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Comparison of the Check-Direct CPE real-time PCR kit to selective agar media for the detection of Carbapenemases in *Enterobacteriaceae*

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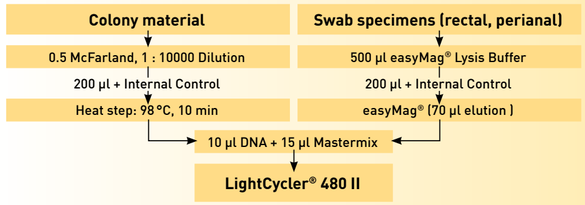
Objectives

Carbapenemase-producing Gram-negative bacteria have become a serious threat to the health care systems worldwide. Specific and reliable detection is crucial to prevent the spread of these organisms. According to this background we validated the real-time PCR kit Check-Direct CPE (Check-Points, The Netherlands) for the detection of KPC, NDM/VIM and OXA-48 in *Enterobacteriaceae*. The test can be applied on rectal and perianal specimens and on overnight grown bacterial colonies (Figure 1). The test was compared to three selective agar media, Brilliance™ CRE agar (Oxoid), CHROMagar™ KPC (MAST) and chromID™ CARBA agar (bioMérieux) (Figure 2). Additionally a first validation of direct swab specimens was performed.

Fig. 1. Workflow of the Check-Direct CPE test



Methods

A test panel of 33 strains of *Enterobacteriacae* was tested with the Check-Direct CPE. These included isolates with known carbapenemases (KPC, OXA-48, VIM and NDM) and carbapenem-resistant isolates where resistance is mediated by combinations of ESBL / AmpC enzymes plus porin loss. All strains were characterized before by specific PCR, MALDI-TOF-carbapenemase test, phenotypic methods (VITEK®2, Etest®, Hodge Test) and sequencing. Subsequently, the assay was validated with direct swab specimens (rectal, perianal). DNA extraction was done with the NucliSENS® easyMag® system (bioMérieux). The Real-Time PCR was performed on the LightCycler® 480 II (Roche). For the testing of Check-Direct CPE and the selective agar media a 1:10000 dilution of a 0.5 McFarland suspension was used. The chromogenic agar plates were incubated for 24 – 48 hours at 35 ± 2°C.

Fig. 2. Selective agar media for carbapenemase detection

Brilliance™ CRE agar | CHROMagar™ KPC | chromID™ CARBA agar

Results

33 *Enterobacteriaceae* strains with characterized carbapenem-resistance mechanism (KPC n=8, NDM-1 n=5, OXA-48 n=11, VIM n=5) were tested with the Check-Direct CPE assay. All (100%) of the carbapenemase-positive strains were correctly differentiated with the assay. The 4 characterized carbapenemase-negative strains also showed a correct-negative result (Table 1). The LoD of the Check-Direct CPE was 25 CFU / PCR reaction. 8 isolates with carbapenem-resistance were directly detected from rectal and perianal swabs (Table 2). With regard to the selective agar media tested, several carbapenemase-positive strains (most OXA-48) were not detected with the chromogenic media (Table 1).

Table 1. Validation of the Check-Direct CPE assay with reference strains with defined carbapenemase-resistance mechanisms.

