ARGENE

# ADENOVIRUS R-gene®

**REF 69-010**
**REF 69-010B**

NEW EXTRACTION PROTOCOL NucliSENS easyMag :
« Whole Blood Viral Extraction Protocol »

CE
IVD

COMPOSITION

| | | |
|---|---|---|
| REF 69-010 | DNA EXTRACTION KIT | Ref. : 67-000 |
| | Quantification and detection kit, ADENOVIRUS R-gene® | Ref. : 69-010B |

ARGENE

# 1. Presentation of the kit

- Human adenoviruses belong to the Adenoviridae family, Mastadenovirus genus. Adenoviruses are medium-sized, double-stranded DNA, non-enveloped icosahedral viruses. At least seven human adenoviruses species (A-G), including 52 serotypes, have been described. Adenoviruses can cause respiratory, ocular or gastrointestinal disease, which mainly appear in children and military recruits as endemic or epidemic outbreaks.
- Adenovirus infections are common, have a worldwide distribution and occur throughout the year. Over the last few 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. These clinical manifestations depend on the underlying disease, affected organ system, patient age, and serotype of the virus.
- In case of a suspected infection by adenovirus, blood specimens should be tested by PCR since the detection of adenovirus DNA from blood samples is usually significative for a disseminating disease. Surveillance in blood samples is currently a common practice among hematopoietic stem cell transplant (HSCT) recipients, especially in the pediatric population. The virus can be detected in blood 2-3 weeks before developing clinical symptoms, which offers the opportunity for intervention. Monitoring and prognosis are better with quantitative PCR methods. Increased viral load measurements are associated with increased risk of death.
- Amplification and detection of the viral genome using real-time PCR are very sensitive and is particularly applicable in case of a non-infectious virus. In this case the viral load is too low to be detected by cell culture and results are needed rapidly. It is recommended to analyse the viral kinetics for each patient.
- The ADENOVIRUS R-gene® kit is designed to measure the viral load of Adenoviruses by Real Time PCR after viral DNA extraction. ADENOVIRUS R-gene® is a user-friendly and complete kit and therefore ideal for routine diagnostics.
- ADENOVIRUS R-gene® allows the quantification of ALL adenovirus serotypes as listed below :
    - Adenovirus A : AdV 12, 18, 31.
    - Adenovirus B : AdV 3, 7, 11, 14, 16, 21, 34, 35, 50.
    - Adenovirus C : AdV 1, 2, 5, 6.
    - Adenovirus D : AdV 8 to 10, 13, 15, 17, 19, 20, 22 to 30, 32, 33, 36 to 39, 42 to 49, 51.
    - Adenovirus E : AdV 4.
    - Adenovirus F : AdV 40, 41.
    - Adenovirus G : AdV 52
- Many types of specimen and numerous DNA purification systems (automatic and manual) have been validated with the kit. Extracted DNA is then amplified and detected by Real Time PCR on the common available platforms.
- Thanks to a general amplification program with the entire range of R-gene® products, the sample analysis can be simultaneously analyzed with the following other targets : HSV1, HSV2, VZV with HSV1 HSV2 VZV R-gene® kit (Ref: 69-004B), CMV with CMV R-gene® kit (ref : 69-003B), CMV, HHV-6, HHV-7 and HHV-8 with CMV HHV6,7,8 R-gene® kit (ref : 69-100B), and EBV with EBV R-gene® (ref : 69-002B), BKV with BK Virus R-gene® kit (ref : 69-013B).
- Results are validated with various controls, including an extraction control, which are all provided with the kit. Results are valid for the diagnosis of early disseminated adenovirus infections as well as for the management of patient therapy.

