

# DEVELOPMENT OF A NEW DIAGNOSTIC TOOL FOR THE QUANTIFICATION OF **ADENOVIRUSES** BY REAL TIME PCR

MAGRO Stéphane,<sup>1</sup> ECHAVARRIA Marcela,<sup>2</sup> BERRIOT Aurélie,<sup>1</sup> MARECHAL Patricia,<sup>1</sup> BARRANGER Côme,<sup>1</sup> and JOANNES Martine<sup>1</sup>.

<sup>1</sup> ARGENE, Parc Technologique Delta Sud, 09340 Verniolle, France

<sup>2</sup> Clinical Virology Laboratory, CEMIC University Hospital, Buenos Aires, Argentina, stephane.magro@argene.com

## Introduction

Human adenoviruses can cause respiratory, ocular or gastrointestinal diseases, mainly occurring in children and recruits as endemic infections or during outbreaks. Adenovirus infections are common, have a worldwide distribution and occur throughout the year.

Over the last years, adenoviruses have been increasingly recognized as significant viral pathogens with high morbidity and mortality among immunocompromised patients. Clinical manifestations in immunocompromised patients include pneumonia, hepatitis, hemorrhagic cystitis, colitis, pancreatitis, meningoencephalitis and disseminated disease, depending on the underlying disease, affected organ system, patient age, and virus serotype.

We propose a new diagnostic tool for the quantitative detection of genome of 52 serotypes of Adenovirus by real time PCR : Adenovirus R-gene™. Adenovirus viral load can be measured in whole blood, plasma, broncho-alveolar liquid, serum, nasopharyngeal samples, urine and stools.

- Analytical sensitivity in whole blood and respiratory samples has been evaluated.
- The sensitivity has been confirmed on the QCMD panel.
- An interfering substances study was carried out to examine whether a panel of endogenous and exogenous potential real time PCR inhibitors affected the performances of the Adenovirus R-gene™ assay.

## Materials and Methods

### Samples:

QCMD Proficiency Panel 2009 samples have been used. Analytical sensitivity was determined using Adenovirus serotype 5 quantitated in TCID<sub>50</sub>/mL (on MRC5). This solution was serially diluted in AdV negative characterised nasal aspirate or whole blood, before extraction. 4 pooled extractions and 30 amplifications had been done for each dilution tested. Endogenous and exogenous interfering substances (see table below) were spiked, before extraction, into two concentrations (10 or 2x Limit of Detection, LoD) simulated AdV3 positive nasal washes samples.

### Extractions:

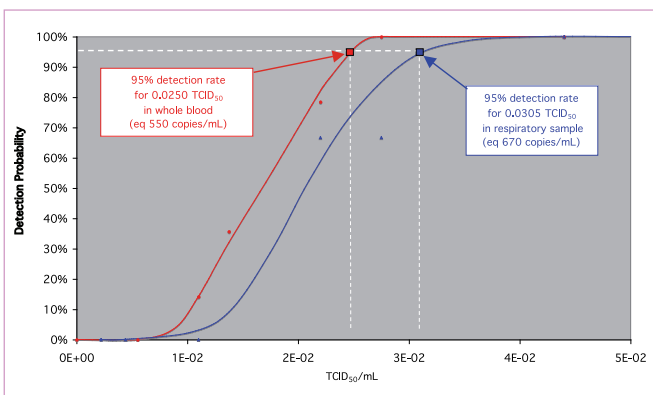
Viral DNA was extracted by using MagNA Pure Compact (Roche) or easyMAG NucliSens (bioMérieux). 200µL of samples were extracted, (proteinase K pre-treatment for respiratory samples), and eluted in 50µL for respiratory samples or 100µL for whole blood.

### Amplifications:

For Adenovirus R-gene™ (69-010 Argene), 10µL of purified nucleic acids were added to 15µL of ready-to-use amplification premix. An Internal Control, added before extraction step, allowed to check both extraction procedure and presence of inhibitory agents. ABI 7500 Fast (Applied Biosystems) was used for QCMD panel and analytical sensitivity. SmartCycler 2.0 (Cepheid) was used for QCMD panel and interfering substances study.

