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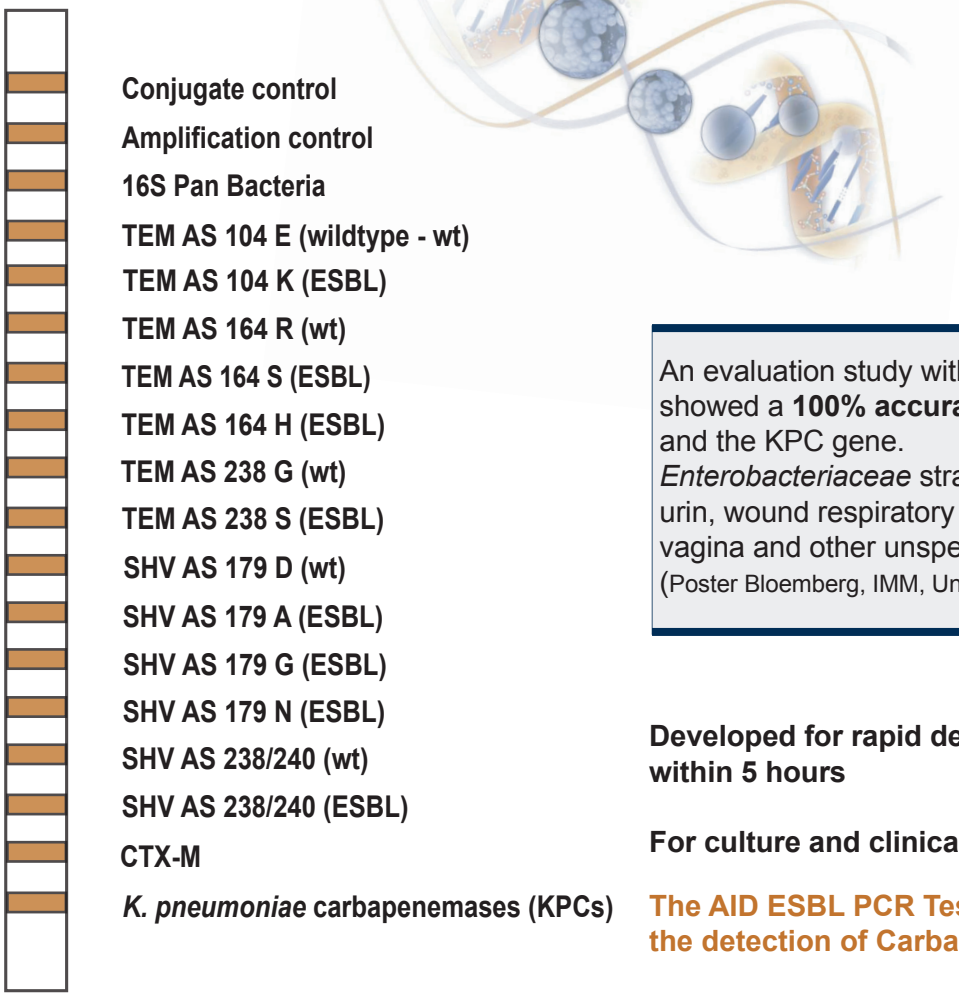
ESBL-Assay

Detection of extended spectrum β-lactamases (ESBL) genes in *Enterobacteriaceae* by PCR

ESBLs (extended spectrum beta-lactamases) positive *Enterobacteriaceae* are isolated world-wide. These enzymes hydrolyse significantly Penicillins, Cephalosporins and Monobactams.

Most ESBLs are related to the TEM, SHV and CTX-M types and are increasing dramatically in Europe.



- **Conjugate control**
- **Amplification control**
- **16S Pan Bacteria**
- **TEM AS 104 E (wildtype - wt)**
- **TEM AS 104 K (ESBL)**
- **TEM AS 164 R (wt)**
- **TEM AS 164 S (ESBL)**
- **TEM AS 164 H (ESBL)**
- **TEM AS 238 G (wt)**
- **TEM AS 238 S (ESBL)**
- **SHV AS 179 D (wt)**
- **SHV AS 179 A (ESBL)**
- **SHV AS 179 G (ESBL)**
- **SHV AS 179 N (ESBL)**
- **SHV AS 238/240 (wt)**
- **SHV AS 238/240 (ESBL)**
- **CTX-M**
- ***K. pneumoniae* carbapenemases (KPCs)**

An evaluation study with 424 clinical strains showed a **100% accuracy** to detect ESBL genes and the KPC gene.
Enterobacteriaceae strains were isolated from: urin, wound respiratory tract, groin, blood culture, vagina and other unspecific body sites
(Poster Bloemberg, IMM, University of Zurich, 2012)

Developed for rapid detection of ESBL genes within 5 hours

For culture and clinical specimens

The AID ESBL PCR Test is now available with the detection of Carbapenemases (KPCs)!

