

Immunochromatography, **Simple, fast, effective and powerful method, for the detection of** **Adenovirus 40/41**

Christophe Depierreux*, Philippe Coppe[§], Emmanuel Pé*, Agnès Paquet*,
Olivier Raimond* et Thierry Leclipteux*

* : Coris BioConcept, rue Marcel Lecomte 199, 5100 Namur (Wépion), Belgium
§ : URBM, Facultés Universitaires Notre-Dame de la Paix, 61, rue de Bruxelles; B-5000 Namur; Belgium

Introduction

Adenovirus are non enveloped RNA viruses responsible for gastroenterites in the young children of less than 2 years. They probably represent the second cause of viral enterites after rotavirus with an incidence which varies from 4 to 12 %. Among the various groups of Adenovirus classified from A to F, 2 serotypes exists, AE-40 and 41, belonging to the sub-group F (Peter, 1998). These are the latter which are more particularly associate with the gastroenterites.

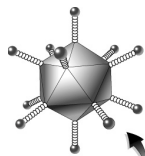
The transmission of the virus is made manner privilegiee by the ways anal-oral as in the case of Rotavirus.

If any person can be infected by Adenovirus, the children of 12 to 19 months form part of the most sensitive hosts and undergo an episode diarrheic of 4 to 5 days after an incubation of 8 to 10 days. This period is frequently associated to light fever, vomiting and abdominal pains. A liquid diarrhea is the symptome met at 97% of the infected patients. Viral excretion in feces is detected for one period of 10 to 14 days or 2 days before the enterite and 5 days after (Denis and al., 1997; Tessa and Van Ranst, 2000).

No seasonal variations is detected in the presence of Adenovirus, however the most significant prevalence is the winter season, season during which the children are regularly largely gathered inside.

The detection of the Adenovirus is usually realised thanks to various techniques such as electronic microscopy, ELISA and Latex. A specificity of 100 % is obtained by electronic microscopy. It is unfortunately, a heavy technique to implement and which requires a good knowledge of the protocols of microscopy, which implies in addition a good knowledge of the viral observations in ultrastructure. In addition, the variability of the taking makes this technique not very sensitive.

ELISA presents the advantage of being the most sensitive method while keeping a very good specificity thanks to



the use of immunological reagents. It is a technique which remains however confined at the laboratory. Conversely, the Latex test is carried out in 5 minutes and does not require any particular hardware.

However, since two to three years a new technique appeared who associates the chromatography in solid phase and the immunological reagents. It is the immunochromatographic technique which profit at the same time from the advantages of the sensitivity and specificity thanks to the use of the immunology reagents and of the advantage of the speed of detection via the solid support. This technique acquires day in day an increasingly large importance and proposes like one of the techniques in becoming in the field of the medical diagnosis. Coris BioConcept was the first company to develop and propose kits of detection of human enteric virus diseases on the Européen market.

The kit " 40/41 Adeno-Strip " makes possible the detection in a specific way any presence of Adenovirus of the enteric stocks 40 & 41 in the stools.

In order to validate this new test, samples coming from a paediatric hospital were analyzed at the same time with method ELISA, recognized as reference method, and with the test immunochromatographic developed by Coris BioConcept.

Materials and methods

Samples

153 human faeces coming from a paediatric laboratory were tested to evaluate the immunochromatographic test " 40/41 Adeno-Strip " compared to ELISA. These samples come from patients suffering of enterites. Parameters of sensitivity and specificity as defined by Bouyer (Bouyer J; et al., 1995) were given for the immunochromatographic test compared to ELISA. The faeces were roughly diluted 5 times in buffer PBS-Tween (0,1%) before their evaluation with the two techniques to be compared.

Elisa

96-wells microplaques were sensitized with anti-Adenovirus antibodies. 100 µl of a dilution 1/3 of the preparation of diluted faeces are incubated 60 minutes at ambient temperature on the sensitized plates. They are then washed three times at PBS-Tween (0,1%) and the conjugate combining anti-Adenovirus the 40/41 marked with peroxidase is distributed on the plates before carrying out new incubation a 60 minutes followed of three washings with PBS-Tween (0,1%). The revelation is carried out in 10 minutes with a solution of TMB-H₂O₂. The reaction is stopped with phosphoric acid and the results are read to 450 Nm. A negative witness is carried out for each fecal matter tested.

