Automated chip-based extraction of HPV mRNA from cervical samples

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- 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.

**Experimental Set-up**

[Diagram showing the process of sample preparation and extraction]

1. **Sample load**: 3 ml PreservCyt™
2. **Concentration**: Nylon filter for cells
3. **Lysis**: Caotropic salt
4. **RNA extraction**: Silica Filter
5. **Wash + Dry + Elute**: Amplification, Detection

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 amplifiable eluate down to 5 cells.
- First results show successful extraction for clinical cervical smear samples also.

<table>
<thead>
<tr>
<th>Cell line / cell count</th>
<th>CaSki</th>
<th>MS751</th>
<th>HeLa</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.000</td>
<td>HPV16: positive</td>
<td>HPV45: positive</td>
<td>HPV18: positive</td>
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<td>HPV18: positive</td>
</tr>
<tr>
<td>5</td>
<td>HPV16: positive</td>
<td>HPV45: negative</td>
<td>HPV18: positive</td>
</tr>
</tbody>
</table>

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

**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**


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