Detection and Identification of Human Pathogens: From Classical DNA Microarrays to Lab-on-a-Chip Systems

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Introduction In clinical practise early on identification of infection causing microbes is the crucial requisite for a fast and optimally targeted infection treatment. In contrast to conventional diagnostic methods lasting at least 24 hours due to their requirement for microbial growth, DNA-based methods meet the needs for a fast, reliable and thereby life-saving diagnosis. A classical (fluorescence-readout) microarray for the identification of the most frequent pathogens in community and hospital acquired infections was established. It includes gram positive cocci, different genera of the family *Enterobacteriaceae*, Non-fermenter and clinical relevant *Candida* species. Efficiency and specificity of the pathogen identification microarray (Path-ID-chip) was tested with numerous clinical isolates. The experimental procedure includes bacterial DNA isolation from blood, multiplex PCR, fluorescence labelling (Cy5-dCTP) by a primer extension step and subsequent microarray hybridization. Relying on the experiences from our Patho-ID-chip we recently started to develop a Lab-on-a-Chip (LOC) device comprising a miniaturized PCR device and an electrode-array enabling label-free capacity measurement.

Procedure (classical microarray)

Isolation of bacterial/fungal DNA from whole blood samples

DNA amplification by multiplex PCR followed by Primer extension

Hybridisation of labeled DNA targets onto Path-ID-chip

Chip Scanning, GenePix image analysis and statistical evaluation

Chip results (pattern recognition algorithm → software "Seps-O-Pert")

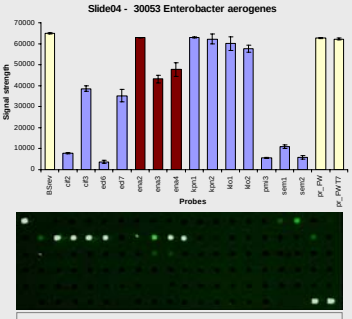

Fig1: Specificity test 16S rRNA probes: The specificity of various probes was examined with the species *E. aerogenes*. Specific probes are shown in red, unspecific in blue and positive controls (for hybridisation and PCR amplification respective) in yellow. All unspecific signals result from probes for members of the family *Enterobacteriaceae*.

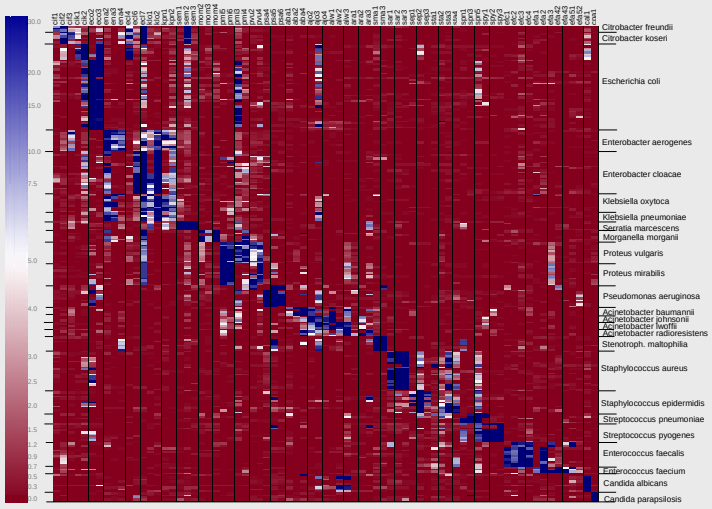

Fig. 2: Results of 241 microarray hybridisation experiments as a heatmap. Columns correspond to probes and rows correspond to hybridizations. Colours correspond to signal values so that red indicates no signal succeeding to white for low signal strengths to blue indicating strong signal values (shown by the colour bar on the left side of the figure).

Microarray Data Analysis

Due to large sequence homologies between some of the species investigated, not every probe yielded a species-specific signal. However, combining the signal information from a combination of different probes, each species was identifiable. We followed a supervised learning approach including the following steps:

- Flag spots below threshold and set the signal as NA
- Calculate median of spot-replicates
- Rank-transformation of the data
- k-Nearest Neighbour Classification

The dataset consisted of 241 hybridisations of 26 different species. In a leave-one-out cross-validation of the rank-transformed dataset, k-NN classified 96,7 % of all species and 100 % of all genera correctly. The misclassified species were those with the largest sequence similarity.

