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# Lab-on-a-chip technology for clinical diagnostics: the fertility chip

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**In the 1990s the term micro total analysis systems (µTAS) was introduced to describe a complete microsystem which integrates sample handling, analysis and detection into a single device, also called Lab-on-a-Chip (LOC) device (1). The LOC concept defines the scaling down of a single or multiple lab processes into a chip format with dimensions as small as a stamp. Scaling down offers many advantages, such as less sample, reagent and waste volumes, faster analysis, integration of many analytical processes within one device, lower cost, to name a few, but first of all an easy handling. These advantages meet the actual demands of clinical laboratories, which are dealing with an increasing workload and decreased funding. Our group showed previously these advantages of the LOC technology for blood electrolyte determinations in clinical diagnostics (2).**

**Furthermore, microfluidic dimensions (10 - 100 µm) equal the size of cells, making these devices very suitable for the analysis of many different biochemical processes even on a single-cell level. Hence, there are many reasons why microtechnology is advantageous compared to existing conventional analysis methods, especially in the case of cellular based assays, to understand how cells react in a certain environment, to a certain drug or in contact with other cell types. Different cell manipulation methods (*e.g.* sorting, detachment, staining, fixing, lysis) can be integrated on one chip, less sample is needed ideally when only a few cells are available (*e.g.,* primary cells) and the dimensions favour single-cell analysis. Furthermore, optical detection techniques can be automated and in some cases be replaced by electrical on-chip detection methods. Moreover, development of cell arrays, which are analogous to DNA or protein arrays, offer the possibility for high-throughput screening. Recent technological developments enable detailed cellular studies, defining a new concept: Lab-in-a-Cell. In this concept the cell is used as a laboratory to perform complex biological operations. Micro- and even nanotechnological tools are employed to access and analyse this laboratory and interface it with the outside world. In the present manuscript we will summarize our recent efforts to demonstrate the advantages of LOC technology to study cells for clinical diagnostics by working on a fertility chip as an example.**

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**Semen analysis**

The fertility chip is developed to improve the care of the couple who is childless by default. Nowadays more than 30 000 couples visit the fertility department of a hospital since they face problems with getting pregnant (3). There the fertility of the man and woman will be determined with an exploratory fertility research. For the man this implies an anamnesis and a semen analysis. For the semen analysis, the man has to bring his semen for assessment to the hospital laboratory, where the motility and concentration of spermatozoa in his semen will be determined. This procedure is not only embarrassing for the man, but it is also time-consuming, labour intensive and not accurate due to the manual assessment (4). Furthermore, before a statement about the fertility of the man can be made, at least three analyses have to be performed (5). A better alternative for the current procedure is a portable system that enables the man to perform several objective and reliable measurements at home. At the moment some at-home tests already exist to determine the fertility of the man, but these rely on subjective interpretation by the man and only give qualitative information about the semen quality (6). For a treatment decision by the gynaecologist quantitative information is necessary. Our fertility chip will give this information and can be a good alternative of the current semen analysis in the laboratory.

**On-chip determination of the spermatozoa concentration**

We currently focus on the development of a LOC for the assessment of the semen quality. Such disposable microfluidic chip will be ultimately used in combination with a handheld measurement system and management software. In our first approach a microfluidic chip has been developed that can be used to determine the concentration of spermatozoa in semen (7). With cleanroom fabrication techniques this glass-glass chip has been made, which comprises a 18 µm deep microfluidic channel. At the tapering of the microfluidic channel to a width of 38 µm two platinum electrodes are positioned at one side of the channel (see figure 1). These electrodes are used for the detection of single spermatozoa in the semen by a technique

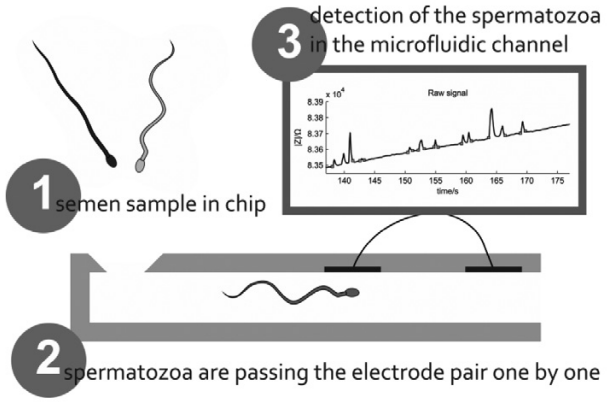


**Figure 1.** Schematic representation of the detection of spermatozoa in semen using a microfluidic chip. The black lines indicate the electrodes in the microfluidic channel.

that is known as microfluidic impedance cytometry. The electrical impedance is measured at a specific frequency between those electrodes and when a cell passes the electrode pair, it changes the average dielectric properties of the measurement volume, resulting in an impedance change. In this way every spermatozoon that passes the electrode pair is counted. Since the impedance change of each event is also dependent on the size of the cell passing the electrode pair, we were able to distinguish between HL-60 cells, spermatozoa and 6 µm polystyrene beads (7). To determine the concentration of spermatozoa in semen, we used a comparable method as used in conventional flow cytometry. A known concentration of polystyrene beads was added to the semen sample and by flowing them through the chip by means of hydrostatic pressure, we showed that we were able to determine the spermatozoa concentration of boar semen in the range from $2{\cdot}10^6$ to $60{\cdot}10^6$ /mL (7).