Immunochromatographic test

The kit "40/41 Adeno-Strip" consists of a nitrocellulose tigette sensitized with immunoreactive directed against the hexon-antigens of Adenovirus (Figure 1). The specificity of the immunological reactions is ensured by the use of a monoclonal antibody, directed against specific antigens of the group F of Adenovirus 40/41 and combined with particles of colloidal gold of 20 Nm. The previously diluted faeces are again diluted 3 times in the buffer of dilution provided with the kit. The sticks are then incubated in 500 µl of this solution during 5 minutes at ambient temperature.

Results and discussions

All 153 faeces was tested at the same time with the two techniques. The comparison of the results observed with the test

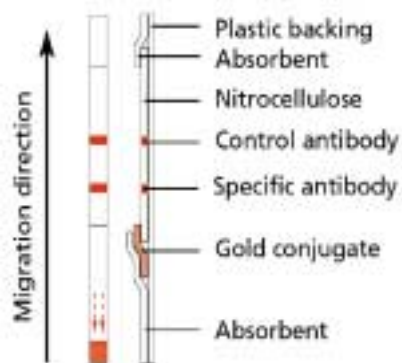


Figure 1 : representation of a stick for face (left) and in side sight (right). The nitrocellulose membrane is stuck on a plastic support. On both sides of this one is laid out of absorbing paper, paper allowing the sample to effectively migrate since the base of the stick (orange arrow) until the top. Between the lower absorbent and the nitrocellulose membrane a polyester membrane impregnated with an antibody coupled with colloidal gold is laid out. This antibody is directed against the hexon-antigens of the group F of Adenovirus 40/41. A sample is regarded as negative when the line control is revealed and positive when two lines are visible.

"40/41 Adeno-Strip" and specific test ELISA for the stocks Adenovirus 40/41 is included in table 1.

Table 1: comparison between the results obtained with ELISA and the test "40/41 Adeno-Strip" for the 153 faeces.

ELISA	40/41 Adeno-Strip	
	Positive	Negative
Positive	9	0
Negative	1	143

The parameters of specificity and sensitivity were given by applying the following formulas:

$$\text{Specificity} = \text{VN}/(\text{VN}+\text{FP}) \times 100 = 143/144 = 99,3 \%$$

$$\text{Sensitivity} = \text{VP}/(\text{VP}+\text{FN}) \times 100 = 9/9 = 100 \%$$

$$\text{Accuracy} = (\text{VP}+\text{VN})/\text{TOTAL} \times 100 = \text{????}$$

The sensitivity is defined as being the proportion of patients identified like patients with the immunochromatographic test among a population of subject really sick. Specificity is defined as being the proportion of healthy subjects identified with the immunochromatographic test among a population of subject indemnes.

According to the results obtained from the comparison, the probability of being mistaken by diagnosing healthy a suffering patient of diarrhoea with virus disease in Adénovirus 40/41 is 0,7 %. In the same way, the probability of emitting a positive diagnosis on a healthy sample is 0 %.

The values of specificity and sensitivity obtained by comparing the reference technique ELISA with our test "40/41 Adeno-Strip" validate our technique. The "40/41 Adeno-Strip" gives values very close to that observed with ELISA. However, compared to the ELISA which remains the most significant test, the "40/41 Adeno-strip" presents several advantages: it is a homogeneous test which is carried out in only one stage without enzymatic washing nor revelation. It does not require any equipment and a reliable answer is obtained in 5 minutes. The simplicity of the test is its principal asset, while presenting an insurance as for the diagnosis posed which is close to that of the ELISA.

Bibliography :

Peter, James B. (Mai 1998). Use and interpretation of laboratory tests in infectious disease. Specialty Laboratories (USA). Fifth Edition.

Denis, F ; Barriere, E ; Venot, C ; Ranger-Rogez, S ; Durepaire, N ; Martin, C ; Ploy, MC (1997). Virus and gastrointestinal infections. Ann Biol Clin (Paris) Jul-Aug;55(4):275-87.

Tessa, C. and Van Ranst, M. (2000). Evaluatie van de Immunochromatografische Isolatie van Rota- en Adenovirus. Thesis, UZ Gasthuisberg Leuven.

In : Epidemiologie : Principes et méthodes quantitatives.

Bouyer J. et al. 1995

Edition Inserm : ISBN : 2-85598-640-0