Genetic Signatures
Transforming Molecular Diagnostics

Evaluation of *EasyScreen™* ESBL/CPO Detection Kit Using Direct-PCR from Patient Culture and Broth Samples

Dilshan M. Abeysekera[1], Elaine McGrath[2], Martin Cormican[2], Damien J Stark[3], John R Melki[1] and Douglas S Millar[1]
1 Genetic Signatures, 7 Eliza St, Newtown, 2042
2 National CPE Reference Laboratory, Galway Ireland
3 Microbiology Department, St. Vincent's Hospital, Darlinghurst, NSW 2010

Introduction

Beta-lactam and carbapenem antibiotics are the most commonly used worldwide in the treatment of bacterial infections. The recent emergence of Extended-Spectrum Beta-Lactamases (ESBL) and Carbapenemase Producing Organisms (CPO) are a significant global concern in healthcare settings, as these enzymes may render standard treatments ineffective. Thus, accurate and rapid detection of these resistant organisms will have a significant impact on patient management.

We have developed a novel **3base™** real-time multiplex-PCR assay to detect most significant and commonly encountered bacterial resistance genes TEM, CTX-M, SME, GES, IMP, NDM, OXA-23 like, OXA-48 like, OXA-51 like, MCR-1, DHA, SHV, VIM, IMI, CMY, KPC and their subtypes.

The **3base™** assay is a simple and rapid molecular method that utilises **3base™** technology to modify the 4 base wild-type DNA sequence (A, C, T, G) into a 3 base sequence (A, T, G) via a novel, patented **3base™** conversion step. The conversion process simplifies the design of multiplex PCR reactions by eliminating the large Tm differences that can be present when targeting multiple pathogens and increasing the homology between sequences. (table1).

	Before	After
Seq 1	GATGGCGATA TGGTTGACAC	GATGGTGATA TGGTTGATAT
Seq 2	GATGGTGACA TGGTAGATAC	GATGGTGATA TGGTAGATAT
Seq 3	GATGGTGATA TGGTGGACAC	GATGGTGATA TGGTGGATAT
Seq 4	GATGGTGATA TGGTAGATAT	GATGGTGATA TGGTAGATAT
Seq 5	GATGGTGATA TGGTGGACAC	GATGGTGATA TGGTGGATAT
Seq 6	GATGGCGACA TGGTTGATAT	GATGGTGATA TGGTTGATAT
Seq 7	GATGGTGATA TGGTGGACAC	GATGGTGATA TGGTGGATAT
Seq 8	GATGGTGACA TGGTAGATAC	GATGGTGATA TGGTAGATAT
Seq 9	GATGGTGATA TGGTAGATAC	GATGGTGATA TGGTAGATAT
Seq 10	GATGGTGATA TGGTGGATAC	GATGGTGATA TGGTGGATAT
Consensus	GATGGYGAYA TGGTDGAYAY	GATGGTGATA TGGTDGATAT
	75% homology over 20 bases	95% homology over 20 bases
	48 possible primer combinations	3 possible primer combinations

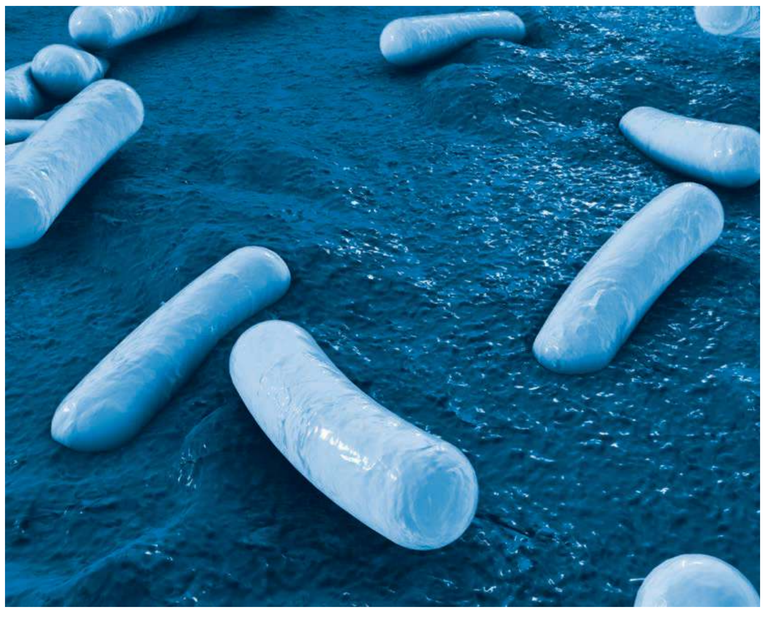
Table1. Example of the **3base™** mechanism. The example sequences above show the increase in homology from 75% ("Before") to 95% ("After") via the **3base™** conversion where all C bases are detected as T bases.

