The Coris BioConcept OXA48 K-SeT Immuno-Chromatographic Assay Detects OXA48-type Carbapenemases with High Sensitivity and Specificity

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ABSTRACT

Background: Clinical microbiology laboratories face significant challenges in identifying and distinguishing carbapenemase-producing organisms (CPO) from epidemiologically non-significant carbapenem-resistant non-CPO. The class D CPO, which may carry a diversity of OXA48-related sequence types, are particularly challenging to detect further complicating the control of rapidly emerging CPO. Rapid tests are needed to improve recognition of highly-significant CPO. This retrospective study evaluated a novel low-complexity lateral-flow line assay, the Coris BioConcept OXA48 K-SeT that was designed to detect OXA48-type carbapenemases in 15 minutes.

Methods: 259 highly-characterized (phenotypically and PCR/sequencing) species-diverse Gram-negative isolates were blinded to prevent bias. Of these, 33 (19 *Klebsiella pneumoniae*, 14 *Escherichia coli*) had class D genotypes: 26 had class D alone (15 OXA48; 6 OXA181, 4 OXA232, 1 OXA244) and 7 had class D in combination with class B genotypes (4 NDM+OXA181, 3 NDM+OXA232). The 226 non-class D isolates included 108 class A (99 KPC, 4 SME, 3 NMC/IMI, 2 GES), 80 class B (73 NDM, 6 VIM, 1 IMP7) and 38 non-CPO. Most non-class D contained multiple mechanisms that together contributed to varying degrees of carbapenem and/or oxyimino-cephalosporin resistance. Notably at least 14 carried OXA1/30, 1 OXA51, and 1 OXA252, while derepressed or plasmid-mediated *ampC*, diverse ESBL, *ompC*/*ompF* or *ompK35*/*ompK36* mutations were common and 1 had intrinsic *cphA*. The OXA48 K-SeT assay was performed as directed using a single colony from each isolate to inoculate the test. For this pick, growth closest to selective ertapenem discs on MacConkey, Columbia Sheep Blood or Mueller-Hinton agars (Oxoid) was used. After 15 minutes at room temperature, results were documented independently by 5 readers. Detection of the control band only was considered a negative result whereas two bands for test and control were considered a positive result. Consensus data were analyzed for sensitivity and specificity for class D CPO detection; 95% confidence intervals (CI) were calculated using www.graphpad.com.

Results: The OXA48 K-SeT detected two clear bands in 26/26 strains carrying single class D carbapenemase genes (OXA48, OXA181, OXA232, 1 OXA244) as well in 7/7 strains carrying a class D carbapenemase genes accompanied by NDM (OXA181 and OXA232). The resulting sensitivity was 100% (95% CI: 87.6-100). In contrast, the OXA48 K-set detected only the single control band in 226/ 226 non-class D isolates including the single *Shewenella putrifaciens* with the progenitor OXA252 gene and all isolates that co-carried the common OXA1/30 gene. The resulting specificity was 100% (95% CI: 98-100).

Conclusions: This study found the low-complexity Coris Bioconcept OXA48 K-SeT assay to be extremely easy to use and simple to interpret. It provided highly accurate results (100% sensitivity and 100% specificity) when used directly from colonies grown on MacConkey, Columbia Sheep Blood or Mueller-Hinton based agars within 15 minutes of set-up.

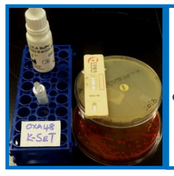
INTRODUCTION

Rapid accurate detection of pan-resistant CPO is crucial for risk-reduction in patient care and to prevent outbreaks. Culture is typically used over PCR in clinical laboratories as the primary means to detect CPO given the lower cost of culture and the limited targets associated with PCR. CPO are typically detected in clinical specimens when routine susceptibilities indicate resistance to ≥1 carbapenems. Surveillance specimens typically first undergo selective culture, followed by algorithms that in many laboratories include overnight meropenem disc diffusion using the sensitive screen breakpoint of ≤25mm to maximize specificity.