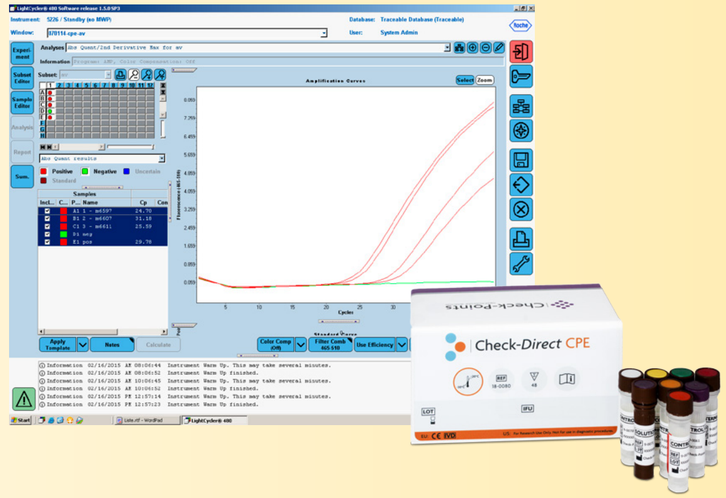
Study-No.	Species	Type	Check-Direct CPE PCR	Selective agar media			V2[1]	HD [1]	ML[1]	MIC[1] (mg/l)[1]		
			Result	Brilliance™ CRE	CHROMagar™ KPC	chromID™ CARBA				IPM[1]	MEM[1]	ERTA[1]
LL5	*E. coli*	KPC	KPC	+	0	+	R	+	+	4	1	3
LL7	*K. pneumoniae*	KPC	KPC	+	+	+	R	+	+	3	4	32
LL28	*K. pneumoniae*	KPC	KPC	+	+	+	I	+	+	32	6	8
LL29	*K. pneumoniae*	KPC	KPC	+	+	+	R	+	+	32	32	32
LL30	*K. pneumoniae*	KPC	KPC	+	+	+	R	+	+	32	8	16
LL36	*K. pneumoniae*	KPC	KPC	+	+	+	I	+	+	32	32	32
LL56	*K. pneumoniae*	KPC	KPC	+	+	+	R	+	+	32	32	32
LL57	*K. pneumoniae*	KPC	KPC	+	+	+	R	+	+	32	16	16
LL11	*K. pneumoniae*	NDM-1	NDM / VIM	0	+	+	R	+	+	32	32	32
LL12	*K. pneumoniae*	NDM-1	NDM / VIM	+	+	+	R	+	+	32	8	32
LL14	*P. rettgeri*	NDM-1	NDM / VIM	+	+	0	R	+	+	>32	>32	>32
LL43	*E. coli*	NDM-1	NDM / VIM	+	+	+	R	+	+	32	32	32
LL44	*K. pneumoniae*	NDM-1	NDM / VIM	+	+	+	R	+	+	32	32	32
LL8	*E. coli*	OXA-48	OXA-48	+	+	0	R	+	+	4	3	12
LL9	*K. pneumoniae*	OXA-48	OXA-48	+	+	+	R	+	+	32	32	32
LL10	*K. pneumoniae*	OXA-48	OXA-48	+	+	+	R	+	+	32	4	32
LL27	*K. pneumoniae*	OXA-48	OXA-48	+	0	0	S	+	+	2	2	0.12
LL35	*K. pneumoniae*	OXA-48	OXA-48	+	+	0	S	+	+	32	4	32
LL38	*E. coli*	OXA-48	OXA-48	0	+	+	I	+	+	32	1	32
LL40	*K. pneumoniae*	OXA-48	OXA-48	+	+	+	R	+	+	32	16	32
LL41	*K. pneumoniae*	OXA-48	OXA-48	0	0	0	R	+	+	8	3	2
LL45	*E. coli*	OXA-48	OXA-48	0	0	0	R	+	+	4	1.5	32
LL47	*K. pneumoniae*	OXA-48	OXA-48	0	0	0	R	+	+	6	1	4
LL48	*K. pneumoniae*	OXA-48	OXA-48	+	+	0	I	+	+	0.38	0.03	0.12
LL1	*E. coli*	VIM	NDM / VIM	+	0	+	R	+	+	4	0.75	1
LL3	*K. pneumoniae*	VIM	NDM / VIM	+	+	+	R	+	+	32	32	32
LL58	*K. pneumoniae*	VIM	NDM / VIM	+	+	+	R	+	+	32	16	32
LL59	*K. pneumoniae*	VIM	NDM / VIM	+	+	+	R	+	+	32	16	32
LL34	*K. oxytoca*	VIM	NDM / VIM	+	0	+	R	+	+	32	1.5	4
LL19	*E. coli*	-	negative	0	0	0	S	0	0	0.5	0.02	0.12
LL22	*E. coli*	porin loss + AmpC	negative	0	0	0	S	0	0	2	0.25	0.04
LL42	*K. pneumoniae*	porin loss + ESBL	negative	0	0	0	S	0	0	1.5	4	32
LL60	*E. coli*	-	negative	0	0	0	S	0	0	2	0.25	0.5

[1] V2: VITEK® 2, R: resistant, I: intermediate, S: sensitive, HD: Hodge Test, ML: MALDI-TOF, MIC: Minimal Inhibitory Concentration, IMP: Imipenem, MEM: Meropenem, ERTA: Ertapenem

Table 2. Direct detection of carbapenemase-positive isolates with the Check-Direct CPE assay.

Study-No.	Specimen Type	Species	Carbapenemase Type	Check-Direct CPE	
				Result	C_t-value
194-439696	stool swab	*K. pneumoniae*	KPC	KPC	24.69
194-439701	stool swab	*E. coli*	KPC	KPC	31.23
194-439703	stool swab	*C. freundii*	KPC	KPC	25.58
194-443325	anal swab	*K. pneumoniae*	NDM-1	NDM / VIM	39.91
093-859110	anal swab	*K. pneumoniae*	NDM-1	NDM / VIM	35.81
098-532081	skin swab	*K. pneumoniae*	OXA-48	OXA-48	17.70
194-446300	rectal swab	*K. pneumoniae*	OXA-48	OXA-48	18.86
193-206547	groin swab	*E. coli*	OXA-48	OXA-48	25.16

Fig. 3. Check-Direct CPE assay



Conclusions

The Check-Direct CPE (Check-Points) showed fast and reliable results for the detection of KPC, NDM/VIM and OXA-48 when tested with characterized reference strains and proved the capability for the use on direct swab specimens. The PCR method proved to be superior to selective agar media.