Methods/Materials

The sensitivity of each target was determined by using synthetic DNA constructs. In addition, validation target organisms and panels were obtained from Vircell, Zeptometrix and QCMD. The performance of the assay was tested on known reference material and Clinical isolates obtained from St. Vincent's Hospital (Sydney, Australia) and University Hospital Galway (Galway, Ireland).

DNA extraction and PCR setup were performed on a GS-mini automated extraction platform using SP006 Sample Processing kits. PCR was performed on a CFX96/384 TouchTM real-time PCR instrument.

To evaluate direct-PCR, where no extraction is required, 30 known clinical isolates were obtained from University Hospital Galway. Nine of these isolates were re-cultured in BHI-broth. Both isolates and broth sample were lysed, converted into **3base™** form at 95°C for 15min and amplified. The results were compared with results obtained using the GS-mini, and data collected previously using a conventional extraction and PCR method.



Validation results

The sensitivity of each component in the multiplexed assay was found to be less than 50 copies of target template per PCR. Mixed infections could be easily detected using the different channels of the PCR instrument. The specificity of the assay was assessed by a cross-reactivity panel, and showed no cross-reactivity. Results from the validation panels yielded 100% concordance with the expected resistance patterns (table 2). In addition ESBL and non-ESBL strains could be differentiated using a novel PCR method in development.

Content	ESBL/ CARBA Status	TEM	SHV	CTX-M	CARBA	*EasyScreen™*
K. pneumoniae	CPO	Negative	SHV-1 (non-ESBL)	Negative	KPC-3, OXA-48	KPC, OXA-48, SHV (non-ESBL)
E. coli	CPO	Negative	Negative	Negative	OXA-244	OXA-244
E. coli	CPO	TEM-1 (non-ESBL)	Negative	Negative	IMP-1	IMP, TEM (non-ESBL)
E. coli	Negative	Negative	Negative	Negative	Negative	Negative
E. cloacae	ESBL/CPO	Negative	SHV-12 (ESBL)	CTX-M-9 (ESBL)	OXA-48	OXA-48, CTX-M, SHV (ESBL)
C. brakii	CPO	Negative	Negative	Negative	VIM-1	VIM
K. pneumoniae	ESBL/CPO	TEM-1 (non-ESBL)	SHV-1 (non-ESBL)	CTX-M-2 (ESBL)	KPC-2	KPC, CTX-M, TEM (non-ESBL), SHV (non-ESBL)
K. pneumoniae	ESBL/CPO	TEM-1 (non-ESBL)	SHV-12 (ESBL)	CTX-M-15 (ESBL)	NDM-1	NDM, CTX-M, TEM (non-ESBL), SHV (ESBL)

Table 2. 2017 QCMD validation.

Clinical Validation

The results of 45 clinical isolates from a previous clinical trial is shown in Table 3. Two-thirds of the clinical isolates tested were found to have mixed infections. In summary, the ***EasyScreen™* ESBL/CPO Detection Kit** when used with the direct-PCR method and compared against a conventional in-house method had a 97% concordance with culture and 100% agreement with Broth samples.

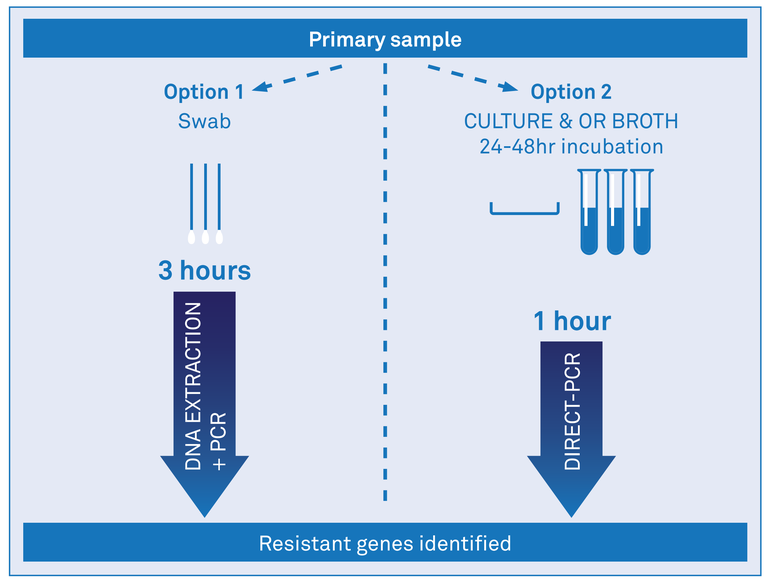
Target	GSL Result	University Hospital Galway Result
CTX-M	19	19
Oxa-48 like	8	8
NDM	6	6
VIM	7	8
IMI	1	1
IMP	5	5
CMY	6	6
GES	1	1
DHA	4	3
KPC	5	5

Table 3. Results obtained using clinical isolates demonstrating 96% concordance between the results.

