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The MEDICALIP Project: Toward the screening of the cytomegalovirus

Le projet MEDICALIP : vers le dépistage du cytomegalovirus

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Abstract

Since rubella vaccine was established, cytomegalovirus infection has become the most frequent cause of congenital infections. The French Health Authority is urging a diagnosis at birth for newborns. Since no screening device is commercially available, a consortium has been established to set-up an original device. The project has been funded by the French National Research Agency. The device consists of a disposable cartridge containing the biological sample and the reactive liquids required for immuno-fluorescence detection on a functionalized surface. It also consists of a mobile reader used to drive the fluids onto the biosensor and to ensure the optical measurement. Positive and negative samples can be discriminated with a fluorescence intensity ratio of 3.

Keywords: Cytomegalovirus; Immuno-fluorescence; Mobile medical device; Screening

Résumé

Depuis la mise en place de la vaccination contre la rubéole, l'infection à cytomegalovirus est devenue une des principales causes d'infection congénitale. La Haute Autorité de santé préconise un diagnostic des nouveau-nés. Puisque aucun système de dépistage n'existe, un consortium a été établi pour concevoir dispositif original. Le projet a été financé par l'Agence nationale de la recherche. Le dispositif consiste en une cassette jetable contenant l'échantillon biologique et les réactifs nécessaires à une réaction d'immunofluorescence sur une surface fonctionnalisée. Il consiste également en un lecteur/actuateur qui gère les fluides et assure la lecture optique. L'intensité de fluorescence détectée pour des échantillons positifs est trois fois supérieure à celle obtenue avec des échantillons négatifs.

Mots clés : Cytomegalovirus ; Dépistage ; Immunofluorescence ; Dispositif médical nomade

1. Introduction

Since the rubella vaccine was established, the cytomegalovirus infection has become the most frequent cause of congenital infections, particularly in premature babies.

Before deciding on the benefit of screening in this population, the HAS is urging "a study in newborns (diagnosis at birth) with a long-term follow-up of infected children" to be carried out. One of the obstacles to carry out such a study lies in the diagnostic means currently available [1]. Apart from CMV detection kits for the laboratory, numerous lines of research concern virus detection and Microsystems (μ TAS, MEMS, etc.) [2,3]. These microsystems are generally dedicated to detecting genetic material after preparing samples, most often by PCR

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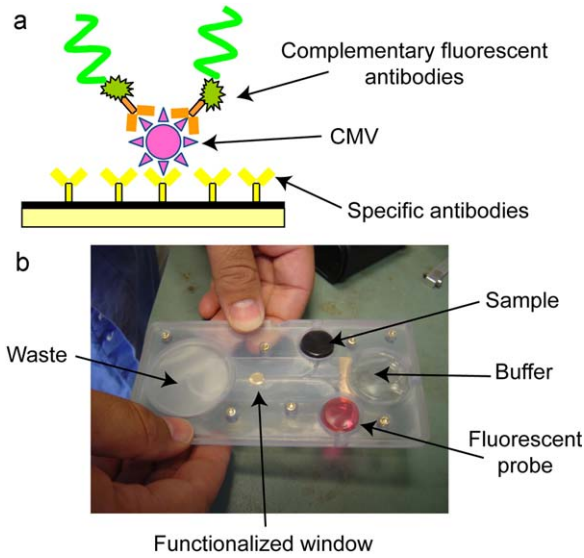


Fig. 1. a: schematic representation of the biosensor architecture; b: picture of the disposable cartridge. Coloured water is used for demonstration purpose.

or RT-PCR [4,5]. Concerning the biological fluid used and/or the type of analysis, most examine blood cells or other types of cells, which require virus extrusion operations of the cells and a blood puncture for collecting biological fluid. Indeed, a mobile device is required.

The microsystem presented here and developed under the coordination of the FEMTO-ST Institute in the framework of a 2006 ANR TecSan project, is an embedded detection device, which uses a microsystem including the functionalized surface for CMV. The detection of the viral material is based on immunofluorescence techniques, with materials and micro-manufacturing processes compatible with a low-cost industrial production. Among the medical acts carried out at birth, gastric aspiration allows a biological fluid combining foetal urine with amniotic fluid to be obtained easily. We therefore chose to use this biological fluid as a screening medium.

