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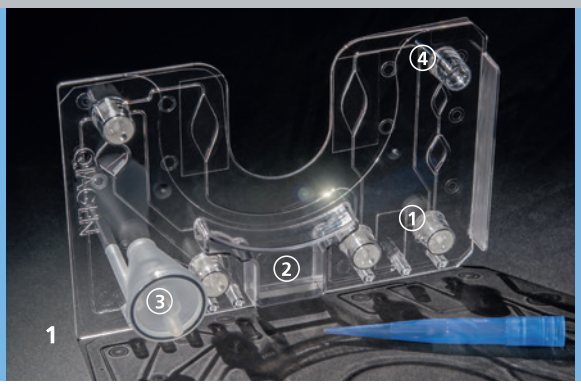

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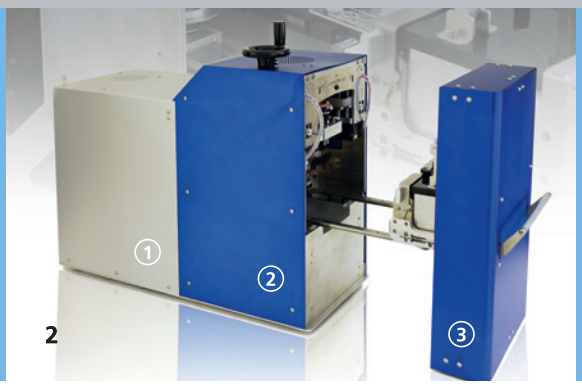

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1 *Chip molecular diagnostics. (1) turning valves, (2) waste, (3) sample inlet and (4) outlet.*
2 *Instrument. (1) electronic control unit, (2) operating device and (3) drawer with chip and reagent container.*

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IN COOPERATION WITH

QIAGEN GmbH

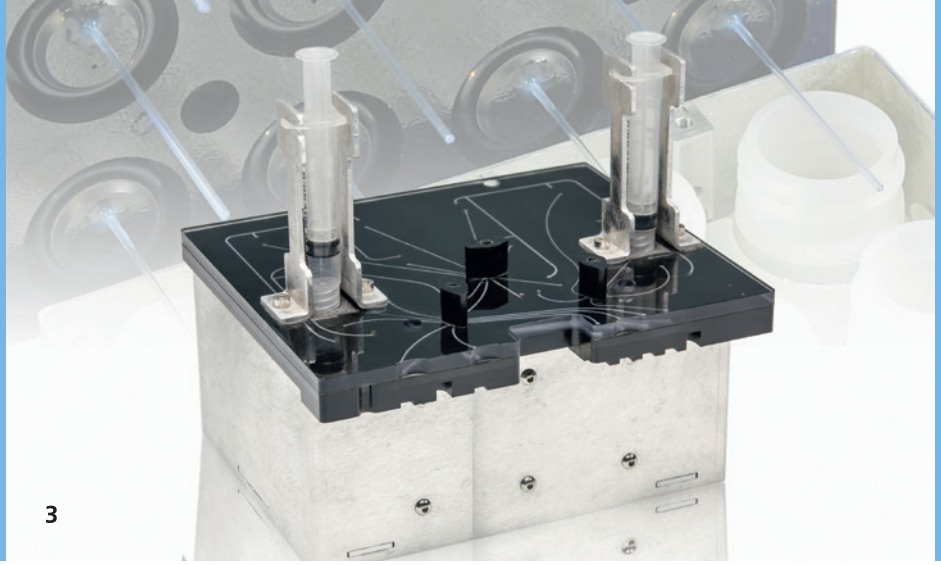
POINT-OF-CARE DIAGNOSTIC SYSTEM FOR NUCLEIC ACIDS TESTING

Introduction

Early diagnosis followed by personalized efficient therapy of infectious diseases (e.g. respiratory diseases, meningitis, sepsis) can lead to considerable reduction of costs in health care. Point-of-care testing (POCT) can provide early detection since this kind of decentralized analysis can be done by untrained personnel at any time. Other advantages of such automated miniaturized Lab-on-a-Chip (LoC) systems are reduction of time and reagents, elimination of cross-contamination and enhanced reproducibility due to eliminated user interference. These new systems will establish themselves on market only when sensitivity and specificity meet clinical requirements.
The goal of the joint "Stormbreaker" project was to develop a new microfluidic-based system which allows performing complex molecular diagnostic process steps (sample extraction, target concentration, amplification, and detection) with a cost-efficient LoC system. Pathogens of various infectious diseases like e.g. atypical pneumonia can be analyzed from nasopharyngeal swab samples within this system.