# 2. Intended use

- The ADENOVIRUS R-gene® kit enables the detection and/or quantification of the genome of all adenovirus serotypes in whole blood, plasma, CSF, biopsies, stool, bronchoalveolar lavage (BAL), nasal aspirate, swab samples and ocular samples. The viral load can be measured using a quantification range supplied with the kit. Viral DNA must be extracted prior to the Real Time PCR amplification.
- This kit cannot be used for screening donors.
- Combined with other biological investigation methods (medical examination, biochemical and immunological analysis, etc.), the results obtained with the ADENOVIRUS R-gene® kit allow an earlier detection of infections caused by adenoviruses and improve the follow-up on immunosuppressed patients in treatment. In respiratory samples, many adenovirus serotypes can be detected with an increased level of sensitivity, which can not be detected by immunofluorescence techniques.
- The detection of adenoviruses with the ADENOVIRUS R-gene® kit can be carried out simultaneously with EBV viral load measurement using the EBV R-gene® kit (ref.: 69-002), HSV-1, HSV-2, VZV viral load measurement using the HSV1 HSV2 VZV R-gene® kit (ref. : 69-004) and the CMV, HHV-6, HHV-7, HHV-8 viral load measurement with the CMV HHV6,7,8 R-gene® kit (ref. : 69-100), BKV viral load measurement using the BK Virus R-gene® kit (ref.: 69-013). All R-gène® Real Time PCR kits follow the same amplification procedure and thus can be simultaniously used in the same experiment.

ARGENE

# 3. Principle of the test

### 3.1. Sample type:

- The Adenovirus R-gene® kit enables the detection of adenovirus genomes in whole blood, CSF, plasma samples and ocular samples using the quantification range supplied with the kit.
- For stool samples, biopsies or respiratory samples (aspirations and nasal swabs, BAL), the response is qualitative. In addition, the amount of buffer is usually not standardised. Therefore it is not possible to monitor the progress of a patient from one sample to another. Moreover, high viral loads are frequently found in this type of samples, leading to inhibition of amplification due to an excess DNA. Therefore, even though the value obtained may be quantifiable, we recommend performing a qualitative assay. If however the amount of stool (heavy stool) or the amount of cells (the number of cells in relation to the volume) is standardised and the quantification standard range has been used, the results may be reported in number of copies per PCR. We suggest to systematically test the sample both undiluted and diluted to 1/100 (including the extraction phase) in order to avoid false-negative amplification results due to an excessmaterial. In the event the sample is found to be both positive and inhibited, we recommend performing a new qualitative assay with an additonal dilution of the DNA extract.
- The quantitative results are reported in copies/mL or copies/PCR according the type of sample. The quantification range for adenovirus is linear between 500 copies/mL to 107 copies/mL i.e. 10 copies/PCR to 200 000 copies/PCR. The results are validated with an extraction, inhibition, positive and negative control provided with the Adenovirus R-gene® kit.

### 3.2. DNA purification:

- The following DNA extraction methods are validated with the ADENOVIRUS R-gene® kit, Ref.: 69-010B:
  - NucliSENS® easy MAG®
  - MagNA Pure LC System® Instrument
  - MagNA Pure Compact Instrument
  - MagNA Pure 96 System
  - BioRobot® M48
  - m2000sp®
  - Versant® kPCR Molecular System SP
  - QIAcube
  - Manual extraction Kit QIAamp® DNA Blood Mini Kit
  - Manual extraction QIAamp® DNA Stool Mini Kit
  - Manual extraction Kit DNA EXTRACTION KIT
- The target DNA, present in the sample and in the extraction + inhibition control (**IC2**), is extracted using one of the extraction methods above.
- The technique used by the DNA EXTRACTION KIT (Ref.: 67-000) associates the selective binding properties of silica gels with a microcentrifugation speed. The sample and internal control (**IC2**) are first lysed with protease in order to optimize the DNA binding capacities on the membrane. The use of the silica column allows, after DNA coating, efficient washing of the sample to eliminate contaminants. After elution, DNA is suitable for direct use in amplification techniques.