At this point, an increasing number of options have become available for confirming suspected CPO. Molecular tests have been considered "gold standard" yet no single assay detects all genotypes, and inevitable evolutionary diversification has lead to genetic drift at key primer sites that, unknowingly in certain assays, reduce PCR sensitivity. Low-complexity commercial PCR assays are costly and don't cover all types; conversely, cheaper more-flexible conventional assays are too labour-intensive for today's busy clinical lab settings, especially in low prevalence areas. Although PCR remains an important tool for selective use in high-risk situations to enable direct-from-swab detection of more common genotypes, it is not available to all laboratories.

Newly recommended same-day phenotypic tests, such as the CARBA-NP-based assays, while sensitive for class A and B CPO, have proven to produce false-negatives with class D CPO in many laboratories. Also problematic is that these and similar phenotypic tests require very large inoculums and cannot be done from MacConkey-based agars, which is frequently the primary medium on which single colonies of a suspect CPO are typically immediately available.

Thus, this retrospective study aimed to determine the class D CPO detection accuracy using the newly available OXA48 K-SeT assay (Coris BioConcept, Belgium; Figure 1). It is an inexpensive, low-complexity, rapid immuno-chromatographic lateral flow test specifically designed to detect only class D OXA48 and closely related genotypes. Advantages are that it may be done from any agar and requires only 1 colony of *Enterobacteriaceae* to produce a reputedly highly sensitive and specific easily interpreted visual result within 15 minutes of set-up.



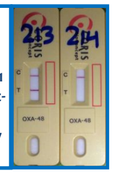


Figure 1 . The Coris BioConcept OXA48 K-Set test is a low-complexity immuno-chromatographic lateral-flow line assay, for highly sensitive and specific detection/confirmation of OXA48-related carbapenemases in *Enterobacteriaceae*. Tests require only 1 colony from any common laboratory agar. Within 15 minutes of set-up, brick-red lines become visible at "C" (control) and "T" (test) positions in a OXA48-positive test organism (see 213 right), or only at the "C" position in a negative test organism (see 214 right).

METHODS

Table 1 (below) describes 259 species-diverse clinical isolates selected for study. All isolates were characterized by conventional PCR for genes encoding *ampC*/ESBL/CPO, etc., and sequenced to determine allelic variations (i.e. for all *blaOXA48*-like genes) when needed. Isolates were identified to species-level by MALDI-TOF (bioMérieux's VITEK MS Plus). Phenotypic expression of CPO had been measured by disc diffusion to meropenem using the screen breakpoint of ≤25mm. Reactions to inhibitors (boronic and dipicolinic acids) were ascertained alongside temocillin susceptibility testing (ROSCO KPC+MBL+OXA48 Confirm kit). Only 1 isolate/patient was included, except if ≥1 genotype or ≥1 genus/species was confirmed as CPO from a patient. Isolate identities were blinded to prevent bias.

The OXA48 K-SeT test (Coris BioConcept, Belgium) is an immuno-chromatographic assay that is performed directly from colonies on any agar to provide a rapid specific confirmation of absence/presence of class D carbapenemases common to *Enterobacteriaceae*. The test relies on antigen capture via a set of specific monoclonal antibodies raised from mice to react with the specific types only (OXA48, OXA162, OXA181, OXA204, OXA232, OXA244). Antibodies cross-reactive with other OXA-genotypes (non- carbapenemases: OXA163, OXA247, OXA405; *Acinetobacter* carbapenemases: OXA23, OXA24, OXA51, OXA58, OXA143, etc.) are excluded. Colloidal gold antibody nanoparticles are attached to nitrocellulose strips housed in a plastic lateral-flow chamber (Figure 1.).