In the next part of the paper, we describe the immunofluorescence detection scheme as well as the device we developed. The third part is devoted to the biochemistry of the biosensor and to the first experimental results concerning CMV detection. Then a conclusion will be proposed to this work.

2. Immunofluorescence scheme and experimental device

The device we set-up is based on an immunofluorescence biosensor. A schematic drawing of the biosensor is given on Fig. 1(a). A gold surface is coated with CMV specific antibodies. A biological sample is then applied onto the surface of the biosensor. If CMV is present in the biological sample, it is trapped onto the surface by means of the antibodies. Then, after washing the surface with buffer, a fluorescent probe is injected. The latter consists of complementary Cy5 labelled antibodies. Therefore, if CMV is present in the sample, a fluorescent signal is detected.

This biosensor is integrated into a disposable cartridge (Fig. 1(b)). The latter contains all the fluids required for the

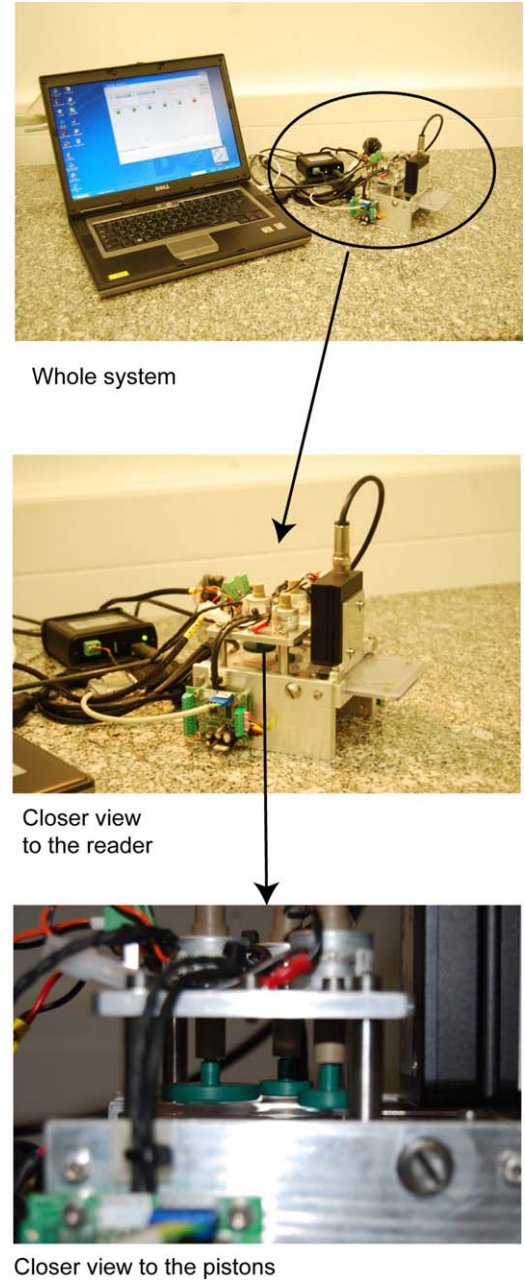


Fig. 2. Different views of the whole system. The protection cover is not shown.

immunofluorescence reaction. In the figure, we can see the gold coated functionalized surface as well as different deformable balloons. Four balloons are used. One contains the biological sample to be tested, a second one contains the fluorescent probe, a third one contains the buffer and the last one is used for waste. In this example, the biological sample is injected into the disposable cartridge with a conventional syringe. Micro-channels are used to drive the fluids from the balloons to the functionalized window where the reaction takes place.