### 3.3. Real time amplification and quantification:

- Amplification is performed using the 5' nuclease TaqMan® technology (patent n°: 5210015, 5487972) also called hydrolysis probes. The ready-to-use amplification mixture includes: primers, dNTPs, amplification buffer, Taq Polymerase, specific Adenovirus primers and probe as well as the primers and probe specific for the internal control (**IC2**) which also must be subjected to the entire extraction procedure (including lysis).
- The following range of Real Time PCR platforms are validated with the ADENOVIRUS R-gene® kit, Ref.: 69-010B:
  - LightCycler®
  - Applied Biosystems
  - SmartCycler® 2.0
  - Rotor-Gene®
  - Stratagene®, Agilent or Versant® kPCR Molecular System AD
  - Dx Real-Time System (Bio-Rad)
- Extracted samples are amplified and quantified at the same time.
- The size of the amplified fragment is 138 bp and is located in the Hexon gene coding for hexagonal capsomeres which form the sub-units of the adenovirus capsid protein.
- A range of 4 quantification standards is provided with the ADENOVIRUS R-gene® kit (**QS1, QS2, QS3, QS4**). The quantification standards are ranged from 5 000 copies/µL to 5 copies/µL corresponding to 50 000 copies to 50 copies per PCR. The quantification standards are used to generate a new standard curve in the software provided with the thermocycler. The quantification of Adenovirus genome in unknown samples is extrapolated from this standard curve (the necessary calculation software is provided with the PCR instrument).
- **QS3** is a quantification standard that contains 500 copies of standard DNA per PCR. This standard can be used to import a previously created standard curve (provided that conditions described in chapter 8.2 are met). It is also used as a positive control for qualitative detection of CMV.
- **SC** is a sensitivity control that contains 1 copy plasmid DNA per µL, corresponding to 10 copies/PCR. This control (SC) validates the performance of the assay and should be considered as a run-control.
- An extraction and inhibition control (**IC2**) is included in the ADENOVIRUS R-gene® kit in order to check, starting from the lysis step if the sample has been well extracted and to verify the presence of amplification inhibitors in the sample.

ARGENE

**WARNING :**

**The performances described have been validated and are guaranteed using the technical extraction/amplification unit combinations recommended in the technical information sheet..**

**To monitor the evolution of viral load in the samples of whole blood, CSF, plasma and ocular samples from one patient test after test, it is essential for successive sample analyses to be taken strictly according to the same protocol and using the same combination of extraction /amplification units.**

**For samples such as stools, biopsies or respiratory samples, a qualitative response is recommended, the quantity of stools (weighed stools) or the number of cells (brought to the sample volume) are not standardized. For quantitiative analysis, it is recommended to test the sample undiluted and diluted to 1/100 (including the extraction phase) in order to avoid false-negative amplification results due to an excess of material. In the event the sample is found to be both positive and inhibited, we recommend performing a new qualitative assay with an additional dilution of the DNA extract.**

# 4. Content of the kit and storage

*Package Insert : 1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib*

## 4.1. DNA Extraction kit 67-000

- Number of extractions per kit: 50

| | | |
|---|---|---|
| **A** | QIAamp® Mini column | 5 x 10 |
| **B** | Collection tubes (2 mL) | 2 x 50 |
| **C** | AL buffer Xn - HARMFUL | 12 mL |
| **D** | AW1 buffer (concentrated) Xn - HARMFUL | 19 mL |
| **E** | AW2 buffer (concentrated) | 13 mL |
| **F** | AE buffer | 12 mL |
| **G** | QIAGEN protease Xn - HARMFUL | 24 mg |
| **H** | Protease solvent | 1.2 mL |

- The kit can be stored before and after opening at +2°C/+8°C until the expiration date written on the box. Storage at higher temperatures should be avoided. The reconstituted QIAGEN protease can be stored in aliquots at -18°C/-22°C to avoid successive freezing.

## 4.2. Detcetion and Quantification Kit ADENOVIRUS R-gene® 69-010B

- Number of tests: 90

| | | |
|---|---|---|
| **W0** | Water for extraction (Molecular grade) | 2 x 1.8 mL |
| **IC2** | Internal Control 2 | 1 mL |
| **R0** | Water for amplification | 300 µL |
| **QS1** | Quantification standard | 300 µL |
| **QS2** | Quantification standard | 300 µL |
| **QS3** | Quantification standard | 300 µL |
| **QS4** | Quantification standard | 300 µL |
| **SC** | Sensitivity control | 300 µL |
| **R10** | Adenovirus and **IC2** Amplification premix | 3 x 450 µL |

- Keep the kit (ref. 69-010B) in the dark before and after first opening frozen at -18°C/-22°C until the expiration date printed on the box.
- Before and after opening of the kit (ref. : 69-010B), Internal control 2 (**IC2**), reagent (**W0**), quantification standards (**QS1**, **QS2**, **QS3**, **QS4**) and sensitivity control (**SC**) must be stored in the extraction room at -18°C/-22°C.
  The reagents **R10** and **R0** must be stored in the room reserved for the preparation of the premix at -18°C/-22°C.
- Each premix cannot undergo more than 7 freezing/defrosting cycles.
- Replace the amplification premixes (**R10**), quantification standards (**QS**) and sensitivity control (**SC**) at -18°C/-22°C immediately after use.

