DETECTION OF THE CYTOMEGALOVIRUS
A Mobile Device and a Disposable Cartridge for Detection at the Patient's Bed

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Abstract: Recently, cytomegalovirus (CMV) infection has become the most frequent cause of congenital infections. The French Health Authority (HAS) 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. It consists of a disposable cartridge containing the biological sample and the reactive liquids required for immunofluorescencence 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. Up to now, positive and negative samples can be discriminated with a fluorescence intensity ratio of 3.

1 INTRODUCTION

Since the rubella vaccine was established, the cytomegalovirus infection has become the most frequent cause of congenital infections, particularly in premature babies (prevalence between 2 and 10% according to studies). Before deciding on the benefit of screening in this population, the HAS (French Health Authority) 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 (Demler-Harrisson, 2009).

Congenital infections are the result of transplacental transmission of CMV. Transmission to the fetus may occur because of primary or secondary maternal infection. The frequency of intrauterine transmission following primary infection during pregnancy is 30% to 40%, compared with only 1% following secondary infection (Stagno, 1986 – Raynor, 1993). Ten to fifteen percent of congenitally infected infants will have symptoms at birth, and 20% to 30% of them will die (Raynor, 1993 – Nigro, 1999 – Pass, 2002). Most of the congenitally infected infants (85–90%) have no signs or symptoms at birth, but 5% to 15% of them will develop sequelae such as sensorineural hearing loss, delay of psychomotor development, and visual impairment (Boppana, 1992 – Pultoo, 2000 – Lazzarotto, 2000).

Routine serologic screening for pregnant women is rarely recommended by public health authorities (Revello, 2002 – Yinon, 2010). The screening, if done, should be performed at the beginning of pregnancy or even prior to a planned pregnancy in order to identify a seroconversion during pregnancy. Instead of screening for pregnant women, some authors recommend to screen babies at birth, but reliable methods to screen newborns for congenital cytomegalovirus (CMV) infection are still needed (Fowler, 1999 – Boppana, 2010).

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The diagnosis of infection in newborns depends on finding the virus in the different biological liquids and more specifically urine which concentrates the virus. Apart from CMV detection kits for the laboratory, numerous lines of research concern virus detection and Microsystems (μTAS, MEMS, etc.) (Huikko, 2003 – Anderson, 2003). These microsystems are generally dedicated to detect genetic material after preparing samples, most often by PCR or RT-PCR (Liao, 2005 – Park, 2004). 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, approved by a microtechnics competitive cluster, is an embedded detection device which uses a microsystem including the functionalized surface for CMV trapping (patent request submitted in September 09). The detection and dosage of the viral material are based on immunofluorescence techniques, with materials and micromanufacturing processes compatible with a low-cost industrial production. Among the medical acts carried out at birth, in particular in premature babies, gastric aspiration allows a biological fluid combining foetal urine (excreted as from the 5th month) 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 bio-chemistry of the bio-sensor and to the first experimental results concerning CMV detection. Then a conclusion will be proposed to this work.

2 DESCRIPTION OF THE DEVICE

The detection relies on the use of an immunofluorescence biosensor depicted in figure 1. The biosensor 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.

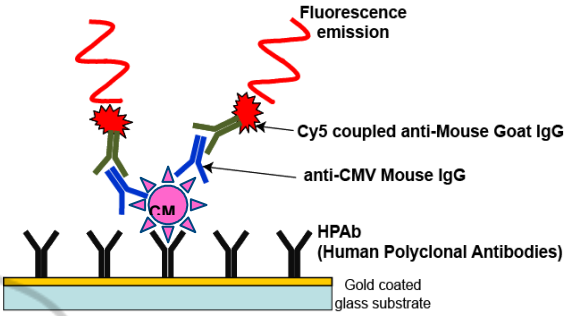


Figure 1: Immunofluorescence detection of CMV.