Driving the fluid and detecting the possible fluorescence signal is performed into the mobile device, hereafter the reader, shown in Fig. 2. The disposable cartridge is inserted into the reader manually. Then the measurement process starts. As previously mentioned, the fluids are contained in deformable

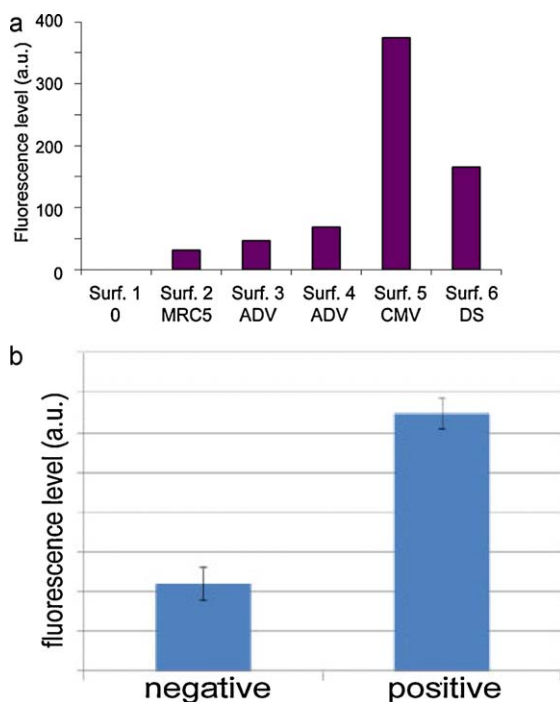


Fig. 3. a: immunofluorescence detection of CMV proteins - comparison with non infected MRC5 cells, AD169 (ADV) and commercial CMV antigens (DS). Experiment on microscope slides; b: immunofluorescence detection of CMV proteins with the disposable cartridge and the mobile reader/actuator.

balloons. Pistons are used to press the balloons and put the fluids to movement. The pistons motions, and therefore the fluids flows, are driven thanks to a computer program. The program also controls the incubation times and washing duration. When the biochemical reaction is finished, an ESE fluorescence measurement unit is used to detect the possible presence of CMV.

It must be noted that the disposable cartridge was designed using materials and micromanufacturing processes compatible with a low-cost industrial production.

3. Biosensor biochemistry and experimental results

3.1. Biochemistry aspects

The biosensor itself relies on the thiols chemistry. A description of the functionalization is given in Fig. 3. Before functionalization, gold surfaces are rinsed by ethanol and ultra-pure water. The chips are incubated in a solution of 11-mercapto-1-undecanol (97%)/16-mercaptohexadecanoic acid (3%) overnight at room temperature (RT).

Surfaces are rinsed by ethanol and ultra-pure water. Then, 40 μ l of EDC/NHS (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride/N-hydroxysuccinimide) are added on each surface and incubated during 30 min at RT. This step is necessary to activate C11/C16 layer. It is used to graft the antibodies to the surface.

The surfaces are then rinsed by 1X PBS and human polyclonal antibodies (PAbH) are incubated on the chips during 1 hour at room temperature in an ultrasonic bath. To ensure optimal

grafting of the PAbH, antibodies are diluted in an acetate buffer at 0,1 mg/ml, pH 5.

After PAbH grafting, surfaces are rinsed with 1X PBS and C11/C16 layer is deactivated using 40 μ l Ethanolamine-HCl (1 M pH 8.5) during 30 min at RT.

The fluorescent probe is composed of an anti-CMV Mouse IgG coupled to a Cy5 - anti Mouse Goat IgG.

3.2. Experimental results

Immunofluorescence detection of CMV antigen was experimented in two steps. In a first time, functionalized microscope slides were used. Six round gold surfaces were deposited onto the slides and various viral proteins were tested as depicted in Fig. 3(a). It can be seen from this figure that when the complete antibodies-antigen combination is used, the fluorescent signal is rather high. However, the different fluids were applied by means of conventional syringes and the measurements were not perfectly reproducible. In a second time, disposable cartridges were used together with the mobile reader. This time, measurements show that the ratio between positive and negative sample was of the order of 3 as it can be observed from Fig. 3(b).

4. Conclusion

In this paper, we have presented a mobile device used to screen CMV at the newborn's bed. Experimental results show a signal to noise ration of about 3, which is enough for screening purposes. The fact that all the required fluids are contained in a stand alone disposable cartridge make the system easy to transpose to the detection of various pathology vectors. Our present work deals with the study of such detections together with the set up of an ergonomic biological sampling system that fulfil the requirements of clinical use.

Conflict of interest statement

There is no conflict of interest.

Acknowledgements

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