On recovery from -80°C and on 2nd subculture, ertapenem discs were placed on agars (Oxoid) whether CPO or not to maintain selective pressure. OXA48 K-SeT tests were performed as per package insert. A single colony picked from growth closest to ertapenem on MacConkey CV, Columbia 5% Sheep blood or Mueller-Hinton agars, was inoculated into reagent in a tube provided in the kit. When mixed, a dispensing cap, also provided in the kit was placed on the tube and inverted to act as a dispenser to transfer the inoculated reagent into the lateral flow device. After 15 min incubation at room temperature, each OXA48 K-SeT was examined independently by 5 readers for absence/presence of brick-red lines at control (C) and test (T) positions (Figure 2), where OXA48-positives had 2 lines (C+T+), while in negatives, only the "C" line developed. Individual results were later correlated to identify error. PCR was used to confirm genotypes in discrepancies. Consensus K-SeT data was analyzed for sensitivity and specificity for detecting OXA48-like genes from suspect CPO. 95% confidence intervals (CI) were calculated using www.graphpad.com.

Table 1. Characteristics of 259 Gram-negative bacilli used to evaluate Class D OXA48-like CPO detection using the Coris BioConcept OXA48 K-SeT immuno-chromatographic assay

Ambler (No.)	CPO Genotypes (No.)	Species identification	No. Inoculated to OXA48 K-SeT	No. POSITIVE by OXA48 K-SeT
Class A CPO (108)	*blaKPC (99)*	*Citrobacter freundii*	2	0
		Enterobacter aerogenes	3	0
		Enterobacter cloacae	35	0
		Escherichia coli	12	0
		Klebsiella oxytoca	1	0
		Klebsiella pneumonia	46	0
	blaGES5 (2)	*Klebsiella oxytoca*	2	0
	blaIMI1 (1)	*Enterobacter cloacae*	1	0
	blaNMCA (2)	*Enterobacter cloacae*	2	0
	blaSME (4)	*Serratia marcescens*	4	0
Class B CPO (80)	*blaIMP7 (1)*	*Pseudomonas aeruginosa*	1	0
	blaNDM (73)	*Acinetobacter baumannii*	1	0
		Citrobacter freundii	1	0
		Enterobacter cloacae	3	0
		Escherichia coli	30	0
		Klebsiella pneumoniae	33	0
		Morganella morganii	4	0
		Proteus mirabilis	1	0
	blaVIM (6)	*Citrobacter freundii*	1	0
		Enterobacter cloacae	4	0
		Pseudomonas putida	1	0
Classes B + D CPO (7)	*blaNDM+blaOXA181 (1)*	*Escherichia coli*	1	1
	blaNDM+blaOXA181 (3)	*Klebsiella pneumoniae*	3	3
	blaNDM+blaOXA232 (1)	*Escherichia coli*	1	1
	blaNDM+blaOXA232 (2)	*Klebsiella pneumoniae*	2	1
Class D CPO (26)	*blaOXA48 (15)*	*Escherichia coli*	8	8
		Klebsiella pneumoniae	7	7
	blaOXA181 (6)	*Escherichia coli*	2	2
		Klebsiella pneumoniae	4	4
	blaOXA232 (4)	*Escherichia coli*	1	1
		Klebsiella pneumoniae	3	3
	blaOXA244 (1)	*Escherichia coli*	1	1
Non-CPO (38)	*ompC-ompF (3)*	*Enterobacter cloacae*	1	0
		Escherichia coli	3	0
	ompK35-ompK36 (6)	*Klebsiella pneumoniae*	6	0
	Weak OXY promoter (1)	*Klebsiella oxytoca*	1	0
	Other mechanisms (26)	*Enterobacteriaceae*	26	0
	blaOXA252 (1)	*Shewanella putrifaciens*	1	0

