Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Primer design and testing for RPA

The design for haRPA primers specific for resistance gene *bla*$_{CTX-M}$ cluster 1 followed the protocol described elsewhere with few adjustments [1]. Five primer pairs were designed based on *E. coli bla*$_{CTX-M-1}$ gene (GenBank number: X92506.1) and an additional pair was based on published qPCR primers for detection of *bla*$_{CTX-M}$ [2]. All FWD primers could be combined with all REV primers to maximize primer pair options within the primer test phase. Homogeneous RPA reactions were performed according to the manual. Briefly, 2.4 µL of each primer (10 µM), 29.5 µL rehydration buffer, 5 µL DNA sample, and 7.2 µL H_2O were added to the freeze dried pellet and the reaction was started by addition of 2.5 µL MgAc (280 mM). After incubation for 40 min at 39 °C in a Thermomixer (Eppendorf, Hamburg, Germany) the RPA product was purified using GeneJet PCR Purification Kit (Thermo Scientific, Waltham, USA) and analysed on an 3% agarose gel (w/v). The agarose gel was stained with 5 µL Stain Clear G (Serva Electrophoresis GmbH, Heidelberg, Germany) The amplicons were checked for appropriate size and ranked based on DNA amount. In total, 20 different combinations of FWD and REV primers were tested and the two most promising ones were chosen for further testing on the haRPA system. One primer pair showed good amplification in the haRPA assay with FWD-sequence 5'-CTGATGAGCGCTTTGCGATGTGCAGCACCAG-3' and REV-sequence 5'-TCACGCGGATCGCCCGGAATGGCGGTGTTTAACG-3' and an amplicon length of 352 bp.

DNA chip production

DNA primers were immobilized on the surface using the SciFLEXARRAYER S1 (Scienion, Berlin, Germany), an inkjet microdispensing system, with an unmodified piezo dispense capillary (PDC 80, P-2040, ID-No. 15842 and 54868) at 20 °C and 55% humidity.

Heterogeneous asymmetric recombinase polymerase amplification

The running and washing buffer was casein (0.5% (w/v)) in phosphate buffered saline (PBS). The modified REV-primer (5'-NH_2-C_{12}-tag) was immobilized on the chip surface and the biotinylated FWD-primer was added to the bulk phase to enable CL reaction with streptavidin-labelled horseradish peroxidase (strep-HRP). The reaction mix, containing nuclease-free water (10.5 µL), primer mix (5 µL), and buffer (29.5 µL) was added to the lyophilized reagents. Subsequently, 5 µL of DNA extract was added and the reaction was started using 4 µL of magnesium acetate (250 µM). 52 µL of the mixture was injected into the microarray flow cell (using a pipette (Eppendorf, Hamburg, Germany)). Afterwards, the chip was inserted into the Munich Chip Reader (MCR) 3 (further developed from the original, including a heatable flow cell as published elsewhere [3], provided by GWK Präzisionstechnik GmbH, Munich, Germany) and a dark frame image was taken with the integrated CCD camera for 60 s. The measurement

was carried out as published elsewhere [1]. The dark frame image was subtracted from the obtained CL image before automated analysis using the microarray analysis software MCR Image Analyzer (GWK Präzisionstechnik, Munich, Germany). The ten brightest pixels of each spot were analysed. Spots with a deviation of more than 15% were not included and marked as outliers.

Bacteria characterization for qPCR testing

Table 1: Overview of bacteria for qPCR specificity tests with the ESBL gene and positive or negative characterization for cluster 1.