ARGENE

# 5. Material and reagents required but not supplied

**5.1. For sample extraction:**

**5.1.1. With DNA EXTRACTION KIT (Ref.: 67-000)**

- Ethanol 96-100%
- Centrifuge (6 000 g / 12 000 g)
- Vortex
- Polypropylen test tubes for microcentrifugation (1.5 mL, 2 mL)- Water bath +56°C
- Micropipettes with plugged tips
- Single use gloves

**5.1.2. Other extraction methods validated**

- Follow the manufacturer's instructions

**5.2. For the detection and quantification ADENOVIRUS R-gene® kit, ref.: 69-010B**

- Micropipettes (P20) with plugged (aerosol barrier) tips or positive displacement tips ;
- Thermocyclers validated with ADENOVIRUS R-gene®
- LC Carrousel Centrifuge for LightCycler® or benchtop microcentrifuge convenient for 2mL reaction tubes, or plate centrifuge for Applied Biosystems, Dx Real-Time System and Stratagene®, Agilent or Versant® kPCR Molecular System AD.
- Single use gloves ;
- Capillaries, tubes or microplates for real time PCR platforms validated for ADENOVIRUS R-gene® ;
- Cooling block suitable for the thermocycler of choice ;
- U.V light ;
- Workstation or plexiglass screen for samples and premix distribution ;
- Colour Compensation r-gene® (ref.: 71-103) for result interpretation on LightCycler® 2.0 ;
- **DP2** premix of DICO Extra r-gene® (ref.: 71-101) to obtain an extraction + inhibition control on LightCycler® 1.0.

# 6. Reagents reconstitution

***ONLY rconstitute reagents supplied with the "DNA Extraction kit" (ref.: 67-000).***

**6.1. Protease stock solution preparation**

- Add 1.2 mL of protease solvent (**H**) to the 24 mg of lyophilized protease (**G**).
- Store aliquots at -18°C/-22°C (repeated freezing and thawing must be avoided).

**6.2. AL buffer (C) preparation**

- Mix AL buffer (**C**) thoroughly by shaking before use.
- Do not store protease mixed with AL buffer (**C**).
- If precipitate is observed in AL buffer (**C**), warm at +70°C to dissolve it.
- AL buffer (**C**) is stable when stored tightly closed at +2°C/+8°C.

**6.3. AW1 buffer (D) preparation**

- AW1 buffer (**D**) is supplied as a concentrate.
- Prior to first time use, add a volume of 25 mL of ethanol (96-100%) to the 19 mL of concentrated buffer.
- AW1 (**D**) buffer is stable when stored tightly closed at +2°C/+8°C.

**6.4. AW2 buffer (E) preparation**

- AW2 buffer (**E**) is supplied as a concentrate.
- Prior to first time use, add a volume of 30 mL of ethanol (96-100%) to the 13 mL of concentrated buffer.
- AW2 (**E**) buffer is stable when stored tightly closed at +2°C/+8°C.

ARGENE

# 7. Warnings and precautions

- This kit is intented for *in vitro* use only. The kit must be handled by a qualified staff member, in accordance with Good Laboratory Practice and handling instructions for molecular biology.
- Read all the instructions before starting the manipulation.

7.1 **General warnings and precautions:**

- Wear protective clothing, i.e: Disposable gloves, lab coat, safety goggles, mask.
- Avoid contact between the reagents and the skin. Wash immediately with copious amounts of water if contact occurs.
- Samples must be prepared under a biological safety hood.
- Never pipet by mouth.
- Do not smoke, eat or drink in dedicated work areas.
- Handle and dispose all specimens and materials as potentially infectious. After use: material, reagents and waste must be handled as potentially infectious.

