

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

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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**TM 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**TM assay is a simple and rapid molecular method that utilises **3base**TM 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**TM 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	GATGGCGAIA TGGTIGACA C	GATGGTGATA TGGTIGATAT
Seq 2	GATGGIGACA TGGTAGATA C	GATGGTGATA TGGTAGATAT
Seq 3	GATGGIGATA TGGTGACA C	GATGGTGATA TGGTGATAT
Seq 4	GATGGIGATA TGGTAGATA I	GATGGTGATA TGGTAGATAT
Seq 5	GATGGIGATA TGGTGACA C	GATGGTGATA TGGTGATAT
Seq 6	GATGGCGACA TGGTIGATA I	GATGGTGATA TGGTIGATAT
Seq 7	GATGGIGATA TGGTGACA C	GATGGTGATA TGGTGATAT
Seq 8	GATGGIGACA TGGTAGATA C	GATGGTGATA TGGTAGATAT
Seq 9	GATGGIGATA TGGTAGATA C	GATGGTGATA TGGTAGATAT
Seq 10	GATGGIGATA TGGTGACA C	GATGGTGATA TGGTGATAT

Consensus	GATGGYGAYA TGGTDGAYAY	GATGGTGATA TGGTDGATAT
	75% homology over 20 bases	95% homology over 20 bases
	48 possible primer combinations	3 possible primer combinations

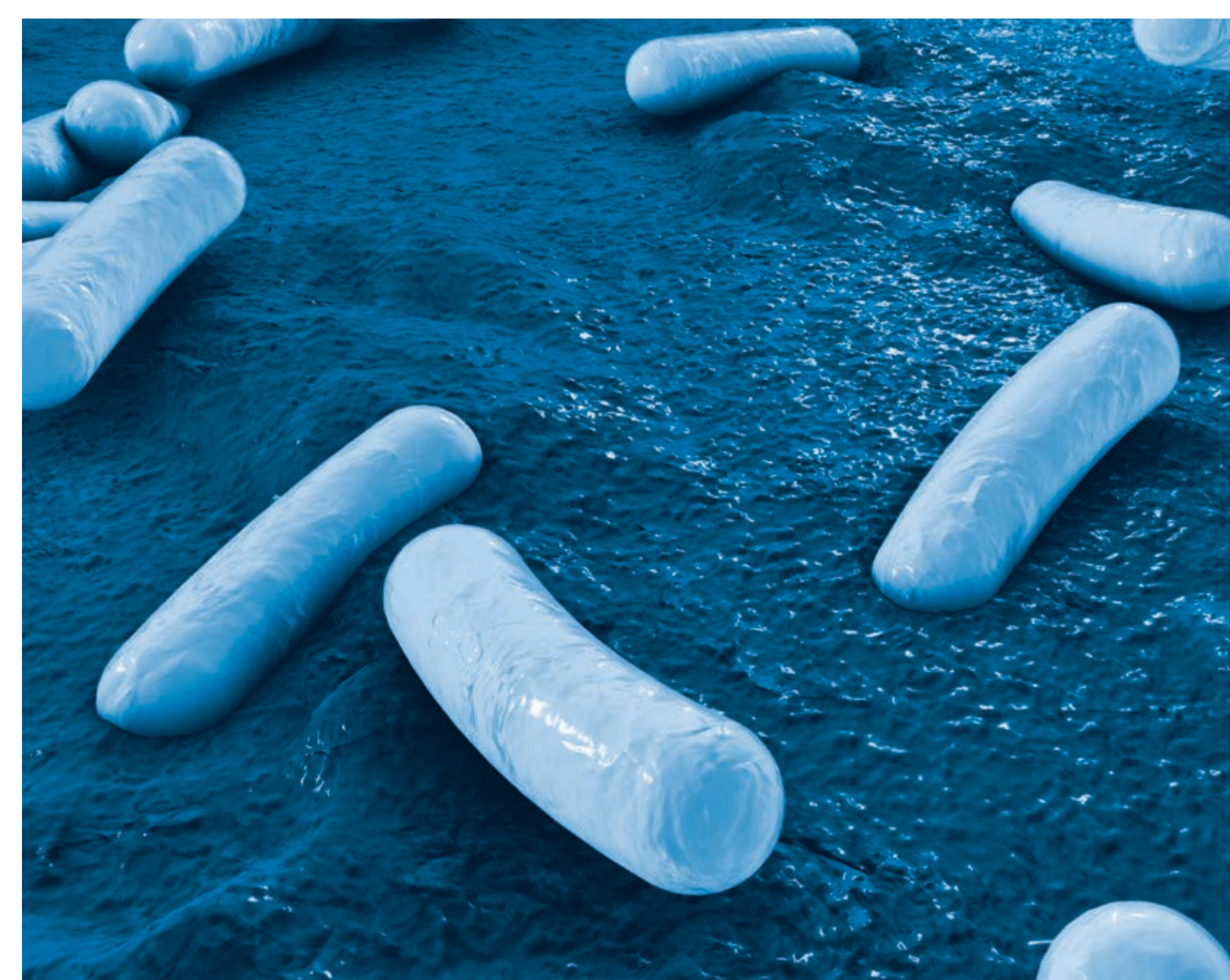
Table 1. Example of the **3base**TM mechanism. The example sequences above show the increase in homology from 75% ("Before") to 95% ("After") via the **3base**TM conversion where all C bases are detected as T bases.