Seps-O-Pert, an in-house developed analysis software, automatically generates the output shown in Fig. 3 from a GenePix result file after applying the analysis steps described above.

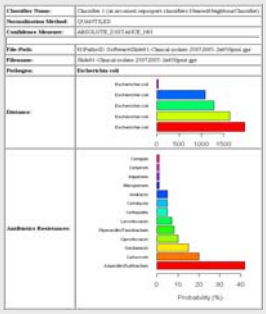

Fig. 3: Final output of the Seps-O-Pert software including the identified pathogen and probabilities for its antibiotic resistance.

PCR-Chip (LOC)

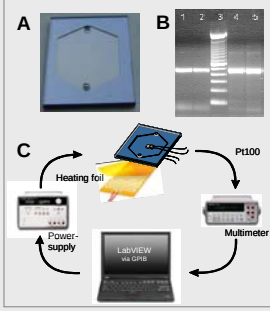

Fig. 5: **A** - photograph of the silicon-based PCR chip which was fabricated using photolithography; chip dimensions 15x20x1mm with a reaction volume of 25 µL. **B** – Agarose gel electrophoresis of 16S rRNA PCR product (497 bp) amplified from *E. coli* DNA.. PCR chip cycled in *in-situ* PCR device (lane 1+2), conventional PCR reaction in tubes (lane 4+5). **C** – PCR setup scheme: On the PCR chip a thermofoil heater and 2 temperature sensors (PT100) are packaged. The heater is powered with the programmable DC voltage supply and the 2 resistance values of the temperature sensors are read out with two measurement instruments. The instruments are connected via GPIB to a PC, where a LabView program controls the PCR.

Lab-on-a-Chip (LOC)

In the field of on-chip biological analysis systems a lot of effort is put into the development of integrated microfluidic systems. Such micro scale devices allow point-of-care diagnostics. To achieve an easy-to-use and cheap handheld device optical detection methods are replaced by label-free capacitive DNA sensing.

We demonstrate the implementation of a hybrid analysis system which consists of a DNA amplification – and DNA detection module in separated chambers which are connected via a simple fluidic channel.

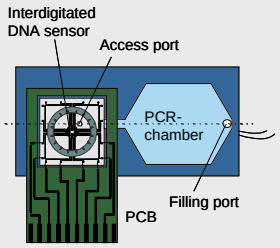

Fig. 4: Scheme of the finally aimed total microsystem for DNA amplification and label-free DNA detection. Details on PCR chamber + DNA sensor are shown in Fig.5, Fig. 6 resp.

Electrode-Chip (LOC)

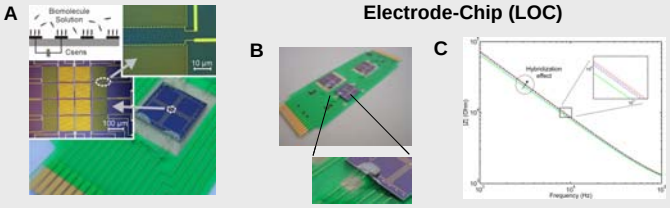

Fig. 6: **A** – Electrode chip bonded on a PCB: On the chip (size:8x8mm²) are 16 interdigitated gold electrodes integrated with a line-space distance of 500 nm. The IDEs are functionalized with diffferent bacterial-species specific DNA probes. Insert scheme (top left) illustrates the capacitive sensor principle. **B** – Electrode chip bonded on a PCB (detail). **C** – Measured impedance spectrum during hybridisation of *E. coli* target on an electrode chip functionalized with an *E. coli* probe. Measurements at 0 mins (green), 9 mins (blue), 18 mins (red), 27 mins (black).

CONCLUSION From our results we conclude that DNA microarrays are useful tools for rapid detection of infectious bacteria particularly when the method is adapted to specific clinical scenarios. In combination with a direct isolation of bacterial DNA from human blood samples this method will represent a serious alternative to conventional determination procedures used in hospitals. Experiments with pathogen-spiked blood showed that 10 *E. coli* and 10^5 *Staphylococcus aureus* cells per mL can be detected on our fluorescence-based Path-ID-chip. The used pathogen probes were specific for the desired species and gave typical hybridisation patterns which allow precise identification of microbes using an automated software platform (see "Microarray Data Analysis"). Experiences gained along establishing our Path-ID microarray have been the basis for the recent Lab-on-a-Chip development, comprising a total microsystem for DNA amplification and label-free DNA detection.

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