collection and Transport System (#480C). Ship Eswabs at 15-25 °C within 24 hours of collection. Eswab specimens are stable for 48 hours at 4-25 °C.
- **Culture isolates and other specimen types:** Contact OpGen for specific information.

Results:

Results from the Acuitas MDRO Gene Test are electronically reported next day after specimen receipt.

Performance:

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

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OXA-51	*A. baumannii*	125
CTX-M	*K. pneumoniae*	79-151
VanA	*E. faecium*	250

Analytical Specificity was determined against 100 clinical isolates with known MDRO genes and 143 clinical isolates without MDRO genes that are detected by the Acuitas MDRO Gene Test. The assay showed excellent specificity by detecting all MDRO genes. 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 MDR genes covering all Acuitas MDRO gene targets with multiple representative genotypes. The positive perianal swabs were prepared in a manner such that at least one-third of the swabs contained MDRO's 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 is presented below in two tables to demonstrate the sample level accuracy and reaction level accuracy.

Sample Level Accuracy			Reaction Level Accuracy		
	MDRO Pos	MDRO Neg		MDRO Pos	MDRO Neg
Acuitas Positive	108	0	Acuitas Positive	42	2
Acuitas Negative	0	10	Acuitas Negative	0	1596
Sensitivity		100%	Sensitivity		100%
Specificity		100%	Specificity		99.87%
Positive Predictive Value		100%	Positive Predictive Value		95%
Negative Predictive Value		100%	Negative Predictive Value		100%

Limitations:

The Acuitas MDRO Gene Test may detect the presence of antibiotic resistant genes in non-viable organisms present in clinical specimens. The test does not identify the bacterial species harboring the resistance gene. A negative test result does not exclude the presence of MDROs because not all forms of antibiotic resistance are detected.

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