

# EVALUATION OF 40/41 ADENO-STRIP QUICK TEST® FOR THE DETECTION OF ENTERIC ADENOVIRUSES

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## Introduction

Adenoviruses type 40&41 belong to the F sub-group and are often called enteric Adenoviruses because their involvement in diarrhoea pathologies has been clearly settled. In Europe, Asia and both North and South America, they have been associated to infant diarrhoea with prevalences ranging from 3,1 to 13,5 %. Under age of 2, they are responsible for long-term diarrhoea and mild fever, coming or not with vomiting, which can last for two weeks. Secondary cases are frequent and asymptomatic carriers have been described. Infections are a all year round problem with a higher incidence during summer time.

*In vitro* culture of enteric Adenoviruses is difficult while other non-F Adenoviruses are easily cultivated. Nevertheless, since these latter could be pointed out either in healthy samples and diarrhoeic ones, they are not relevant for diarrhoea diagnostics.

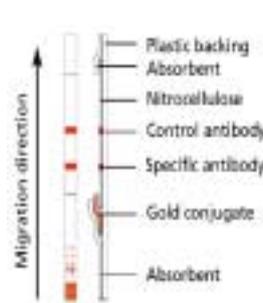
Enteric Adenovirus could be cultivated in specialised laboratories on Graham 293 cells or Chang conjunctival ones. Routine diagnostic should then be carried out by alternative techniques such as :

- Electronic microscopy allows a very specific diagnostic of enteric Adenoviruses since non-F groups are in such a low quantity that they cannot be detected, but sensitivity is rather weak. Moreover, this technique is difficult to handle in routine laboratories.
- ELISA is also available under commercial kits like Adenoclone Type 40/41 (Cambridge Bioscience, Worcester, Mass.), but this method is time consuming and does not allow a rapid diagnostic.
- Quick latex tests are also convenient (Adenolex, Orion Diagnostica, Espoo, Finland), but unfortunately, they detect all Adenovirus groups. This test could be followed by more specific tests.
- PCR molecular techniques are more and more useful and could be highly specific for enteric Adenoviruses provided followed by restriction enzymes digestions on amplified DNA.

## Purpose of the study

40/41 Adeno-Strip Quick test is a new test manufactured by Coris BioConcept (Namur - Wépion, Belgium). This test is based on the immunochromatographic method (see figure 1). Given the high simplicity and rapidity of this kit, we compared it with the agglutination Adenolex kit routinely used for the detection of enteric Adenoviruses in our laboratory. These procedures were followed by a PCR method with restriction enzymes digestion carried out on the amplified DNA. We are presenting hereafter the results of this evaluation.

Figure 1 : Immunochromatographic technique



Representation of a stick for face (left) and in side sight (right). The nitrocellulose membrane is stuck on a plastic support. Absorbing paper allowing the sample to effectively migrate since the base of the stick (arrows) until the top is placed on both sides of this one. A polyester membrane impregnated with an antibody coupled with colloidal gold is placed between the lower absorbent and the nitrocellulose membrane.

## Materials and methods

### Samples

109 faeces samples have been used. Some of them (mostly positive samples in Adenolex) were stored at - 20°C while the others were tested immediately after their reception in the laboratory.

### Adenolex (Orion Diagnostica, Espoo, Finlande)

Diagnostic was performed according to manufacturer's indications. In short, after centrifugation in Adenolex buffer, supernatant is incubated onto a slide with both control and reactive latex.

### PCR and identification with restriction of amplified DNA

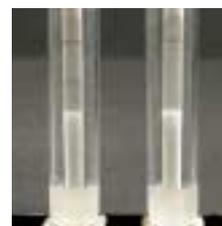
The technique described by Kidd and al. (J. Clin. Microbiol. 1996, 34 : 622-627) was used to samples giving discrepant results. VA3a, VA3b and VA6 primers were used to amplify a 260 bp fragment in sub-group F Adenovirus samples. These fragments are not digested by *Sfu1* and *Ava1* restriction enzymes.

### 40/41 Adeno-Strip Quick test

Diagnostic was performed according to manufacturer's indications. In short, test strips were dipped into 0,5 ml suspension faeces in dilution buffer (provided with the kit). Reaction's results were evaluated after samples had effectively migrated up to the top of the stick (see figure 2). Incubation never exceeded 15 minutes.

Results were interpreted with manufacturer's indications : 1 line = negative result ; 2 lines = positive ; no lines = invalid result.

Figure 2 : 40/41 Adeno-Strip Quick test



Positive result is shown on the left stick with two lines appearing while a negative result is illustrated on the right side with only the control band.

## **Results**

Both techniques gave expected results without misinterpretations since the control line was always appearing on the 40/41 Adeno-Strip test as control latex never agglutinates.

### **Sensitivity**

22 positive samples with Adenolex test and confirmed with PCR were tested positive with 40/41 Adeno-Strip Quick test.

Serial dilutions of one positive sample was performed to fix the absolute sensitivity. A positive reaction was observed up to a 1/64 dilution for 40/41 Adeno-Strip Quick test whereas Adenolex was positive only up to a 1/16 dilution.

### **Specificity**

87 fresh samples were processed in parallel with the two techniques : two were tested positive with 40/41 Adeno-Strip Quick test. Only one was tested positive with the Adenolex. The PCR procedure followed by the restriction enzyme digestion confirmed the results for the two positive samples.

In a routine use, 99 samples have been analysed and the five positive samples detected were PCR confirmed (this result is not mentioned in the table below as Adenolex was not tested with these samples).

Table 1 : Comparison between 40/41 Adeno-Strip Quick test and Adenolex

		40/41 Adeno-Strip		
		Positive	Negative	Total
Adenolex	Positive	23 *	0	23
	Negative	1 *	85	86
	Total	24 *	85	109

\* samples confirmed with PCR and identification with restriction of amplified DNA.

Sensitivity : 100%

Specificity : 95,8% (100% if we take into account PCR and identification with amplified DNA)

## **Discussion**

Both 40/41 Adeno-Strip Quick test sensitivity and specificity are excellent. By comparing the 40/41 Adeno-Strip test results with the whole PCR procedure, no one sample has been misdiagnosed. The high simplicity and rapidity of the method let us decide to use this diagnostic tool for our routine analysis.

*Comment : 40/41 Adeno-Strip Quick test is distributed in France by Servibio (BP 88, 92193 Meudon)*

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