This biosensor is integrated into a disposable cartridge (figure 2). It contains all the fluids required for the 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 figure 3. The disposable cartridge is inserted into the reader manually. Then the measurement starts. As previously mentioned, the fluids are contained in deformable balloons. Pistons are used to press the balloons and put the fluids to movement. In our case, we have 3 pistons: "sample piston", "buffer piston" and "probe piston". 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.

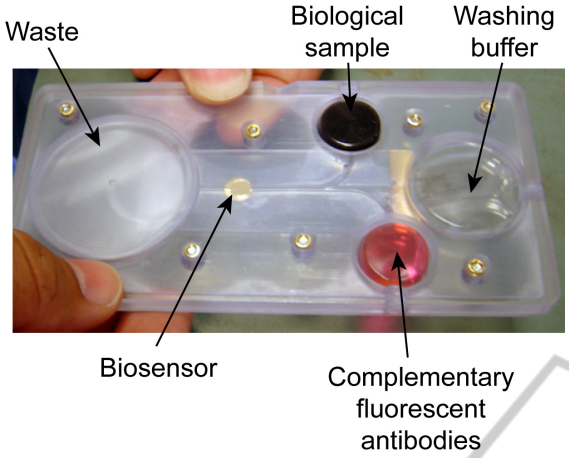


Figure 2: View of the disposable cartridge.

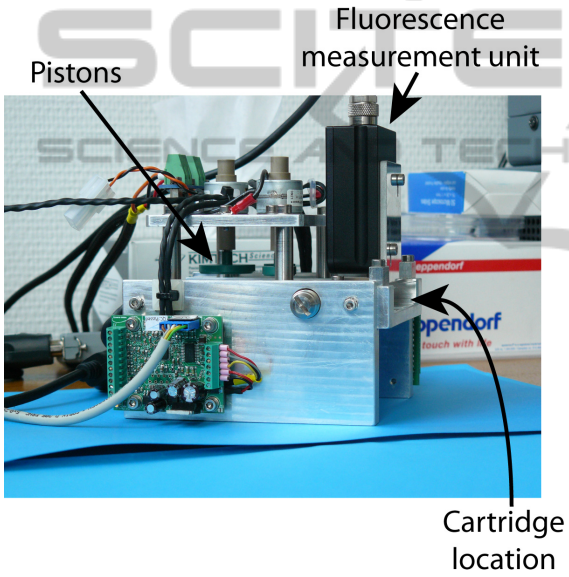


Figure 3: View of the reader/actuator.

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

3 IMMUNOFLUORESCENCE DETECTION OF CMV

3.1 Bio-functionalization

Chips are incubated in a solution of 11-mercapto-1-undecanol (97%) / 16-mercaptohexadecanoic acid (3%) overnight at room temperature (RT).

Then, 40µl of EDC/NHS (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride/ N-hydroxysuccinimide) are added on each surface and incubated during during 30 min at RT. This step is necessary to activate C11/C16 layer.

The surfaces are then rinsed by 1X PBS (phosphate buffer saline) and human polyclonal antibodies (PAbH) are incubated on the chips during 1 hour at room temperature. To ensure optimal grafting of the PAbH, antibodies are diluted in an acetate buffer at 0,1mg/ml, pH 5.

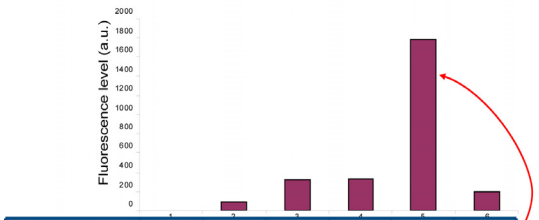
Surfaces are then rinsed with 1X PBS and C11/C16 layer is deactivated using 40µl Ethanolamine-HCl (1 M pH 8.5) during 30 min à RT. After a last rinsing by 1X PBS, biochips can be used.

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

3.2 Experimental Detection of CMV

Immunofluorescence detection of CMV antigen was experimented in 3 steps.