**Motility assessment on-chip**

Another parameter that is important to assess the semen quality is the motility of the spermatozoa. For the purification of the 'best' spermatozoa out of a semen sample for assisted reproductive technologies (*e.g. in-vitro* fertilisation, intracytoplasmic sperm injection) Cho and co-workers developed a LOC approach (8, 9). In this approach two microchannels combine to one separation channel, where the two laminar flows join from both channels. Only motile spermatozoa have the ability to cross the flow barrier and will end up in

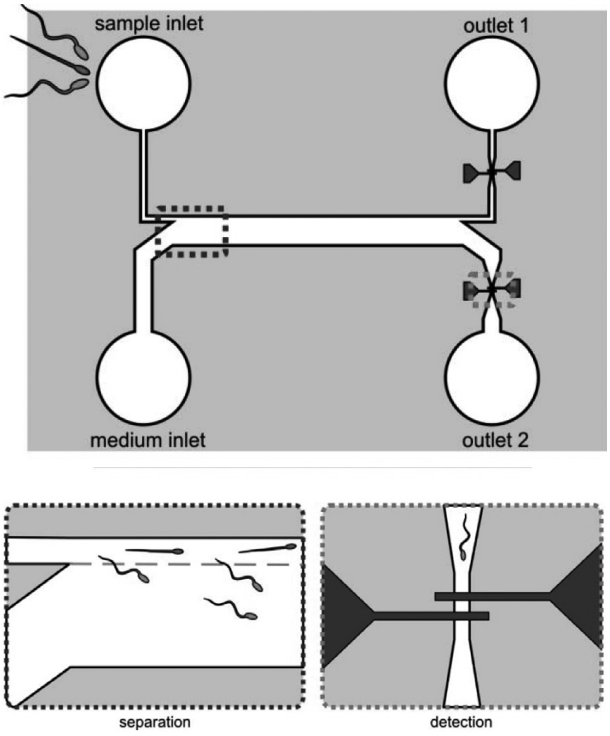


**Figure 2.** The LOC device that was used for the motility determination. It consists of two parts: separation and detection. In the 5 mm long, 18 µm deep separation channel the motile spermatozoa are able to cross the laminar flow barrier and arrive at outlet 2, while the immotile cannot cross and end up at outlet 1. At both detection regions the cells are detected using electrical impedance measurements (10).

the other channel, thereby creating a sample of motile spermatozoa which can be used. For the determination of the motility we use the same principle as mentioned before and we combine this with the electrical detection of spermatozoa at the two outlet channels (see figure 2). The detection of the spermatozoa in both outlet channels is done with the same configuration as used for determining the concentration on-chip. We propose a new model for the determination of the separation efficiency of motile spermatozoa from the semen sample (10) and compare these simulated results with experimental data, which show good agreement. In this way we were able to distinguish between samples with motile and immotile spermatozoa.

**Outlook**

Parameters of the semen quality that are normally determined in the hospital laboratory can be measured with LOC devices in an objective way making point-of-care diagnostics possible. With LOC devices a shift toward at-home analysis can be made, thereby reducing the costs and making it more patient friendly. Additionally, several measurements can easily be performed such that a better statement of the semen quality is obtained. This information can lead to a better treatment decision of the gynaecologist, thereby improving the care of the couple who are childless by default.

The detection of cells with electrical impedance measurements in a microfluidic chip is not only restricted to spermatozoa in semen, but also other cells suspended in a fluid can be counted. The only condition is that the dielectric properties of the cell are different than those of the medium. Therefore microfluidic impedance cytometry can also be used for other medical diagnostic tests, like for instance a 3-part differential count of the leukocyte population (11, 12) and the detection of infected cells (13).

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VUmc

# Pyridoxine afhankelijke epilepsie

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**Andrew Hunt *et al* rapporteerden in 1954 over een jongetje die kort na zijn geboorte sterke convulsieve aanvallen kreeg (1). Deze aanvallen reageerden niet op gebruikelijke anti epileptica, maar wel op de intra musculaire toediening van pyridoxine (vitamine B6). Het kind bleef vrij van aanvallen door dagelijkse orale inname van pyridoxine, en deze klinische entiteit werd pyridoxine afhankelijke epilepsie (PDE) genoemd. Lange tijd is PDE een puur klinische diagnose gebleven, waarbij het heroptreden van aanvallen na het stoppen van de pyridoxine suppletie, een van de diagnostische criteria was. In 2005 is ontdekt, in onderzoek waarin onze groep een belangrijke rol speelde, dat voor de overgrote meerderheid van patiënten, hun PDE werd veroorzaakt door een defect in de lysine afbraak met een autosomaal recessief overervings patroon (2).**

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