Bacterial species	ESBL gene	Cluster 1 positive/negative
Enterobacter asburiae	$bla_{\text{CTX-M-1}}$	Positive
Enterobacter cloacae	$bla_{\text{CTX-M-15}}$	Positive
E. coli	$bla_{\text{CTX-M-1}}$	Positive
E. coli	$bla_{\text{CTX-M-2}}$	Negative
E. coli	$bla_{\text{CTX-M-3}}$	Positive
E. coli	$bla_{\text{CTX-M-9}}$	Negative
E. coli	$bla_{\text{CTX-M-14}}$	Negative
E. coli	$bla_{\text{CTX-M-15}}$	Positive
E. coli	$bla_{\text{CTX-M-27}}$	Negative
E. coli	$bla_{\text{TEM-3}}$	Negative
K. pneumoniae	$bla_{\text{CTX-M-15}}$	Positive
K. pneumoniae	$bla_{\text{CTX-M-55}}$	Positive
K. pneumoniae	$bla_{\text{SHV-18}}$	Negative
P. aeruginosa	No ESBL	Negative

Specificity test for qPCR

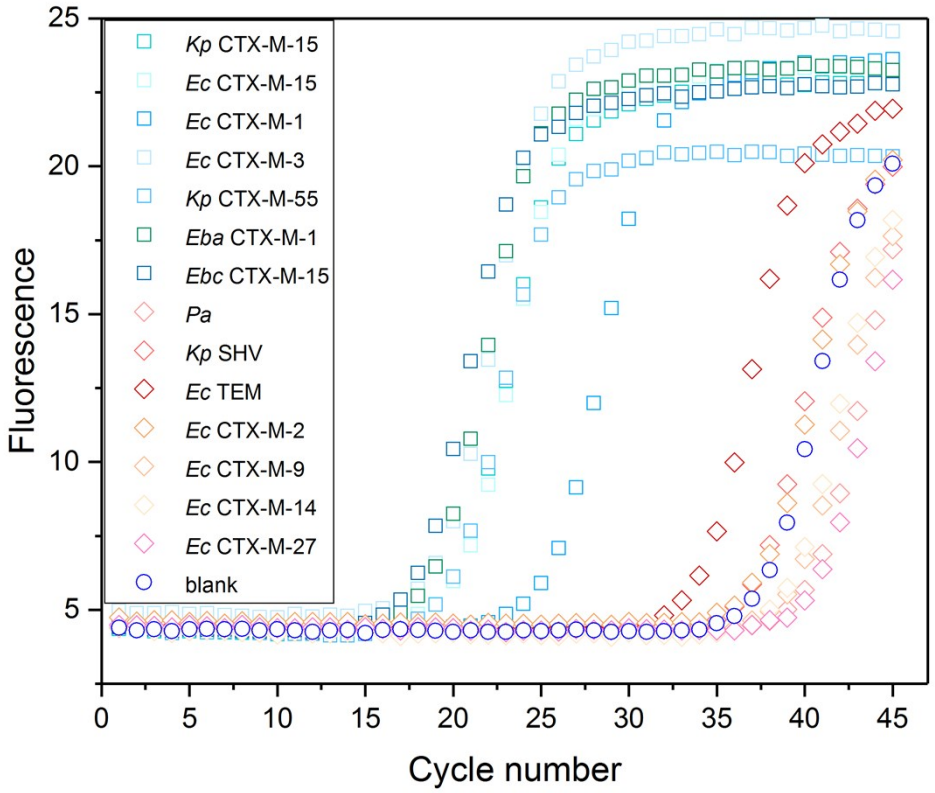

Figure 1: Specificity test for qPCR with DNA in 0.1 ng μL^{-1}. *Kp*: *K. pneumoniae*; *Ec*: *E. coli*; *Eba*: *E. asburiae*; *Ebc*: *E. cloacae*; *Pa*: *P. aeruginosa*; CTX-M-number: with $bla_{\text{CTX-M-number}}$ gene; SHV: with bla_{SHV} gene; TEM: with bla_{TEM} gene; blanl: non target control.

1 C. Kober, R. Niessner and M. Seidel, *Biosens. Bioelectron.*, 2018, **100**, 49–55.

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3 S. Lengger, J. Otto, D. Elsässer, O. Schneider, A. Tiehm, J. Fleischer, R. Niessner and M. Seidel, *Anal. Bioanal. Chem.*, 2014, **406**, 3323–3334.