Automated chip-based extraction of HPV mRNA from cervical samples

T.Baier[1], T.Hansen-Hagge[1], R.Gransee[1], A.Crombé[1], S.Schmahl[1], C.Paulus[1], K.S. Drese[1], P.Grønn[2], L.Solli[2], I.M. Falang[2], A. Karlgard[2], F.Karlsen[2], C.Martin[3], H.Keegan[3], J.O'Leary[3], L.Furuberg[4],
[1]Institut für Mikrotechnik Mainz GmbH, Germany, [2]NorChip AS, Klokkarstua, Norway, [3]Coombe Women&Infants University Hospital, Dublin, Ireland, [4]SINTEF ICT, Oslo, Norway

Institut für Mikrotechnik Mainz GmbH

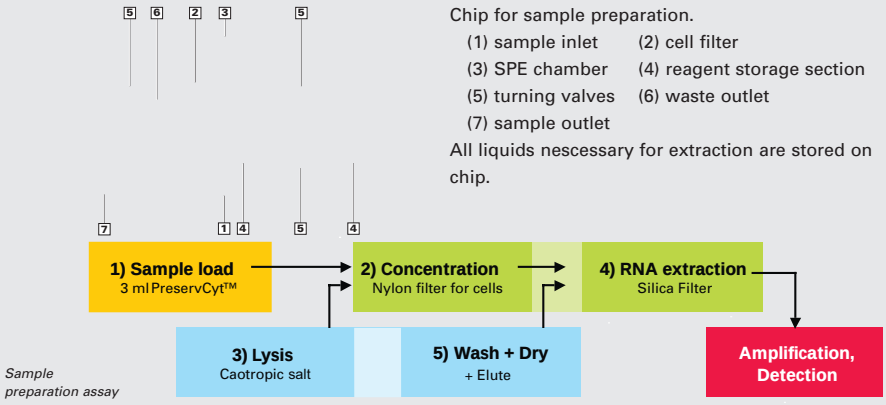
Contact: info@imm-mainz.de
www.imm-mainz.de

- **mRNA is a valuable bio-marker** for detection of many common diseases.
- Presence of mRNA indicates biological activity of an agent and avoids false positive results if marker activity is required.
- mRNA detection allows early detection of cancer and other diseases.
- New biomarkers for various kinds of diseases emerge regularly.

- **Cervical cancer** is one of the most frequent types of cancer among women worldwide [1].
- Nearly all cases of this cancer are directly linked to previous infection with one or more types of human papilloma virus (HPV) [2].
- Early diagnosis of persistent HPV infections and subsequent treatment prevent onset of the disease.

- Against this background, a **chip-based automated platform** for the extraction of nucleic acids, including HPV mRNA from cervical smears as a model system, has been developed.
- The device accepts 3 ml of a suspension of fixated cervical smear cells in PreservCyt™.
- After insertion of sample and chip into the device the extraction procedure proceeds without further user interaction.

Experimental Set-up



Sample preparation assay

Chip for sample preparation.
(1) sample inlet (2) cell filter
(3) SPE chamber (4) reagent storage section
(5) turning valves (6) waste outlet
(7) sample outlet
All liquids nescessary for extraction are stored on chip.

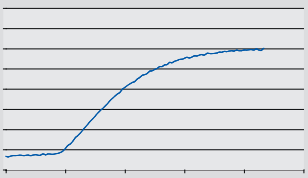
Operating device for the automated extraction of mRNA from cervical samples, containing two syringe pumps for fluid actuation, motors for valve operation, a heater and electronics for device control. The device is operated by a customised LabView program.

Results: Successful Extraction and Validation

- The device has been successfully tested on various cell lines (HeLa, Ms751, CaSki) which express HPV mRNA.
- Device performance was validated by Nucleic Acid Sequence Based Amplification (NASBA) of the eluate, using the PreTect® HPV-Proofer kit (NorChip AS, Klokkarstua, Norway).
- A sensitivity study reveals amplifyable eluate down to 5 cells.
- First results show sucessfull extraction for clinical cervical smear samples also.

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

Results of sensitivity study of the sample preparation procedure.
Samples of different cell lines containing from 5 to 50.000 cells were processed.



Sample NASBA amplification curve for HeLa cell lines showing fluorescence intensity vs. Time.

Conclusions and Outlook

- The sample preparation device presented here was developed within the project MicroActive. It is planned to integrate this device with a second automated instrument for on-chip parallel NASBA amplification and detection of several mRNA targets exemplified by different HPV types [3,4].
- This combined system may thus serve as a point of care system for the detection of gene expression directly in a physician's office, avoiding the often delayed analysis by a specialized laboratory.
- However, the device is not limited to cervical samples and opens the way for a wide range of similar sample preparation applications.
- With small modifications this system can be adapted to other fields of operation where it is desirable to analyse complex biological samples "in the field" and on a short timescale.
- **This includes for example**
  > Foodstuff analysis / animal feed control
  > Personalised Medicine, Point-Of-Care
  > Forensics

References/Acknowledgements

[1] World Health Organization, "Fact sheet No. 297: Cancer", (2006)
[2] J. M. M. Walboomers, et al., "Human papillomavirus is a necessary cause of invasive cervical cancer worldwide.", J. Pathol., 189, 1219, (1999).
[3] A.Gulliksen, et.al., "Parallel nanoliter detection of cancer markers using polymer microchips", Lab Chip, 2005, 5, 416-420
[4] L. Furuberg, et.al., "RNA amplification chip with parallel microchannels and droplet positioning using capillary valves", Microsyst.Technol (2008) 14:673681.

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