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, Zeptomatrix 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**TM 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	<i>EasyScreen</i> TM
<i>K. pneumoniae</i>	CPO	Negative	SHV-1 (non-ESBL)	Negative	KPC-3, OXA-48	KPC, OXA-48, SHV (non-ESBL)
<i>E. coli</i>	CPO	Negative	Negative	Negative	OXA-244	OXA-244
<i>E. coli</i>	CPO	TEM-1 (non-ESBL)	Negative	Negative	IMP-1	IMP, TEM (non-ESBL)
<i>E. coli</i>	Negative	Negative	Negative	Negative	Negative	Negative
<i>E. cloacae</i>	ESBL/CPO	Negative	SHV-12 (ESBL)	CTX-M-9 (ESBL)	OXA-48	OXA-48, CTX-M, SHV (ESBL)
<i>C. brachii</i>	CPO	Negative	Negative	Negative	VIM-1	VIM
<i>K. pneumoniae</i>	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)
<i>K. pneumoniae</i>	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**TM 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.

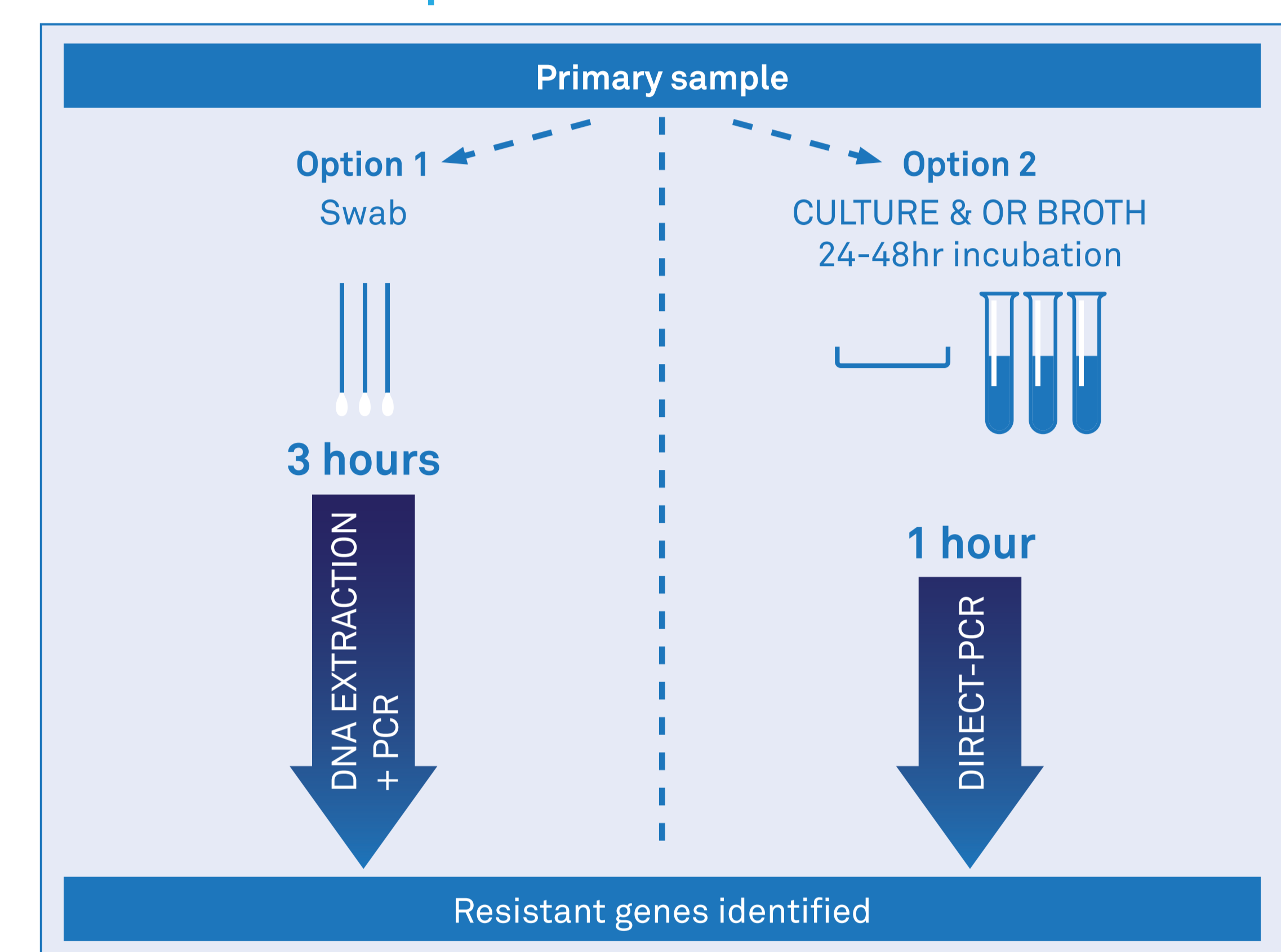
Target	GSL Result	University Hospital Galway Result
CTX-M	19	19
Oxa-48 like	8	8
NDM	6	6
VIM	7	8
IMI	1	1
IMP	5	5
CMY	6	6
GES	1	1
DHA	4	3
KPC	5	5

