Supplemental Table 1: OXA Isolates from the ARISOLATEBANK[a] used in assay development and assay accuracy

AR Bank[b] #	Species	Oxacillinase	MIC[c] for Meropenem (Interpretation)	Assay Development or Accuracy Assessment
0036	*Acinetobacter baumannii*	OXA-24	>8 (R)	Development
0039	*Klebsiella pneumoniae*	OXA-181	2 (R)	Development
0051	*Klebsiella ozaenae*	OXA-181	4 (R)	Accuracy Assessment
0063	*Acinetobacter baumannii*	OXA-24	>8 (R)	Development
0066	*Klebsiella pneumoniae*	OXA-232	>8 (R)	Accuracy Assessment
0074	*Enterobacter aerogenes*	OXA-48	2 (I)	Accuracy Assessment
0075	*Klebsiella pneumoniae*	OXA-232	>8 (R)	Accuracy Assessment
0140	*Klebsiella pneumoniae*	OXA-181	4 (R)	Development
0141	*Klebsiella pneumoniae*	OXA-181	4 (R)	Accuracy Assessment
0142	*Klebsiella pneumoniae*	OXA-181	2 (I)	Accuracy Assessment
0153	*Klebsiella pneumoniae*	OXA-232	>8 (R)	Accuracy Assessment
0160	*Klebsiella pneumoniae*	OXA-48	8 (R)	Accuracy Assessment

[a]https://www.cdc.gov/drugresistance/resistance-bank/index.html
[b]ARISOLATEBANK
[c]MIC (minimum inhibitory concentration) in μg/ml

Supplemental Table 2: Isolates used in specificity assessment #1

Isolate	Isolate Number[a]	Carbapenemase[b]
Klebsiella pneumoniae	MRSN 368320	OXA-48
Escherichia coli	MRSN 368339	OXA-48
Escherichia coli	MRSN 368384	OXA-48
Escherichia coli	MRSN 368393	OXA-48
Enterobacter cloacae complex	MRSN 489809	OXA-48
Klebsiella pneumoniae	MRSN 510756	OXA-48
Klebsiella pneumoniae	MRSN 512213	OXA-48
Klebsiella pneumoniae	MRSN 520939	OXA-48
Escherichia coli	MRSN 20486	OXA-181
Klebsiella pneumoniae	MRSN 368311	OXA-232
Klebsiella pneumoniae	MRSN 479495	OXA-232
Klebsiella pneumoniae	MRSN 520948	OXA-232
Klebsiella pneumoniae	MRSN 546052	OXA-232
Escherichia coli	MRSN 548014	OXA-244
Escherichia coli	ECRO91[c]	KPC
Escherichia coli	ECONIH1[c]	KPC
Enterobacter cloacae complex	ECNIH5[c]	KPC
Citrobacter freundii complex	CFNIH1[c]	KPC
Enterobacter cloacae complex	ECNIH4[c]	KPC
Klebsiella pneumoniae	BAA-2146[d]	NDM
Escherichia coli	CCNIH1[c]	NDM
Acinetobacter baumannii complex	CCNIH2[c]	NDM
Klebsiella pneumoniae	CCNIH3[c]	NDM
Acinetobacter species	CCNIH4[c]	NDM
Enterobacter cloacae complex		
Klebsiella pneumoniae		
Pseudomonas aeruginosa		
Klebsiella oxytoca		
Escherichia coli		
Citrobacter koseri		

[a]Isolates from the Walter Reed Army Institute of Research, Multidrug Resistant Organism Repository and Surveillance Network (WRAIR MRSN)

[b]Presence of carbapenemase was confirmed either by whole genome sequencing or by PCR

[c]Isolates from the National Institutes of Health Clinical Center

[d]Isolate from the American Type Culture Collection

Supplemental Table 3: Isolates used in specificity assessment #2

Isolate	Isolate number[a,b]	Carbapenemase[c]
Klebsiella pneumoniae	MRSN 368320[d]	OXA-48
Escherichia coli	MRSN 368339[d]	OXA-48
Escherichia coli	MRSN 20486[d]	OXA-181
Klebsiella pneumoniae	MRSN 367311[d]	OXA-232
Klebsiella pneumoniae	CDC-076	VIM-1
Pseudomonas aeruginosa	CDC-110	VIM-2
Pseudomonas aeruginosa	CDC-054	VIM-4
Pseudomonas aeruginosa	CDC-103	IMP-1
Klebsiella pneumoniae	CDC-034	IMP-4
Pseudomonas aeruginosa	CDC-092	IMP-14
Klebsiella pneumoniae	CDC-046	VIM-27
Stenotrophomonas maltophilia		
Enterobacter cloacae complex		
Stenotrophomonas maltophilia		
Klebsiella pneumoniae		