Sample spp.	Purified Sample	Direct PCR	Sample spp.	Purified Sample	Direct PCR
E. coli	NDM	NDM	*Citrobacter species*	KPC/DHA	KPC/DHA
K. oxytoca	IMP	IMP	*E. coli*	OXA-48	OXA-48
E. coli	CMY/VIM	CMY/VIM	*E. coli*	CTX-M	CTX-M
K. pnuemoniae	CTX-M/NDM/ OXA-48	CTX-M/NDM/ OXA-48	*E. coli*	CMY	CMY
E. coli	CTX-M/NDM	CTX-M/NDM	*E. coli*	CTX-M	CTX-M
P. aeruginosa	GES	GES	*K. pnuemoniae*	OXA-48	OXA-48
K. oxytoca	VIM	VIM	*E. cloacae*	CTX-M/IMP	CTX-M/IMP
E. coli	CTX-M	CTX-M	*E. cloacae*	VIM/CTX-M	VIM/CTX-M
E. cloacae	NEGATIVE	NEGATIVE	*K. pnuemoniae*	KPC	KPC
E. coli	CTX-M	CTX-M	*Citrobacter species*	KPC/DHA	KPC/DHA
K. pnuemoniae	OXA-48/CTX-M	OXA48/CTX-M	*E. coli*	CTX-M/OXA-48	CTX-M/OXA-48
E. cloacae	VIM	VIM	*E. cloacae*	Negative	NEGATIVE
E. coli	CTX-M/CMY	CTX-M/CMY	*E. cloacae*	CTX-M/IMP	CTX-M/IMP
P. aeruginosa	VIM	VIM	*K. pnuemoniae*	KPC	KPC
K. oxytoca	NDM/DHA/ CTX-M	NDM/DHA/ CTX-M	*E. cloacae*	IMP	IMP
E. coli	NDM/CMY	NDM/CMY	*K. pnuemoniae*	CTX-M/OXA-48/CMY	CTX-M/OXA-48/CMY
E. cloacae	VIM	VIM	*K. pnuemoniae*	NDM	NDM

Table 4. Comparison of direct from culture PCR with automated purification of the sample.

Work Flow options



Assays in development

	MIC			Amino Acid Position		
Enzyme	Ceftax	Cefotax	Aztreo	104	164	240
TEM-1	0.12	0.06	0.12	Glu	Arg	Glu
TEM-10	>256	1	128	Glu	Ser	Lys
TEM-12	16	0.12	1	Glu	Ser	Glu
TEM-26	256	0.5	164	Lys	Ser	Glu

The naturally occurring TEM and SHV genes are not indicative of ESBL production, therefore the following mutations/markers are being targeted in order to differentiate the presence of ESBL from non-ESBL

Target	Mutation/types
TEM	E104K, R164S/H, G238S, E240K mutations
SHV	G238S, E240K mutations
MCR	MCR-2, MCR-3, MCR-4, MCR-5 and MCR-6

Conclusions

The ***EasyScreen™* ESBL/CPO Detection Kit** has been developed as a useful tool in rapidly detecting resistant genes directly from cultures and broths without the need for DNA extraction/purification steps (table 4). The PCR protocol can be significantly shortened to reduce the run-time given the high copy number. The turn around-time is approximately 1 hour for culture and broth samples. This test could be effective in assisting and accelerating patient management strategies.

The increased sub-type homology of the **3base™** technique expands the detection capacity of multiplex-PCR for some target genes (CTX-M). Also, novel variants (such as IMP-14) or new resistant markers can be readily incorporated into existing assays given the properties of the 3baseTM converted DNA sequences, thus improving the throughput of such assays. The optimised assay provides a sensitive and specific alternative for the detection of ESBL and CPO sequences and can be carried out in less than 3 hours for primary patient specimens and in approximately 1 hour for cultured isolates.

References

1. Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. Clin Infect Dis. 1997 Jan;24 Suppl 1:S19-45.
2. Two novel plasmid-mediated cefotaxime hydrolyzing b-lactamases (CTX-M-5 and CTX-M-6) from Salmonella typhimurium. FEMS. Microbiol. Lett. 1998;165:289–293. [PubMed].