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
<i>E. coli</i>	NDM	NDM	<i>Citrobacter species</i>	KPC/DHA	KPC/DHA
<i>K. oxytoca</i>	IMP	IMP	<i>E. coli</i>	OXA-48	OXA-48
<i>E. coli</i>	CMY/VIM	CMY/VIM	<i>E. coli</i>	CTX-M	CTX-M
<i>K. pneumoniae</i>	CTX-M/NDM/OXA-48	CTX-M/NDM/OXA-48	<i>E. coli</i>	CMY	CMY
<i>E. coli</i>	CTX-M/NDM	CTX-M/NDM	<i>E. coli</i>	CTX-M	CTX-M
<i>P. aeruginosa</i>	GES	GES	<i>K. pneumoniae</i>	OXA-48	OXA-48
<i>K. oxytoca</i>	VIM	VIM	<i>E. cloacae</i>	CTX-M/IMP	CTX-M/IMP
<i>E. coli</i>	CTX-M	CTX-M	<i>E. cloacae</i>	VIM/CTX-M	VIM/CTX-M
<i>E. cloacae</i>	NEGATIVE	NEGATIVE	<i>K. pneumoniae</i>	KPC	KPC
<i>E. coli</i>	CTX-M	CTX-M	<i>Citrobacter species</i>	KPC/DHA	KPC/DHA
<i>K. pneumoniae</i>	OXA-48/CTX-M	OXA48/CTX-M	<i>E. coli</i>	CTX-M/OXA-48	CTX-M/OXA-48
<i>E. cloacae</i>	VIM	VIM	<i>E. cloacae</i>	Negative	NEGATIVE
<i>E. coli</i>	CTX-M/CMY	CTX-M/CMY	<i>E. cloacae</i>	CTX-M/IMP	CTX-M/IMP
<i>P. aeruginosa</i>	VIM	VIM	<i>K. pneumoniae</i>	KPC	KPC
<i>K. oxytoca</i>	NDM/DHA/CTX-M	NDM/DHA/CTX-M	<i>E. cloacae</i>	IMP	IMP
<i>E. coli</i>	NDM/CMY	NDM/CMY	<i>K. pneumoniae</i>	CTX-M/OXA-48/CMY	CTX-M/OXA-48/CMY
<i>E. cloacae</i>	VIM	VIM	<i>K. pneumoniae</i>	NDM	NDM

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

Work Flow options



Assays in development

Enzyme	MIC	Ceftax	Cefotax	Aztreo	Amino Acid Position		
TEM-1	0.12	0.06	0.12		104	164	240
TEM-10	>256	1	128		Glu	Arg	Glu
TEM-12	16	0.12	1		Glu	Ser	Lys
TEM-26	256	0.5	164		Glu	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**TM 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**TM 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 **3base**TM 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

- Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. Clin Infect Dis. 1997 Jan;24 Suppl 1:S19-45.
- 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].