7.2 **Warnings and precautions for molecular biology:**

- Amplification procedures require highly skilled techniques to avoid risk of sample contamination:
  - Use separate working places for sample preparation and amplification reactions. Movement in the laboratory must be in one direction only from the reagent preparation area to the amplification area. Allocate a set of lab coats and pipettes to each area. Never introduce an amplified product in reagent and/or sample preparation areas.
  - Pipettes used to handle samples are reserved for this purpose only. These pipettes must be positive displacement pipettes or pipettes equipped with filter tips. All tips must be sterile.
  - The pipettes used to aliquot reagents must be reserved only for this purpose. The necessary reagents for amplification are aliquoted in order to be used during one single experiment.
  - Tubes from different specimens and amplification premix must never be opened at the same time.
  - Used samples must be exclusively reserved for this analysis.

- Do not use reagents after expiration date printed on the labels.
- Do not substitute reagents from kits with different batch numbers or from other manufacturers.
- The reagents must be fully defrosted to room temperature before testing.
- The use of a metal cold block (+2/+8°C) is recommended for the manipulation of the reagents and the samples.
- Always perform preventive maintenance for workstations, for automated extraction, amplification, and centrifuge systems, according to the manufacturer's recommendations.

.

*For more detailed information, see the product safety data sheet which can be downloaded from www.biomerieux.com/techlib*

**7.3. Reagent specific warnings and precautions**

- AL (**C**) buffer and AW1 buffer (**D**) contain guanidinium chloride (chaotropic salt).
  - R22: Harmful if swallowed.
  - R36/38: Irritating for eyes and skin.
  - S13: Keep away from food, drinks and animal feeding supplies.
  - S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
  - S36: Wear suitable protective clothing.
  - S46: If swallowed, seek medical advice immediately and show the container or label.

  This component must not be used with disinfecting agents that contain bleach.

- The AW2 buffer (**E**) and protease diluent (**H**)of kit 67-000 contain 0.04 % of sodium azide as preservative.

- Protease (**G**) contains subtilisin
  - R37/38: Irritating to respiratory system and skin.
  - R41: Risk of serious damage to eyes.
  - R42: May cause sensitisation by inhalation.
  - S22: Do not breathe dust.
  - S24: Avoid contact with skin.
  - S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
  - S36/37/39: Wear suitable protective clothing, gloves and eye/face protection.
  - S46: If swallowed, seek medical advice immediately and show the container or label.

- Avoid contact between the reagents and the skin. Wash immediately with copious amounts of water if contact occurs. Wear gloves when handling the reagents.

ARGENE

# 8. Internal quantification standards and controls

**GLOSSARY :**

***530 nm*** *= the reading channel "FAM" or "Green" or other depending on the real-time PCR platforms. For reasons of simplification, only "**530 nm**" is used.*

***560 nm*** *= the reading channel "VIC", "Hex" or other according to the real-time PCR platforms. For reasons of simplification, only "**560 nm**" is used.*

*CT = Crossing Threshold for most real-time PCR platforms or CP (Crossing Point) for devices in the LightCycler® range. For reasons of simplification, only CT is used.*

**WARNING***: The order for adding samples and controls must be followed. (see: Chapter "Amplification preparation").*

### 8.1. The internal quantification standards (QS1, QS2, QS3, QS4)

- The use of the internal quantification standard range is imperative for sample quantification.
- The quantification standards are used to produce a standard curve in the software provided with the thermocycler.
- The quantification standards range from 5 000 copies/µL (**QS1**) to 5 copies/µL (**QS4**).
- The quantification standards must be designated as « standard »and their values must be entered when samples are defined in the table of data analysis software.
- The **QS** signal is detected at **530 nm**.

### 8.2. The quantification standard (QS3)

- The quantification standard **QS3** allows the importation of the standard curve created in the first run.
- The importation of the standard curve is a quantification method that must only be used from run to run with reagents of the SAME batch. The period between the run defining the standard curve with all four quantification standards and the run using the imported standard curve should not be longer than 3 months.
- Applied Biosystems®, Stratagene® and Versant kPCR Molecular System AD real time PCR instruments do not allow the importation of the standard curve.
- The signal is detected at **530 nm**.