[a]CDC isolates from the CDC and FDA Antibiotic Resistant Isolate Bank (ARISOLATEBANK) https://www.cdc.gov/drugresistance/resistance-bank/index.html

[b]Isolates from the Walter Reed Army Institute of Research, Multidrug Resistant Organism Repository and Surveillance Network (WRAIR MRSN)

[c]Presence of carbapenemase was confirmed either by whole genome sequencing or by PCR

[d]OXA-48 family carbapenemase positive isolates also used in specificity assessment #1

Supplemental Table 4: OXA-48 family as determined from the CARD database[a]

Designation	Accession/Version[b]	Length (AA)	Original Organism
OXA-48	AAP70012.1	265	*Klebsiella pneumoniae*
OXA-54	AAR89917.1	265	*Shewanella oneidensis*
OXA-162	ADG27454.1	265	*Klebsiella pneumoniae*
OXA-163	ADY06444.1	261	*Enterobacter cloacae*
OXA-181	AEP16366.1	265	*Klebsiella pneumoniae*
OXA-199	AFC95894.1	265	*Shewanella xiamenensis*
OXA-204	AFU91598.1	265	*Klebsiella pneumoniae*
OXA-232	AGD91915.1	265	*Escherichia coli*
OXA-244	AGC60012.1	265	*Klebsiella pneumoniae*
OXA-245	AGC60013.1	265	*Klebsiella pneumoniae*
OXA-247	AGC70814.1	261	*Klebsiella pneumoniae*
OXA-370	AHF71363.1	265	*Enterobacter sp.*

[a]Comprehensive Antibiotic Resistance Database

[b]Protein sequences used in the proteomic alignment in which theoretical digestion to identify core peptides conserved throughout the entire OXA-48 family was performed.

Supplemental Table 5: Results of initial assay optimization

Sample[a,b,c,d]	ANQAFLPASTFK (rdotp)[e]/R[f]	YSVVPVYQEFAR (rdotp)/R	Sample	ANQAFLPASTFK (rdotp)/R	YSVVPVYQEFAR (rdotp)/R
S1 (2 μL)	1/3.73	1/3.54	S1 (4 μL)	1/3.83	1/3.7
S2 (2 μL)	0.95/0.03	0.53/0.004	S2 (4 μL)	0.91/0.04	0.61/0.01
S3 (2 μL)	0.74/0.05	0.5/0.01	S3 (4 μL)	0.06/0.08	0.46/0.01
S4 (2 μL)	1/3.49	1/3.02	S4 (4 μL)	1/3.37	1/2.99
N1 (2 μL)	0.85/0.03	0.31/0.02	N1 (4 μL)	0.42/0.06	0.27/0.02
N2 (2 μL)	0.47/0.02	0.71/0.01	N2 (4 μL)	0.54/0.02	0.76/0.01
N3 (2 μL)	0.32/0.01	0.89/.004	N3 (4 μL)	0.63/0.01	0.69/0.01
N4 (2 μL)	0.57/0.01	0.82/0.1	N4 (4 μL)	0.49/0.01	0.79/0.01

[a]S1 (AR Bank #0039), S2 (AR Bank #0063), S3 (AR Bank #0036), S4 (AR Bank #0140)

[b]S1 and S4 (OXA-181 positive)