In a first time, functionalized microscope slides were used in order to test the biosensor alone. For this, commercial CMV antigens were used as biological samples. Six round gold surfaces were deposited onto the slides and various biochemical structures were tested as depicted in figure 4. 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.



	Surf. 1	Surf. 2	Surf. 3	Surf. 4	Surf. 5	Surf. 6
Activation	X	X	X	X	X	X
HPAb			X	X	X	X
Deactivation	X	X	X	X	X	X
CMV antigen					X	X
anti-CMV Mouse IgG				X	X	
Cy5 - anti Mouse Goat IgG		X	X	X	X	X

Figure 4: Biosensor testing with antigen solutions.

In a second time, we tested the specificity of the sensor with various viral proteins obtained from infected MRC5 cells. This experiment was done on microscope slides. The result is shown on figure 5 where ADV, CMV and DS stand for adenovirus, cytomegalovirus and commercial antigen respectively.

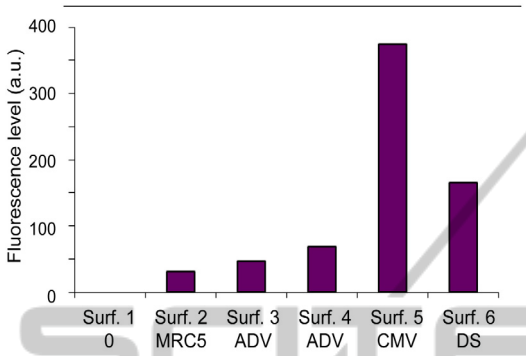


Figure 5: Specificity of the bio-recognotion.

In a third time, disposable cartridges were used together with the mobile reader. The idea was to test the complete device with CMV infected cells. This time, measurements show that the ratio between positive and negative sample was of the order of 3 as it can be observed from figure 6.

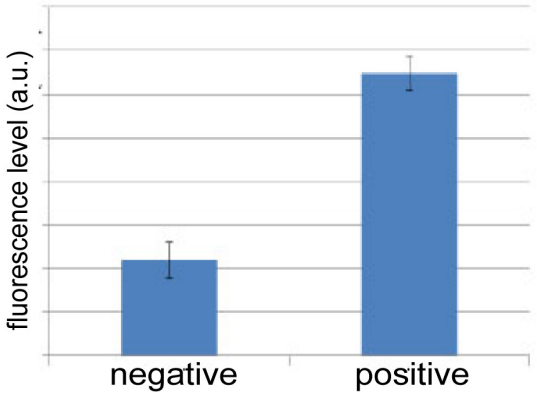


Figure 6: Complete device testing with CMV infected cells.

4 DRIVING SOFTWARE AND FURTHER DEVELOPMENTS

The device presented here is driven by means of a computer. Fluids flows and optical detection can be monitored by the computer. Figure 7 shows the control window of the computer program.

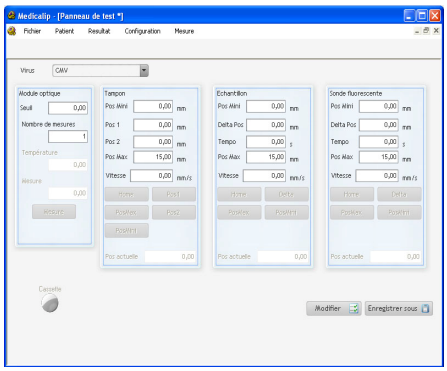


Figure 7: Driving window of the computer program.

In order to enhance the immunofluorescence reaction, fluids are driven as follows. First, the biosensor surface is rinsed with the buffer. The "sample piston" is actuated so that the reaction chamber is filled. We wait a few minutes so that viral proteins can be trapped onto the biosensor surface. Then the "sample piston" is actuated again in order to fill the reaction chamber with a new sample volume. After a few minutes of incubation, the process is iterated until the "sample balloon" is empty. Rinsing the surface with the buffer is performed in one go. Then, the "probe piston" is actuated in the same manner as the "sample piston". At the end, the biosensor's surface is rinsed with the buffer and the fluorescence measurement is performed. The automation of the measurement is depicted in figure 8.