Competences

Within this project Fraunhofer IMM was responsible for the development of the microfluidic system for the analysis of nucleic acids of pathogens within sputum samples. This system comprises a polymeric disposable analysis chip and an instrument that runs the assay on the chip automatically. In close cooperation with QIAGEN Fraunhofer IMM developed a disposable chip that contains structures for sample mixing, lysis, solid phase extraction (SPE) using magnetic beads, nested PCR amplification, and hybridization of nucleic acids with labeled beads for subsequent LiquiChip detection. The chip design, first manufactured and



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tested by rapid prototyping, was finally realized by injection molding technique. Especially the Fraunhofer IMM standard turning valves were transformed to a 2K injection molding compatible design.
In parallel, the instrument to run the chip has been developed. Fluid control via syringe pumps and turning valves was realized to allow precise transport, metering and merging of buffers needed. All chemicals needed are either stored dry on the single use disposable microfluidic chip or in a separate reagent storage cartridge that allows performance of 24 analyses.
Usability and robustness of system handling were being center staged when establishing the LabView controlled electronics and software.
After sample intake by the user the chip is introduced into the system that performs all necessary steps fully automatically.

System design

The developed system comprises of an injection molded disposable chip, a reagent cartridge and the instrument. The process is controlled via LabView on a conventional PC. Various sensors within the system check operations done by the user and inform the control software accordingly. Supported by instructions on the screen and optical signals the user is guided through the whole process. While in this demonstrator the first mechanical movements to connect chip and instrument are still done manually by the user, the next generation will do this also automatically.
Chip and reagent cartridge have to be inserted into the loading drawer of the system by the user after introducing up to 1 ml sample to the lysis chamber on the chip. Loaded drawer is pushed into the system and chip, reagent cartridge and instrument get connected. After heat supported chemical-enzymatic lysis within the lysis chamber, the sample previously mixed with lysis buffer, is transported to the SPE chamber. Within the SPE chamber magnetic beads get mixed with the slowly passing sample to isolate the RNA. After the washing steps, the RNA gets eluted and transported to the first PCR chamber where the first step of a nested RT-PCR takes place. Nested-PCR was chosen to reach high sensitivity. Two µl of this reaction are then transferred to the next PCR chamber for the second amplification step. Finally, an aliquot of this reaction gets mixed and hybridized with fluorescent labelled beads for the subsequent LiquiChip detection.
The core of the device is a construction based on rotating heating bars that allows for fast cycling times. The resistive heaters have constant temperatures, one for each of the three temperatures of the PCR cycle. An additional non-heated position is used for cooling. Heating is done from both sides of the chip. With this concept heating and cooling rates of up to 3.5 K/s can be reached. To further enhance heat transfer within the PCR chambers sample is actively mixed using magnetic stir bars.
Transport of fluids is realized by two syringe pumps integrated on the reagent cartridge. By pushing the loading drawer into the instrument syringes get connected to linear drives. The minimal pumping volume is 0.12 µl which can be dispensed precisely into the chip. All fluids are guided to the addressed reservoirs via on-chip integrated turning valves. For final detection the system is connected via a tube to a QIAGEN LiquiChip Instrument.

Summary

In this joint project Fraunhofer IMM and QIAGEN GmbH developed a Molecular Diagnostic System which allows to perform all diagnostic process steps for pathogen analysis from swab samples within a cost-efficient LoC system.
Parallel development of disposable analysis chip and corresponding instrument led to a system which can be adapted to identify various infectious diseases and therefore will allow to open completely new markets for in-vitro diagnostics.

This work was partly funded by the Bundesministerium für Bildung und Forschung (BMBF (031599B-IMM), 05/2009 to 07 (Qia) oder 04 (IMM)/2012.

3 *Reagent container with syringe pumps.*