References:
Guido V. Bloemberg, Silke Polsfuss, Vera Meyer, Erik C. Böttger, Michael Hombach (2013).
Evaluation of the AID ESBL line probe assay for rapid detection of extended-spectrum β-lactamase (ESBL) and KPC carbapenemase genes in Enterobacteriaceae
J Antimicrob Chemother doi:10.1093/jac/dkt345

Order-No.	Product	
RDB2180	ESBL	12 tests
RDB2180X	ESBL	60 tests

CE

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The AID modular line probe assay for rapid detection of extended spectrum β-lactamases (ESBL) genes in *Enterobacteriaceae*.

The AID ESBL line probe assay was evaluated for the detection of extended spectrum β-lactamases (ESBL) genes in *Enterobacteriaceae.* The line probe assay was shown to detect with a 100% accuracy ESBL genes for which oligonucleotide probes are present in the assay. The line probe assay was successfully implemented in the routine diagnostic laboratory for rapid detection of ESBL genes.

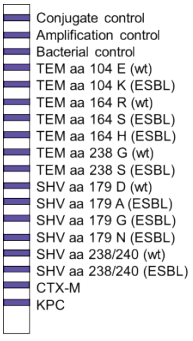
Introduction

Increasing prevalence of multidrug-resistant Gram-negative bacteria has continuously been reported over the past years, in particular *Enterobacteriaceae* producing extended spectrum b-lactamases (ESBLs). ESBLs have the ability to hydrolyse penicillins, first- second- and third-generation cephalosporins and aztreonam (but not cephamycins or carbapenems), and their activity is decreased by inhibitors such as clavulanic acid. Most ESBLs can be classified in TEM, SHV and CTX-M types. TEM and SHV wt genes evolve as ESBL by mutation. ESBL-producing organisms may be responsible for life-threatening infections, leading to increased morbidity, mortality and healthcare-associated costs. A fast and accurate detection of ESBL carrying *Enterobacteriaceae* strains is needed (1). Phenotypic susceptibility testing can be complicated by the presence of multiple b-lactamases, e.g. ESBL`s, AmpC`s and carbapenamases, in one bacterial strain. The purpose of this study was to evaluate an accurate, fast, easy to use and cost efficient molecular line probe assay (AID Autoimmun Diagnostika GmbH, Germany) for the detection of the most prevalent ESBL genes in *Enterobacteriaceae*.

Assay design

Based on epidemiological analyses of ESBL prevelance an ESBL line probe assay (reverse hybridization) was designed (AID Autoimmun Diagnostika GmbH, Germany) to cover TEM-ESBL (E104K, R164S, R164H, G238S), SHV-ESBL (D179A, D179G, D179N, mutant aa 238/240) and CTX-M genes (all known classes). An assay design was developed for screening ESBL genes (Fig. 1). In addition, a probe for one of the most prevalent carbapenamases, e. g. *Klebsiella pneumoniae* carbapenamase (KPC), was included.

Fig.1 Extended spectrum β-lactamases (ESBL) assay (AID).



1. Specificity AID ESBL kit

Following optimization of the assay design, the ESBL assay was evaluated against a series of clinical ESBL culture isolates and PCR products to test the specificity of the assay. (Table 1). All clinical *Enterobacteriaceae* isolates had been characterized in detail for antibiotic susceptibility by phenotypic and molecular testing.

The line probe assay detected ESBL genes and the KPC gene in clinical strains with a 100% accuracy.

