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AUTOMATED SYSTEM FOR MOLECULAR DIAGNOSTIC FROM SWAP SAMPLES

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IN COOPERATION WITH

QIAGEN GmbH

Introduction

The future of diagnostics will lead to systems which can be used in decentralized settings. By speeding up the therapy through early and direct diagnosis the expenses in health care can be decreased. Point-of Care Testing (POCT) systems can be used by untrained personal and do not need special requirements in the testing environment. In the future, such systems may be found in practitioners' rooms, in satellite labs of hospitals, or also a usage in mobile medical units is possible.
The "ne[X]D" project was initiated to allow gynecologists to detect Neisseria and Gonorrhea infections from samples of their patients directly during the examination by taken vaginal swabs.
The final project goal is to develop a microfluidic-based diagnostic system, which covers sample processing, extraction, amplification, and detection. In addition, control concepts for lysis, amplification, as well as self-check of hardware modules and instrument have to be implemented.
Cost-efficiency was the primary focus, while the performance in regards to sensitivity and specificity should remain comparable to the standard reference test systems.

Competences

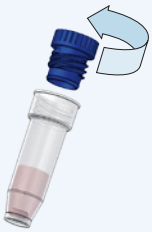
The project was initiated by a consortium of the QIAGEN GmbH Hilden, QIAGEN Lake Constance GmbH (formerly ESE GmbH) and Fraunhofer IMM. Within this project Fraunhofer IMM was responsible for the development of the fluidic components (from initial CAD designs, gap vents, membrane stop structures), device validation (from initial tests to parameter optimization), and aspects of chip production (mask-assisted laser bonding).
Right from the start the project consortium aimed at an injection molded chip for serial integration of the processing structures for

Fig. 3: Handling Workflow for an Assay

1. Sampling

Sample Uptake with a Swab

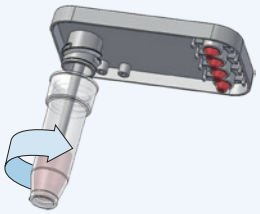
2. Sample Transfer to Collection tube



Open Tube

Sample release in Lysisbuffer

3. Sample to Chip Connection



Screwing the tube to the Chip

4. Device Loading

Placing the chip and start processing

sample mixing, lysis, reverse sample purification using magnetic beads, and multiplex PCR amplification in order to integrate the complete workflow.

The project requirements were straight from the beginning product-oriented for cost-efficient manufacture, robustness in performance, and user-friendly handling. New features in chip functionality like squeeze-valves or inmolded hydrophobic fluid-stop membranes (2k process), where established.

One out-standing feature of the system is that the assay workflow is enabled only by one buffer solution, which is used to lyse the sample and to rehydrate the dried multiplex PCR reactions after magnetic bead mediated lysate clearing (reverse purification).

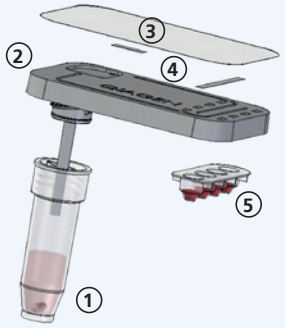


Fig. 2: Chip design

1) Sample collection tube filled with processing buffer, a magnetic stirrer and wax-embedded magnetic beads.

2) Basic chip with inmolded hydrophobic membranes (4).

3) Laser bonded cover foil.

5) 4-well PCR reaction tube strip with dried PCR assay reagents.

Setup

The ne[X]D system comprises of an injection molded disposable polymeric chip, a sample collection tube and an instrument being capable to perform fully automated sample processing in the chip.

The manual handling steps are designed for untrained user to perform the assay (Fig. 3). Several integrated controls will guarantee a valid diagnostic result.

Once the assay is started, the sample transferred in the lysis buffer will get lysed by a boiling step while continuous magnetic stirring. Meanwhile wax-embedded magnetic beads are released by melting. The wax forms an evaporation barrier and the magnetic beads separate into the water phase. After lysis, the solution is actively chilled and sample precipitates are bound to the magnetic beads. In the following magnetic separation the lysate is cleared by magnetic removal of the precipitates. The nucleic acids are remaining in the supernatant, which then is pumped from the collection tube into the basic chip and finally fills the amplification wells. The rehydration of the PCR assay reaction is done actively by magnetic agitation through an incorporated foil magnet. As next step a conventional real time multiplex PCR is carried out in each well and the fluorescence changes for each PCR cycle are measured by ESElog fluorescence detectors attached to a motor-driven linear rail (Fig. 4).

The heating is done by peltier elements. Especially the PCR block was optimized in its design by thermal simulations. Therefore these elaborated Peltier heaters can reach heating and cooling rates of up to 3.5 K/s.

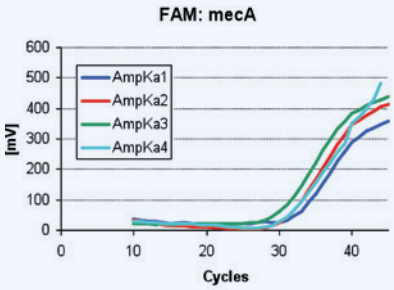


Fig. 4: Amplification plot of a PCR reaction detecting the methicillin gene from Staphylococcus aureus. Fluorescence changes of the four different amplification wells are displayed in dependence of the PCR cycle.

Summary

The QIAGEN GmbH and the Fraunhofer IMM developed in close cooperation a microfluidic-based Molecular Diagnostic System, which allows for performing all diagnostic process steps for pathogen analysis from swab samples to result.

This system reflects outstanding features which range from simple handling by untrained users to very cost-effective design. This platform for microfluidic-based molecular assays may be easily adapted for various infectious diseases and therefore can address the future markets for decentralized in-vitro diagnostics.

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