*Note: In the event of purely qualitative detection, the QS3 serves as a positive control that verifies that the amplification step has been carried out correctly*

### 8.3. Sensitivity control (SC)

- The sensitivity control (**S**C) validates the performance of the assay and should be considered as a run-control.
- The sensitivity control (**SC**) is amplified with **R10** amplification premix.
- Systematically tested, the sensitivity control (**SC**) is the equivalent of a weak positive sample. For this reason, it may occasionally turn out to be negative.
- The signal is detected at **530 nm**.

### 8.4. Extraction + Inhibition controls

#### 8.4.1. The sample extraction+inhibition control (IC2sample)

- This control consists of an internal control (**IC2**) that must be added to the samples prior to extraction and checks both the efficacy of the extraction and detects the presence of possible inhibitors.
- Signal reading at **560 nm**.

#### 8.4.2. The reference extraction+inhibition control (IC2W0)

- This control consists of an internal control (**IC2**) that must be added to the negative extraction control (**W0**) prior to extraction and must be amplified at the same time as the patient samples in order to obtain a reference (**IC2W0**). The results must be compared with the extraction + inhibition control of patient samples (**IC2sample**).
- Signal reading at **560 nm**.
  - ⇒ Comparison of CT (Crossing Threshold) values of both **IC2W0** and **IC2sample** controls at **560 nm** evaluates the efficacy of the extraction and detects the presence of possible inhibitors.

### 8.5. The negative controls

#### 8.5.1. The negative extraction+amplification control (IC2W0)

- This is the same tube as was described in the "Reference extraction+inhibition control" chapter but, once the reading has been made at **530 nm**, it constituted a negative control used to check the absence of contamination on extraction and amplification.
- Signal reading of negative extraction+amplification sample at **530 nm**.

#### 8.5.2. The negative amplification control

- This is the same tube as was described in the "Reference extraction+inhibition control" chapter but, once the reading has been made at **530 nm**, it constituted a negative control used to check the absence of contamination on extraction and amplification.
- This control is optional.
- The signal is detected at **530 nm**.
  - ⇒ The comparison of the CT value at **530 nm** of the negative amplification control (**R0**) and **IC2W0** identifies a possible contamination.

ARGENE

# 9. Sample treatment and transport

*Samples must be collected and transported following instructions of the laboratory.*

### 9.1. Sample transport

- For samples to be transported, check your local legislation for hazardous and infectious material transport.
- Samples must be transported and treated by the laboratory in the shortest possible time (preferably within 24 hours).

### 9.2. Sample treatment

#### 9.2.1. Blood samples

*WARNING : The use of heparinized tubes does not suit genic amplification analysis. Blood collection tubes contain citrate which may be responsible for decreasing signal during the detection of amplified products.*

- Blood must be collected in EDTA.
- Before the extraction step, homogenize blood sample by over turning the sample tubes for 10 minutes with an automatic shaker
- Aliquot each blood sample in small volumes under a biological safety cabinet.
- Samples should be transferred to the laboratory within the shortest possible timeframe (preferably within 24 hours) after the samples are taken.
- Blood samples must be sent to the laboratory at room temperature (+18°C/+25°C).

#### 9.2.2. Plasma sample

- Blood must be collected in a dry tube or a tube containing EDTA.
- Centrifuge tube at 1200 x g for 10 minutes at 20°C. A maximum of 2 mL (200 µL minimum) serum and plasma is decanted under a biological safety cabinet in cryotubes.
- Serum and plasma must be sent to the laboratory preferably at room temperature (+18°C/25°C) or at +2°C/+8 °C. Serum and plasma not directly treated upon arrival must be store at +2°C/+8 °C for one week maximum. In case this delay exceeds one week, store serum and plasma at -18°C/-22°C.
- If serum and plasma samples are sent to the laboratory on dry-ice, they must be stored afterwards at -18°C/-22°C or preferably at-78°C/-82°C.