## Results

### Analytical sensitivity in whole blood and respiratory sample



### Adenovirus QCMD Proficiency Panel (2009)

		Adenovirus R-gene™ - 69-010				
		QCMD Expected Results			EasyMag NucliSENS	MagNA Pure Compact
	Serotype	Copies/mL	Log Copies/mL	Log Copies/mL	Log Copies/mL	Log Copies/mL
ADV09-01	Adeno 1	180	2.26	-	-	-
ADV09-02	Adeno 1	1 023	3.01	3.26	3.61	2.96
ADV09-03	Adeno 4	298	2.47	2.68	2.58	2.29
ADV09-04	Adeno 4	17 742	4.25	4.86	4.85	4.72
ADV09-05	-	-	-	-	-	-
ADV09-06	Adeno 1	21 184	4.33	4.88	5.06	4.59
ADV09-07	Adeno 3	137	2.14	2.14	1.71	2.16
ADV09-08	Adeno 4	1 879	3.27	3.82	3.97	3.67
ADV09-09	Adeno 1	851	2.93	3.32	3.40	2.76
ADV09-10	Adeno 4	279	2.45	2.93	2.73	2.83
IC2W0 (NC)	-	-	-	-	-	-

On 9 positive Adenovirus QCMD samples, 8 were detected with the different extraction /amplification systems tested. Only a very low positive AdV1 sample at 180 cp/mL has not been detected. 4 on 5 very low samples, AdV 3 at 137 copies/mL, AdV4 at 279 cp/mL and 298 cp/mL and AdV 1 at 851 cp/mL were well detected.

The analytical sensitivity for AdV 5 was 0.0305 TCID<sub>50</sub> or 670 copies/mL with a 95% detection rate on respiratory sample and 0.025 TCID<sub>50</sub> or 550 copies/mL in whole blood.

In whole blood, as little as 0.009TCID<sub>50</sub> or 200 copies/mL may be detected in 5% of cases.

### Interfering substances study

Substance Name	Active Ingredient	Concentration Tested	Adv 3 Sample	Adv CT (cycles)	Delta CT (cycles)	Internal Control CT (cycles)
None (Reference)	-	-	10xLoD	27,0	na	24,5
			2xLoD	28,6	na	24,1
Mucin	Purified mucin protein	60µg/mL	10xLoD	26,7	0,3	24,1
			2xLoD	28,9	-0,3	24,1
Human Whole Blood	NA	2% (volume/volume)	10xLoD	26,5	0,5	24,3
			2xLoD	28,3	0,3	24,2
Neo-Syneprine®	Phenylephrine HCl	15% (vol./vol.)	10xLoD	27,4	-0,4	24,5
			2xLoD	29,5	-0,9	24,2
Original Nasal Spray	Chlorhydrate d'oxymetazoline	15% (vol./vol.)	10xLoD	27,3	-0,3	24,5
			2xLoD	29,7	-1,1	24,2
Zicam Homeopathic	Luffa Operculata, Galphimia Glauca, Histaminum Hydrochloricum	5% (vol./vol.)	10xLoD	26,7	0,3	24,4
			2xLoD	28,9	-0,3	24,3
Safine Nasal Spray	Sodium chloride with preservatives	15% (vol./vol.)	10xLoD	27,1	-0,1	24,3
			2xLoD	29,2	-0,6	24,8
Chloraseptic® Sore Throat lozenges	Oral anesthetic / analgesic	0,63mg/mL	10xLoD	26,4	0,6	23,5
			2xLoD	28,7	-0,1	23,9

The table shows the interfering substances tested for this study. Concentrations spiked in the samples ranged from 2% to 15% of recommended dose for some substances, while others were tested at concentrations reported in literature or in references from other IVD package inserts.

Adenovirus R-gene™ showed no cross-reaction with any of exogenous or endogenous interfering substances tested. No inhibition was observed.

## Conclusions

Results presented in this study show the sensitivity, robustness and reliability of Adenovirus R-gene™ kit. This is combined to the capability to detect all serotypes (1 to 52) of the seven human adenoviruses species (A to G).

This high quality, in combination with its compatibility with the major extraction and real-time PCR platforms, allows an immediate integration in most routine diagnostic laboratories.

Furthermore, since the amplification parameters for AdV are the same as for other viruses, the Adenovirus R-gene™ kit can be used in combination with other Argene kits including those for transplant patients.