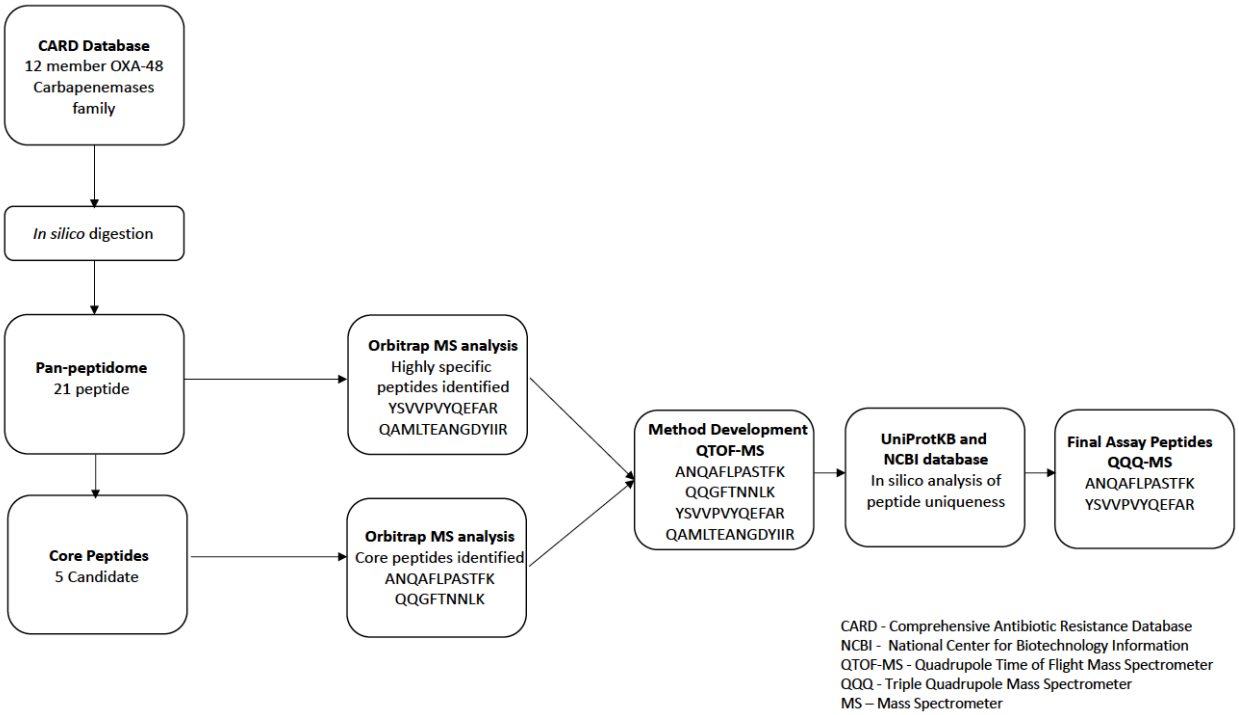
[c]S2 and S3 (OXA-24 positive)

[c]N1-N4 (negative controls)

