

Development of an Automatic Chip-based Detection of mRNA

L. Solli¹, A. Gulliksen¹, T. Baier², R. Gransee², T. Hansen-Hagge², M. Mielnik³, I-R. Johansen³, L. Furuberg³, L. Rigger⁴, P. Koltay⁵, C. Martin⁶, H. Keegan⁶, J. O'Leary⁶ and F. Karlsen¹

¹NorChip AS, Klokkestua, Norway, ²Institut für Mikrotechnik Mainz GmbH, Germany, ³SINTEF ICT, Oslo, Norway, ⁴Department of Microsystems Engineering (IMEK), Albert-Ludwigs-Universität Freiburg, Germany, ⁵BioFluidiX GmbH, Freiburg, Germany, ⁶Coombe Women & Infants University Hospital, Dublin, Ireland

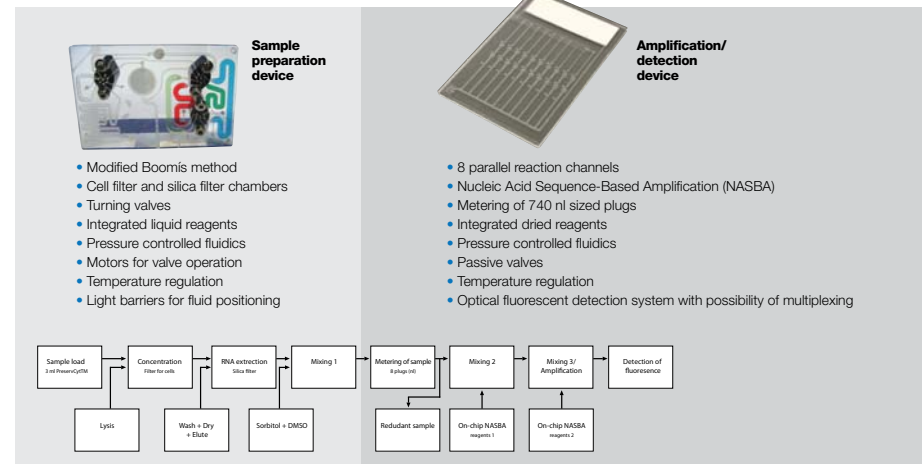


A direct link exists between the different types of disease and the presence or absence of single or multiple mRNA transcripts. Expression patterns of different mRNA transcripts simultaneously may be related to increased risk of cancer, the presence of pre-cancer, lack of immune defense, increased risk of heart failure, specific allergies or the presence of active infection.

This project is developing an automated point-of-care platform for on-chip sample preparation, nucleic acid extraction, amplification and fluorescent detection. All necessary reagents are stored on chip. After insertion of the chip into the device and loading of the sample onto the chip the procedure proceeds without further user interaction.

Two modular devices, one for sample pretreatment and one for amplification/detection are currently being tested individually before integration into a single setup. Molecular biomarkers for Human Papillomavirus (HPV) are used as a model system in the platform.

Description of work:



Results:

- The device has been successfully tested on various cell lines and clinical samples which express HPV mRNA.
- Device performance was validated by NASBA of the eluate using the PreTect® HPV-Proofer kit.
- The device has been successfully tested on dilution series of CaSki cells (table) and clinical samples positive for HPV16 (graph) with primers and probes integrated on chip.

Clinical sample	HPV16	U1A
1	Positive	Positive
2	Positive	Positive
3	Positive	Negative
4 (Neg. control)	Negative	Negative

Cell line/ Cell count	CaSki	MS751	HeLa
50.000	HPV16 Positive	HPV45 Positive	HPV18 Positive
5.000	HPV16 Positive	HPV45 Positive	HPV18 Positive
500	HPV16 Positive	HPV45 Positive	HPV18 Positive
50	HPV16 Positive	HPV45 Positive	HPV18 Positive
5	HPV16 Positive	HPV45 Negative	HPV18 Positive

No. of extracted cells	No. of cells in the macro scale well	No. of cells in the reaction on	Spot volume (nl)	Results singleplex mix on chip	Results macro scale
50.000	5.000	125	125	8/8	1/1
50.000	5.000	125	100	6/6	1/1
5.000	500	12,5	125	4/4	1/1
5.000	500	12,5	100	7/7	1/1
500	50	1,25	125	8/8	1/1
500	50	1,25	100	7/7	1/1
50	5	0,125	125	4/6	1/1
5	0,5	0,0125	100	0/5	1/1

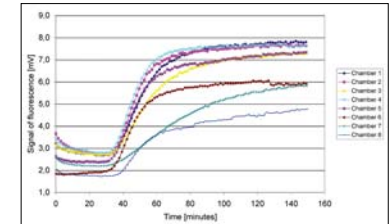
Conclusions and outlook:

A combined system is intended to serve as a point-of-care system for the detection of gene activity directly in a physician's office, thus avoiding time consuming analysis by specialized laboratories. The system is not limited to cervical samples and opens the way for a range of applications.



The system can be adapted to other fields of operation where analyses of complex biological samples in the field in a short time are desirable. This includes for example:

- Food analysis
- Personalized medicine/ POC
- Forensics



Acknowledgements:

This work was partly funded by the European commission under contract no. IST-NMT-CT-2005-017319