Development of novel immunochromatographic tests for rapid identification of OXA-48 and KPC carbapenemases in Enterobacteriaceae

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Introduction

OXA-48 and **KPC** are two of the major carbapenemases expressed in Enterobacteriaceae and their rapid identification presents a serious challenge for diagnostic laboratories.
We developed two monoplex immunochromatographic assays: **OXA-48 *K*-SeT** (already commercially available) and **KPC *K*-SeT**, and initiated investigations for a combi assay: **OXA-48 & KPC *K*-SeT** detecting specifically both OXA-48 and KPC carbapenemases from bacterial colonies in 15 minutes.

KPC antibodies selection

CAPTURE ANTIBODY	DETECTION ANTIBODY (Coating)										
(Gold-Conjugate)	UF4	AA6	BA4	KA5	KB4	MF5	NA3	NE7	PF7	RF8	TA1
UF4	-	-	-	-	-	-	-	-	+	-	-
AA6	-	-	-	-	-	-	-	-	(+)	-	-
BA4	-	(+)	-	-	-	-	+(+)	(+)	+(+)	-	-
KA5	-	-	-	(+)	-	-	+	+	+	-	-
KB4	-	-	-	-	-	-	(+)	+	(+)	-	-
MF5	-	-	(+)	-	-	-	-	-	(+)	-	-
NA3	-	(+)	(+)	+	-	-	-	-	++	-	-
NE7	(+)	+	-	(+)	+	-	-	-	++	-	-
PF7	+(+)	(+)	(+)	+	-	-	++	+++	(+)	-	+
RF8	(+)	-	-	-	-	-	-	-	(+)	-	-
TA1	-	-	-	-	-	-	(+)	-	+	-	-

Signal intensity	
-	Negative
(+)	Very weak positive
+	Weak positive
+(+)	Positive
++	Positive/ strong positive
++ (+)	
+++	
++++	

=> Selection of **NE7 as detection antibody** and **PF7 as capture antibody**

KPC *K*-SeT evaluation

Blind evaluation was performed on collection and clinical bacterial isolates

		Retrospective validation		Prospective validation (ongoing)		
		KPC *K*-SeT		KPC *K*-SeT		
		+	-	+	-	Total
KPC PCR (Eazyplex)	+	12	0	21	0	33
	- (Carba non KPC)	0	20	0	68	88
	- (Non carba)	0	0	0	48	48
Total		12	20	21	116	169

100% sensitivity and 100% specificity

Using pure recombinant protein, detection level of **KPC *K*-SeT** was defined at: **0,625 ng/ml**.

OXA-48 *K*-SeT performances

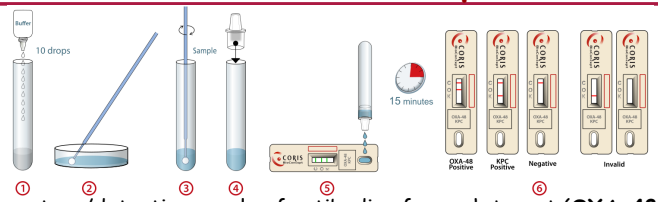
Blind evaluation was performed on collection and clinical bacterial isolates

		Retrospective validation		Prospective validation (ongoing)		
		OXA-48 *K*-SeT		OXA-48 *K*-SeT		
		+	-	+	-	Total
OXA-48 PCR	+	28	0	92	0	120
	- (Carba non OXA-48)	0	20	0	45	65
	- (Non carba)	0	28	0	51	79
Total		28	48	92	96	264

100% sensitivity and 100% specificity

Using pure recombinant protein, detection level of **OXA-48 *K*-SeT** was defined at: **0,125 ng/ml**.

Combi OXA-48 & KPC *K*-SeT development



- The best capture/detection couple of antibodies for each target (**OXA-48** and **KPC**) was selected for further combi tests development.
- Optimization tests were performed to avoid cross reactions between carbapenemases
- Buffer optimizations were done to avoid background signals

Conclusion & Future plans

We developed a new lateral flow assay (**KPC *K*-SeT**) for the **rapid detection** of KPC carbapenemase, and started the set-up of a combined test for the detection of both OXA-48 (and the variants of OXA-48 family) and KPC carbapenemases (**OXA-48 & KPC *K*-SeT**). The **KPC *K*-SeT** test was further evaluated in **prospective studies** in **reference centers**, confirming the **excellent sensitivity and specificity of the test**. The clinical performances of the test will be further assessed on a **larger panel** of clinical bacterial isolates when we will start clinical evaluation of our combi assay.

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