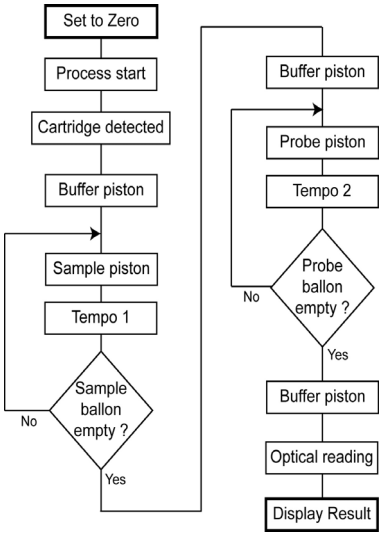


Figure 8: Control of the fluid flows and incubation times.

A measurement window is used to inform the operator on the measurement step being processed. It includes various parameters like the patient's

name, the name of the virus (we will comment later on this aspect), the temperature controls and the virus detection result (see figure 9).

One important aspect concerns the fact that all the fluid required for the immunofluorescence detection are contained in the disposable cartridge. In this way, it is possible to fabricate specific disposable cartridges for specific virus screening. We may also envisage the detection of other kind of biological entities. In fact, we plane to equip the cartridges with bar codes that will be read by the reader/actuator. The latter will then tune itself to the right opto-fluidic parameters such as liquid flows, incubation times and optical detection threshold. The computer program offers the possibility to set-up opto-fluidic parameters for each kind of virus as it can be seen from figure 10.

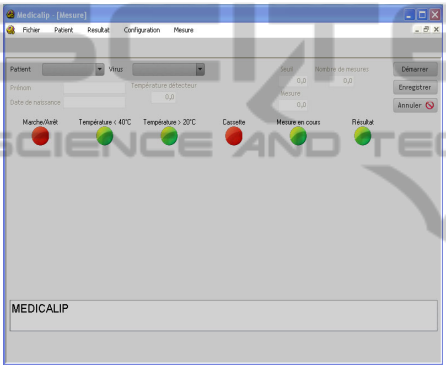


Figure 9: Control window.

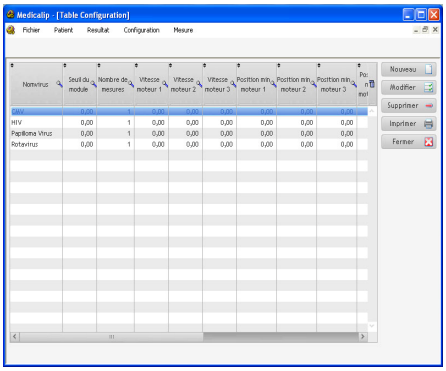


Figure 10: Setting the opto-fluidic parameters for each specific virus.

Although this multi-virus arrangement is particularly interesting in the cases where only one mobile reader can be used, more specific application may require a more compact and single virus detection device. This is the case for CMV screening for which the device will be used at birth in the care room next to the delivery room. We are now working on the ergonomic aspect of this specific application.

The last issue that must be taken into account is the time required for the test to be performed. Up to now, a bit less than 1 hour is required from the moment when the cartridge is inserted in the reader and the moment when the result is displayed. It is much more rapid than a diagnosis in a virology laboratory (we mean taking into account the gastric liquid sampling, the packaging of the sample, the transportation to the virology laboratory and the diagnosis). However, it can still be improved by means of acoustic accelerating techniques we are working on at the moment (Kardous, 2010).

5 CONCLUSIONS

In this conference, 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 fulfill the requirements of clinical use.

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REFERENCES

Anderson H., van den Berg A., Microfluidic, 2003, Devices for cellonics: a review, *Sens. and Act. B*, Vol. 92, pp. 315-325.