[e]rdotp is the ratio dot products

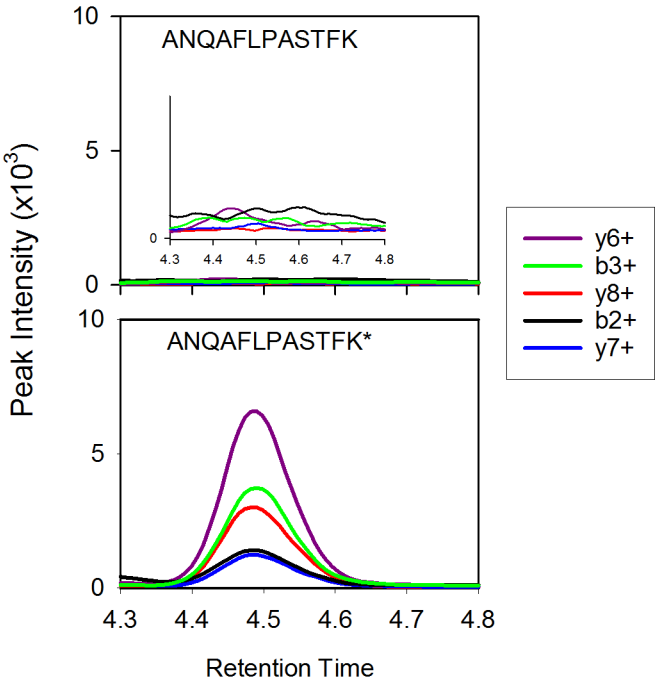
[f]R is the intensity ratio

Supplemental Figure 1: Work flow diagram outlining the assay development methods



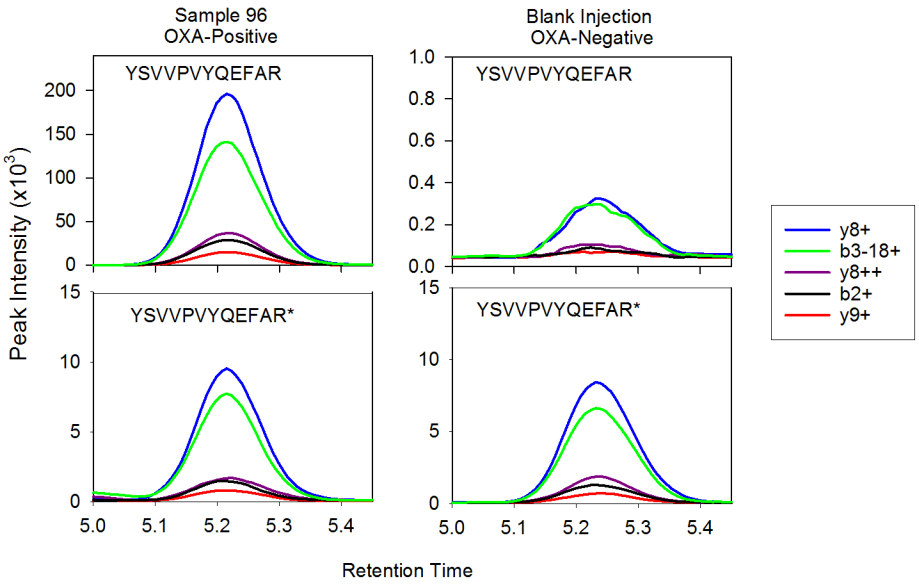
Work flow diagram outlining the assay development methods. The CARD database was used to identify protein sequences for all OXA-48 family enzymes. *In silico* digestion was performed to determine the pan-peptidome and core peptides were identified. Orbitrap LC-MS/MS was used for initial peptide identification, method development was performed on a QTOF LC-MS/MS to identify peptides that were highly responsive and reproducibly detected, and final assay development and accuracy assessment were performed on a QQQ LC-MS/MS

Supplemental Figure 2: LC-MS/MS chromatogram of a sample that was called negative on manual review



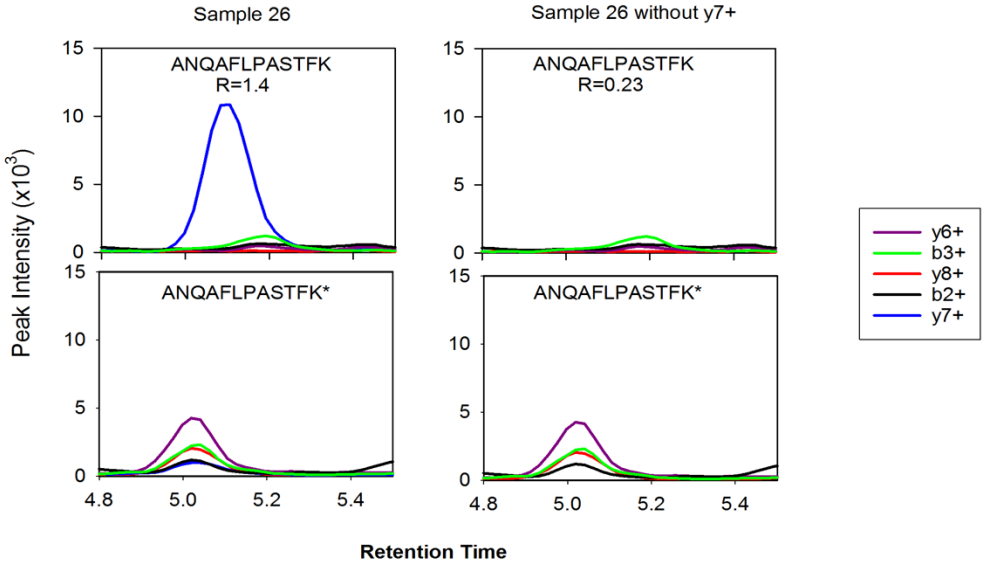
Sample 18 required manual review due of a rdotp of 0.93 for peptide ANQAFLPASTFK. On manual review no clear defined transitions were observed in the unlabelled peptide chromatogram resulting in expert review call of negative.