RESULTS

- Tables 2 and 3 below summarize results obtained using the Coris BioConcept OXA48 K-SeT.
- The OXA48 K-SeT detected two strong brick-red bands in 26/26 strains carrying class D carbapenemase genes only (OXA48, OXA181, OXA232, 1 OXA244)
- Two bands were also detected in 7/7 strains carrying a class D carbapenemase genes (OXA181 and OXA232) plus NDM.
- The resulting sensitivity for detection of OXA48-related genes was 100% (95% CI: 87.6-100).
- Conversely, the OXA48 K-SeT was negative and detected only the single control band in 226/226 non-class D isolates including a *Shewenella putrifaciens* with progenitor OXA252, an *Acinetobacter baumannii* with OXA51, and all isolates that carried the common OXA1 gene.
- The resulting specificity for detecting only OXA48-related genes was 100% (95% CI: 98-100).

Figure 2. As seen in this batch of study isolates tested using the Coris BioConcept OXA48 lateral flow assay, the OXA48-positives (2 brick-red lines at "C" and "T") and OXA48-negatives (1 brick-red line at "C") were readily distinguished as reaction lines clearly visible and uniform for all organisms tested throughout the study

Table 2. Evaluation of the CORIS BioConcept's OXA48 K-SeT for detection of OXA48-like CPO

Genotype composition of challenge isolates	No. (%) tested/No. (%) positive
blaOXA48-like (OXA48, OXA181, OXA232, OXA244)	26 (10)/26 (100)
blaNDM plus *blaOXA48-like* (OXA181, OXA232)	7 (2.7)/7 (100)
Total OXA48-like CPO tested	33 (12.7)/33 (100)
blaKPC	99 (38.2)/0 (0)
blaNDM	73 (28.2/0 (0)
blaVIM	6 (2.3)/0 (0)
blaSME	4 (1.5)/0 (0)
blaNMCA/IMI1	3 (1.2)/0 (0)
blaGES5	2 (0.8)/0 (0)
blaIMP7	1 (0.4)/0 (0)
Total Non-OXA48-like CPO tested	188 (72.6)/0 (0)
Non-CPO (including OXA1, OXA51, OXA252, *cphA*)	38 (14.7)/0 (0)
Total Non-CPO tested	38 (14.7)/0 (0)
Total non-OXA48-like isolates tested	226 (87.3)/0 (0)
Total isolates tested	**259**

Table 3. Performance of the Coris BioConcept's OXA48 K-SeT for detection/confirmation of OXA48-related genes in suspect Carbapenemase-producing organisms (CPO)

Sensitivity (95%CI) for detecting OXA48-like genes only	100% (87.6-100)
Specificity (95%CI) for excluding non-OXA48 CPO and non-CPO	100% (98-100)

CONCLUSIONS & DISCUSSION

Use of Coris BioConcept's OXA48 K-SeT as a rapid Class D CPO confirmatory test

The Coris BioConcept's OXA48 K-SeT assay provided highly accurate results (100% sensitive and specific) as it detected all allelic variants related to blaOXA48 that produced carbapenemases (OXA48, OXA181, OXA232, OXA244) and excluded closely related non-carbapenemases as evidenced by a clearly negative result for *Shewanella putrifaciens* with chromosomal *blaOXA252*

Experience from this study found the Coris Bioconcept OXA48 K-SeT assay readily qualified as a low-complexity test as there was minimal hands-on time and required no laboratory equipment; it was extremely easy to use and was simple to interpret.

The OXA48 K-SeT was also found to be a very practical test since it could be used directly from colonies grown on any common primary medium including MacConkey, Columbia Sheep Blood or Mueller-Hinton based agars

Furthermore, from a patient management perspective, the K-SeT would be able to provide reliable results within 15 minutes of test set-up, and thus would enable immediate infection control attention to a newly identified patient positive for an OXA48-type CPO

The only drawback is that the evaluated test only targets a single CPO class. However, Coris BioConcept has already produced another K-SeT specific for KPC which is also reportedly 100% sensitive and specific (Glupczynski *et al*, JAC 2016).

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