Validation of the new real-time PCR kit Check-Direct CPE for the detection of KPC, NDM/VIM and OXA-48 in *Enterobacteriaceae*

PRP20

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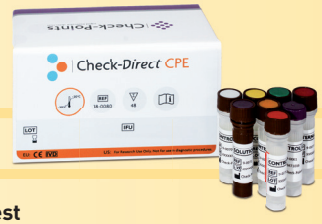
Introduction

The emergence and dissemination of carbapenemase-producing Gram-negative bacteria poses a serious impact on the health care system. Specific and reliable detection is crucial to prevent the spread of these organisms. According to this background we validated the new 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.

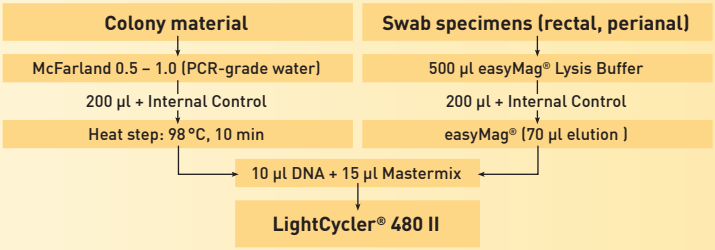


Methods

The Check-Direct CPE assay was validated for 28 reference strains of *Enterobacteriaceae*. These strains were characterized by PCR, MALDI-TOF and phenotypic methods (VITEK® 2, Etest®, Hodge Test) and sequencing. The panel 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 as well as carbapenem-susceptible isolates.

DNA was extracted from overnight colonies using a crude DNA extraction method with a heating step at 98 °C. Subsequently, the assay was validated with swabs spiked with reference strains (500-50000 CFU/ml) to assess the capability of the test for use with direct swab specimens (e.g. rectal swabs). DNA extraction was performed with the NucliSENS® easyMag® system (bioMérieux). The real-time PCR was carried out on the LightCycler®480 II (Roche).

Workflow of the Check-Direct CPE test



Results

25 *Enterobacteriaceae* reference strains with characterized carbapenem-resistance mechanism (KPC: n=6, NDM: n=5, OXA-48: n=11, VIM: n=3) were tested with the Check-Direct CPE assay. All (100%) of the carbapenemase-positive strains were correctly detected with the assay.

The characterized carbapenemase-negative strains also showed a correct-negative result (Table 1). The LoD of the Check-Direct CPE assay was determined by using spiked swab samples and ranged from 18 to 37 CFU/PCR for 8 reference strains tested (Table 2). First direct testing of patient specimens showed promising results (Table 3).

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

Study-No.	Species	Type	V2[1]	HT[1]	ML[1]	MIC (mg/l)[1]			Check-Direct CPE	
						IPM[1]	MEM[1]	ERTA[1]	Result	C_t-value
LL5	*E. coli*	KPC	R	+	+	4	1	3	KPC	25.11
LL7	*K. pneumoniae*	KPC	R	+	+	3	4	32	KPC	24.28
LL28	*K. pneumoniae*	KPC	I	+	+	32	6	8	KPC	22.69
LL29	*K. pneumoniae*	KPC	R	+	+	32	32	32	KPC	22.64
LL30	*K. pneumoniae*	KPC	R	+	+	32	8	16	KPC	22.56
LL36	*K. pneumoniae*	KPC	I	+	+	32	32	32	KPC	25.35
LL11	*K. pneumoniae*	NDM	R	+	+	32	32	32	NDM/VIM	24.90
LL12	*K. pneumoniae*	NDM	R	+	+	32	8	32	NDM/VIM	25.03
LL14	*P. rettgeri*	NDM	R	+	+	>32	>32	>32	NDM/VIM	23.40
LL43	*E. coli*	NDM	R	+	+	32	32	32	NDM/VIM	25.79
LL44	*K. pneumoniae*	NDM	R	+	+	32	32	32	NDM/VIM	26.29
LL8	*E. coli*	OXA-48	R	+	+	4	3	12	OXA-48	21.46
LL9	*K. pneumoniae*	OXA-48	R	+	+	32	32	32	OXA-48	21.52
LL10	*K. pneumoniae*	OXA-48	R	+	+	32	4	32	OXA-48	21.28
LL27	*K. pneumoniae*	OXA-48	S	+	+	2	2	0.12	OXA-48	21.84
LL35	*K. pneumoniae*	OXA-48	S	+	+	32	4	32	OXA-48	21.50
LL38	*E. coli*	OXA-48	I	+	+	32	1	32	OXA-48	25.33
LL40	*K. pneumoniae*	OXA-48	R	+	+	32	16	32	OXA-48	19.67
LL41	*K. pneumoniae*	OXA-48	R	+	+	8	3	2	OXA-48	20.73
LL45	*E. coli*	OXA-48	R	+	+	4	1.5	32	OXA-48	22.57
LL47	*K. pneumoniae*	OXA-48	R	+	+	6	1	4	OXA-48	23.64
LL48	*K. pneumoniae*	OXA-48	I	0	+	0.38	0.03	0.12	OXA-48	21.88
LL1	*E. coli*	VIM	R	+	+	4	0.75	1	NDM/VIM	21.78
LL3	*K. pneumoniae*	VIM	R	+	+	32	32	32	NDM/VIM	22.35
LL34	*K. oxytoca*	VIM	R	+	+	32	1.5	4	NDM/VIM	22.61
LL19	*E. coli*	–	S	0	0	0.5	0.02	0.12	negative	–
LL22	*E. coli*	porin loss + AmpC	S	0	0	2	0.25	0.04	negative	–
LL42	*K. pneumoniae*	porin loss + ESBL	S	+	+	1.5	4	32	negative	–

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

Table 2. LoD of reference strains using spiked swabs.

Study-No.	Species	Type	Check-Direct CPE	
			CFU/PCR	CFU/ml
LL1	*E. coli*	VIM	29	2,900
LL3	*K. pneumoniae*	VIM	37	3,700
LL5	*E. coli*	KPC	27	2,700
LL7	*K. pneumoniae*	KPC	21	2,100
LL8	*E. coli*	OXA-48	24	2,400
LL9	*K. pneumoniae*	OXA-48	28	2,800
LL11	*K. pneumoniae*	NDM	19	1,900
LL12	*K. pneumoniae*	NDM	18	1,800
LL22	*E. coli*	porin loss + AmpC	–	–

Table 3. 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	NDM/VIM	39.91
093-859110	anal swab	*K. pneumoniae*	NDM	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

Conclusion

The Check-Direct CPE for the detection of KPC, NDM/VIM and OXA-48 showed fast and reliable results when tested with characterized reference strains and proved the capability for the use on direct swabs specimens.

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