References:
1. Polsfuss S, Bloemberg G, Giger J, Meyer V, Böttger E, Hombach M. Evaluation of a diagnostic flow-chart for detection and confirmation of extended spectrum beta-lactamases (ESBL) in *Enterobacteriaceae*. Clin Microbiol Infection. (in press)

Table 1. Evaluation of the ESBL line probe assay using clinical *Enterobacteriaceae* strains and fusion PCR amplicons from the Institute of Medical Microbiology (IMM).

		TEM								SHV							CTX-M		KPC	
		AID							Seq.	AID						Seq.	AID	Seq.	AID	Seq.
		WT	ESBL	WT	ESBL	ESBL	WT	ESBL		WT	ESBL	ESBL	ESBL	WT	ESBL					
Isolate	Species	104 E	104 K	164 R	164 S	164 H	238 G	238 S		179 D	179 A	179 G	179 N	238/240	238/240					
101082	*E.coli*	+	-	+	-	-	+	-	TEM wt	-	-	-	-	-	-	nd	-	nd	-	nd
259917	*P. mirabilis*	-	+	+	-	-	-	+	TEM 104K, 238 S	-	-	-	-	-	-	nd	-	nd	-	nd
	TEM 164 S PCR	+	-	-	+	-	+	-	TEM 164 S	-	-	-	-	-	-	na	-	na	-	na
	TEM 164 H PCR	+	-	-	-	+	+	-	TEM 164 H	-	-	-	-	-	-	na	-	na	-	na
87778	*K. pneumoniae*	-	-	-	-	-	-	-	nd	+	-	-	-	+	-	SHV wt	-	nd	-	nd
	SHV 179A PCR	-	-	-	-	-	-	-	na	-	+	-	-	+	-	SHV 179A	-	na	-	na
	SHV 179G PCR	-	-	-	-	-	-	-	na	-	-	+	-	+	-	SHV 179G	-	na	-	na
	SHV 179N PCR	-	-	-	-	-	-	-	na	-	-	-	+	+	-	SHV 179N	-	na	-	na
67545	*K. pneumoniae*	-	-	-	-	-	-	-	nd	+	-	-	-	+	+	SHV 238S/240	-	nd	-	nd
259296	*E.coli*	-	-	-	-	-	-	-	nd	+	-	-	-	-	+	SHV 238S/240K	-	nd	-	nd
251055	*E.coli*	+	-	+	-	-	+	-	TEM wt	-	-	-	-	-	-	nd	+	CTX M gr I	-	nd
251860	*E.coli*	+	-	+	-	-	+	-	TEM wt	-	-	-	-	-	-	nd	+	CTX M gr 8	-	nd
47303	Klebsiella sp.	-	-	-	-	-	-	-	nd	+	-	-	-	+	-	SHV wt	+	CTX M gr 9	-	nd
100716	*K. pneumoniae*	+	-	+	-	-	+	-	TEM wt	+	-	-	-	+	-	SHV wt	-	nd	+	KPC

AID: ESBL line probe assay
Seq.: detected by PCR and sequencing
na: not applicable
nd: not detected

2. Implementation of AID ESBL kit in routine diagnostics.

To implement the genetic resistance kit in a diagnostic work-flow we evaluated its use to rapidly detect ESBL genes and KPC in clinical culture strains. Sensitivity was determined using a well characterized (molecular and phenotypic) set of *Enterobacteriaceae* clinical strains (n=**424;** 227 *E. coli*, 55 *K. pneumoniae*, 19 *K. oxytoca, 61 Enterobacter cloacae* and 62 other species) obtained in our routine diagnostic laboratory, containing 148 TEM positive strains of which 2 ESBL TEM , 86 SHV positive strains of which 26 ESBL SHV, 134 CTX-M positive strains and 3 KPC containing strains (1). Results show an excellent performance of the ESBL line probe assay, with a specificity of 100 % for TEM, SHV and CTX-M detection, and a sensitivity of 100% for TEM and CTX-M detection and of 94.4% for SHV, due to an SHV sequence variant of *K.variicola*, which is not detected by the SHV PCR primers used (Table 2). The assay will be optimized to detect the latter SHV sequence variants. One *E. coli* strain contained a wt-TEM and ESBL-TEM gene, as shown by clear double signals in the line probe assay. As a negative control group 170 clinical strains characterized phenotypically as non-ESBL and genetically confirmed as TEM, SHV and CTX-M negative. None of these strains produced false positive signals. In addition, we did not observe false positive signals from *K. oxytoca* strains with a K1 beta-lactamase gene.