#### 9.2.3. Stool dilution

**a) For an automatic extraction with NucliSENS® easyMAG® :**

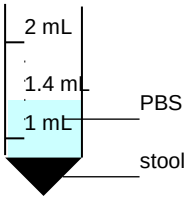
- Add a large lens of stools at 800 µL of NucliSENS lysis pad.
- Vortex vigorously.
- Incubate for 10 minutes at room temperature (+18°C/+25°C).
- Centrifuge for 30 minutes at 3000 x g.

- Transfer the supernatant into the control well of the corresponding easyMAG® loader.

**b) For other extraction methods:**

- Make a stool suspension of 10 to 50% in 1 mL of sterile PBS in the following way:
  - In a 2 mL marked centrifuge tube pipet 1 mL of sterile PBS. Add the stool specimen described to obtain a suspension of which the total volume may vary from 1.1 mL (stool suspension at 10%) to 1.5 mL (stool suspension at 50%).
  - Mix well.

*Example: Stool suspension at 40%*




- Centrifuge at 6000g for 30 minutes. Collect the supernatant.
- Centrifuge at 12000g for 15 minutes. Collect the supernatant.
- This supernatant contains the sample to be extracted and can be preserved at -78°C/-82 °C.

#### 9.2.4. Respiratory samples

- The respiratory specimens (solid or swab samples) should be suspended again, if necessary, in a small volume of normal saline solution and centrifuged at 4000-6000 rpm for 6 minutes before starting the test.
- In the event of using NucliSENS® easyMAG® automated system, sample + **IC2** a pre-treatment with Proteinase K is necessary. In this case, add 10 µL of Proteinase K to 20 mg/mL and incubate for 15 minutes at 56°C.

ARGENE

# 10. Sample extraction protocol

**WARNING:** **Before starting the extraction procedure, make sure samples and reagent IC2 have been homogenized.**

**Identificaton of the adenovirus subgroup responsible for the infection may be carried out with the ADENOVIRUS CONSENSUS kit (ref: 67-065). In that case, keep an aliquot of the corresponding sample.**

**For samples such as stools, biopsies or respiratory samples, a qualitative response is recommended, the quantity of stools (weighed stools) or the number of cells (brought to the sample volume) are not standardized. For quantitiative analysis, it is recommended to test the sample undiluted and diluted to 1/100 (including the extraction phase) in order to avoid false-negative amplification results due to an excess material. In the event the sample is found to be both positive and inhibited, we recommend performing a new qualitative assay with an additional dilution of the DNA extract.**

**In the room reserved for sample extraction**

## 10.1. With « DNA EXTRACTION KIT » (Ref. : 67-000 + IC2+ W0)

- Equilibrate samples, IC2 and **W0** to room temperature +18°C/+25°C.
- Equilibrate AE buffer (**F**) to room temperature +18°C/+25°C.
- Make sure that AL buffer (**C**), AW1 buffer (**D**), AW2 buffer (**E**), and reconstituted protease solution have been prepared according the instructions given in section "Reagents reconstitution".
- Redissolve any precipitate in AL buffer (**C**) by heating it at +70°C if necessary. Cool to room temperature before use.
- All centrifugation steps must be carried out at room temperature.

### 10.1.1. Lysis

- Prepare and identify (on the lid) an equal number of 1.5 mL microcentrifuge tubes to samples being analyzed. Add ONE tube dedicated to the extraction of the mix (**W0**+IC2).
- Heat the water bath to +56°C.
- Pipette 200 µL of AL buffer (C) into each 1.5 mL microcentrifuge tube previously identified.
- Add 20 µL of protease.
- Add 10µL of internal control (IC2).
- Add 200 µL of **W0** in the tube identified for the mix **W0**+IC2.
- Add 200 µL of sample in the tubes identified for sample extraction. PBS may be added to the sample if the sample volume is less than 200µL. In this case the result obtained will be only qualitative.
- Mix by pulse-vortexing for 15 seconds. To ensure efficient lysis, it is essential that the sample is mixed thoroughly to yield a homogeneous solution.
- Incubate at +56°C for 10 minutes.
- Lysis is completed after a 10 minute incubation. Longer incubation times have no effect on the yield or quality of the purified DNA. Potentially infectious agents can be inactivated by incubating the sample at +95°C for 15 minutes after the lysis step. However, extending this incubation time gives rise to a degradation of DNA.
- Briefly centrifuge the 1.5 mL microcentrifuge tube to remove any droplets from the inside of the lid.