Supplemental Figure 3: LC-MS/MS chromatogram of carryover to blank sample



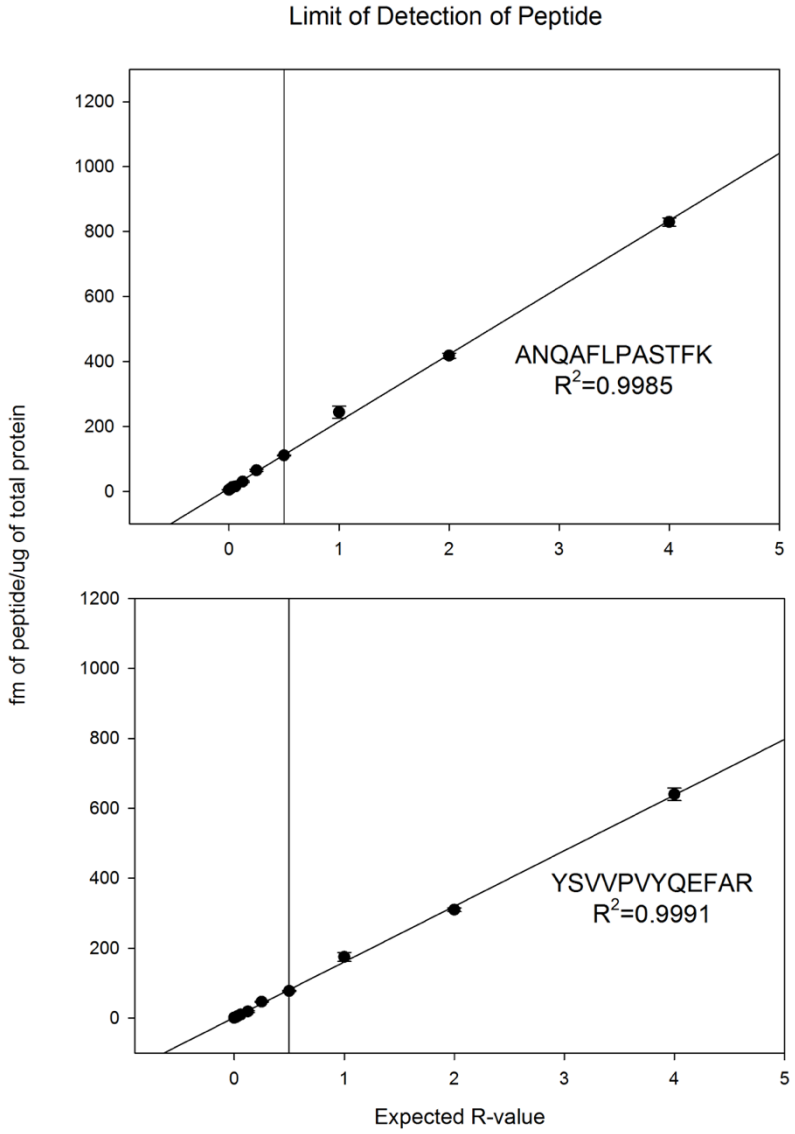
Carryover was noted following sample 96 in the first assay accuracy assessment in which the blank injection had low level peaks that correlated with the correction transitions and transition order for a positive identification.

Supplemental Figure 4: LC-MS/MS chromatogram of Sample 26 in the second accuracy assessment demonstrating interfering peak.



LC-MS/MS chromatogram of Sample 26 in the second accuracy assessment demonstrating interfering peak. Removal of the y7+ peak results in a change in the R-value of 1.4 to 0.23.

Supplemental Figure 5: Limit of detection quantification with serial dilutions



Serial dilutions of an OXA-48 positive sample from an expected R-value of 4 to an expected R-value of 0.015 was performed in duplicate. The peptide concentration/μg of total protein that correlates with a rdotp of >0.95 and an R value of 0.5 was measured. Peptide concentrations were 110.9 fmol/μg of total protein for peptide ANQAFLPASTFK and 77.7 fmol/μg of total protein for peptide YSVVPVYQEFAR. Trend line represents an R-value of 0.5.