Table 2. Testing of 424 clinical *Enterobacteriaceae* strains using the ESBL line probe assay. The *Enterobacteriaceae* strains were isolated from : urin, wound respiratory tract, groin, blood culture, vagina, miscellaneous and other unspecified body sites.

	TEM								SHV							CTX-M		KPC	
	AID							Seq.	AID						Seq.	AID	Seq.	AID	Seq.
Isolates	WT	ESBL	WT	ESBL	ESBL	WT	ESBL		WT	ESBL	ESBL	ESBL	WT	ESBL					
(n)	104 E	104 K	164 R	164 S	164 H	238 G	238 S		179 D	179 A	179 G	179 N	238/240	238/240					
12	+	-	+	-	-	+	-	TEM wt	+	-	-	-	+	-	SHV wt	+	CTX M gr I	-	nd
1	+	-	+	-	-	+	-	TEM wt	+	-	-	-	+	-	SHV wt	-	nd	+	KPC
9	+	-	+	-	-	+	-	TEM wt	+	-	-	-	+	-	SHV wt	-	nd	-	nd
1	+	-	+	-	-	+	-	TEM wt	+	-	-	-	+	+	SHV 238S/240	+	CTX M gr I	-	nd
2	+	-	+	-	-	+	-	TEM wt	+	-	-	-	+	+	SHV 238S/240	-	nd	-	nd
2	+	-	+	-	-	+	-	TEM wt	+	-	-	-	+	+	SHV 238S/240	-	nd	+	KPC
2	+	-	+	-	-	+	-	TEM wt	+	-	-	-	-	+	SHV 238S/240K	-	nd	-	nd
46	+	-	+	-	-	+	-	TEM wt	-	-	-	-	-	-	nd	+	CTX M gr I	-	nd
1	+	-	+	-	-	+	-	TEM wt	-	-	-	-	-	-	nd	+	CTX M gr 8	-	nd
11	+	-	+	-	-	+	-	TEM wt	-	-	-	-	-	-	nd	+	CTX M gr 9	-	nd
58	+	-	+	-	-	+	-	TEM wt	-	-	-	-	-	-	nd	-	nd	-	nd
1	+	+	+	-	-	+	+	TEM wt **and** TEM104K, 238 S											
2	-	+	+	-	-	-	+	TEM 104K, 238 S	-	-	-	-	-	-	nd	-	nd	-	nd
1	-	-	-	-	-	-	-	nd	+	-	-	-	+	-	SHV wt	+	CTX M gr I	-	nd
1	-	-	-	-	-	-	-	nd	+	-	-	-	+	-	SHV wt	+	CTX M gr 9	-	nd
30	-	-	-	-	-	-	-	nd	+	-	-	-	+	-	SHV wt	-	nd	-	nd
1	-	-	-	-	-	-	-	nd	+	-	-	-	+	+	SHV 238S/240	-	nd	-	nd
11	-	-	-	-	-	-	-	nd	+	-	-	-	-	+	SHV 238S/240K	+	CTX M gr 9	-	nd
7	-	-	-	-	-	-	-	nd	+	-	-	-	-	+	SHV 238S/240K	-	nd	-	nd
1	-	-	-	-	-	-	-	nd	-	-	-	-	-	-	SHV wt	+	CTX M gr I	-	nd
5	-	-	-	-	-	-	-	nd	-	-	-	-	-	-	SHV wt	-	Nd	-	nd
30	-	-	-	-	-	-	-	nd	-	-	-	-	-	-	nd	+	CTX M gr I	-	nd
19	-	-	-	-	-	-	-	nd	-	-	-	-	-	-	nd	+	CTX M gr 9	-	nd
170	-	-	-	-	-	-	-	nd	-	-	-	-	-	-	nd	-	nd	-	nd

AID: ESBL line probe assay
Seq.: detected by PCR and sequencing
na: not applicable
nd: not detected

Conclusions

The ESBL Resistance line probe assay (AID) is a rapid tool for accurate detection of ESBL genes in *Enterobacteriaceae*. The assay can be implemented readily in the diagnostic laboratory without major equipment investments and is easy to use.

This study was conducted by the Institute of Medical Microbiology, University of Zurich, 8006 Zurich, Switzerland