Boppana S. B., Pass R. F., Britt W. J., Stagno S, Alford C. A., 1992, Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality, *Pediatr. Infect*. Dis. J. Vol. 11, pp. 93–99.

Boppana S. B., Ross S. A., Novak Z, Shimamura M, Tolan Jr R. W., Palmer A. L., Ahmed A, Michaels M. G., Sánchez P. J., Bernstein D. L., Britt W. J., Fowler K. B., 2010, Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection, *JAMA*. Vol.14, pp. 1375-1382.

Demmler-Harrisson G. L., 2009, Congenital cytomegalovirus: public health action towards awareness, prevention, and treatment, *J. of Clin. Virol*., Vol. 46 Suppl 4, pp. S1-5.

Fowler K. B., Dahle A. J., Boppana S. B., Pass R. F., Newborn hearing screening: will children with hearing loss caused by congenital cytomegalovirus infection be missed?, 1999, *J. Pediatr*. Vol.135, pp. 60-64.

Huikko K., Kostiainen R., Kotiaho T., Introduction to micro-analytical systems: bioanalytical and pharmaceutical applications, 2003, *Eur. J. of Parmaceutical Sciences*, Vol.20, pp.149-171.

Kardous F., Simon B., Yahiaoui R., Manceau J. F., Boireau W., Improving immunosensor performances using acoustic mixer on droplet microarray, 2010, *Biosens. And Bioelec. In Press*, doi:10.1016/j.bios.2010.09.007

Lazzarotto T., Varani S., Guerra B., Nicolosi A., Lanari M., Landini M. P., Prenatal indicators of congenital cytomegalovirus infection, 2000, *J. Pediatr*. Vol.137, pp. 90–95.

Liao C. S., Lee G. B., Liu H. S., Hsieh T. M., Luo C. H., Miniature RT-PCR system for diagnosis of RNA-based viruses, 2005, *Nucleic Acids Res*. Vol. 33, pp. e156.

Nigro G., Mazzocco M., Anceschi M. M., La Torre R., Antonelli G., Cosmi E. V., Prenatal diagnosis of fetal cytomegalovirus infection after primary or recurrent maternal infection, 1999, *Obstet. Gynecol*. Vol.94, pp. 909-914.

Park J. C., Park Y. S., Kim E. H., Oligonucleotide chip composition for analyzing hepatitis c virus (hcv) genotype and detecting method thereof, 2004, Patent US2004170957.

Pass R. F., Cytomegalovirus infection, 2002 Pediatr. Rev. Vol.23, pp. 163–170.

Pultoo A., Jankee H., Meetoo G., Pyndiah M. N., Khittoo G., Detection of cytomegalovirus in urine of hearing-impaired and mentally retarded children by PCR and cell culture, 2000, *J. Commun. Dis*. Vol. 32, pp. 101–108.

Raynor B. D., Cytomegalovirus infection in pregnancy, 1993, Semin. Perinatol. Vol.17, pp. 394-402.

Revello M. G., Gerna G., Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant, 2002, *Clin. Microbiol. Rev*. Vol. 15, pp. 680–715.

Stagno S., Pass R. F., Cloud G., Britt W. J., Henderson R. E., Walton P. D., Veren D. A., Page F., Alford C. A., Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome, 1986, *JAMA* Vol. 256, pp. 1904–1908.

Yinon Y., Farine D., Yudin M. H., Gagnon R., Hudon L., Basso M., Bos H., Delisle M. F., Menticoglou S., Mundle W., Ouellet A., Pressey T., Roggensack A., Boucher M., Castillo E., Gruslin A., Money D. M., Murphy K., Ogilvie G., Paquet C., Van Eyk N., Van Schalkwyk J., Cytomegalovirus infection in pregnancy, 2010, *J. Obstet. Gynaecol*. Can. Vol. 32, pp. 348-354.