### 10.1.2. Column loading

- Add 200 µL of 96-100% ethanol to the sample, and mix by pulse-vortexing for 15 seconds.
- Centrifuge the tubes briefly to remove any droplets present in the cap.
- Prepare and identify the same number of spin columns as the samples to be tested. Carefully apply the above mixture to the spin column (in a 2 mL collection tube) without wetting the rim.
- Close each spin column in order to avoid aerosol formation during centrifugation and centrifuge at 6 000xg for 1 minute. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the spin column is empty.
- Place the spin column into a clean 2 mL collection tube (provided), and discard the tube containing the filtrate.

### 10.1.3. Washing

- Carefully open the spin column and add 500 µL of AW1 buffer (**D**) without wetting the rim. Close the cap and centrifuge at 6 000xg for 1 minute.
- Place the spin column in a clean 2 mL collection tube (provided), and discard the tube containing the filtrate.
- Carefully open the spin column and add 500 µL of AW2 buffer (**E**) without wetting the rim.
- Close the cap and centrifuge at full speed (12 000xg) for 3 minutes.
- Place the columns on a clean 2 mL tube (not provided) and eliminate tubes containing filtrates.
- Centrifuge for 1 minute at full speed (12 000 x g) prior to elution. This step eliminates any trace of AW2 buffer (E).
- Place each column on a clean, identified 1.5 mL tube (not provided) and eliminate tubes containing filtrates

ARGENE

### 10.1.4. Elution

- Carefully open the spin column.

**WARNING: The elution volumes vary depending on the nature of the samples:**

- For samples of whole blood, respiratory samples or stool, add **100 µL** of balanced elution buffer AE(**F**) at room temperature.
- For samples of plasma add **50 µL** of balanced elution buffer AE (**F**) at room temperature.

- Incubate at room temperature for 5 minutes.
- Centrifuge at 6000xg for 1 minute.
- The DNA extracted is in the eluate.
- Extracted DNA is stable for up to one year when stored at -18°C/-22°C.

### 10.2. With extraction instruments and/or kits validated with ADENOVIRUS R-gene®

- These extraction instruments must be regularly maintained as recommended by the manufacturer by a qualified and trained staff.

| Instrument | Kit | Sample + IC2 Volumes | Sample Type | Protocol | Elution Volume |
|---|---|---|---|---|---|
| | QIAamp® DNA Blood Mini kit<br>Ref: 51 104 / 51 106 | 200 µL of sample + 10 µL IC2 | Whole blood<br>Respiratory samples<br>Biopsies | | 100 µL |
| | | | Plasma<br>CSF | | 50 µL |
| | | | Stool | | 100 µL |
| | QIAamp DNA Stool Mini kit<br>Ref: 51504 | | Stool | | 100 µL |
| QIAcube<br>QIAGEN<br>ref. : 9001292 / 9001293 | QIAamp® DNA Blood Mini kit<br>Ref: 51 104 / 51 106 | | Whole blood<br>Respiratory samples<br>Biopsies | Blood and body fluid spin protocol V3 | 100 µL |
| | | | Plasma<br>CSF | | 50 µL |
| | | | Stool | | 100 µL |
| | QIAamp DNA Stool Mini kit<br>Ref: 51504 | | Stool | Isolation of DNA from stool for pathogen detection V1 | 100 µL |
| MagNA Pure Compact<br>Roche Diagnostics<br>ref. : 03 731 146 001 | MagNA Pure Compact Nucleic Acid Isolation Kit I<br>Ref 03 730 964 001<br>32 isolations | | Plasma<br>Respiratory samples | Total_NA_Plasma_100_400 | 50 µL |
| | | | Whole blood | DNA_Blood_100_400 | 100 µL |
| MagNA Pure LC System®<br>Roche Diagnostics<br>Ref 12 236 931 001 | MagNA Pure DNA Isolation Kit I<br>Ref 03 003 990 001<br>192 isolations | | Plasma<br>Respiratory samples | Total NA Variable_elution_volume | 50 µL |
| | | | Whole blood<br>Stool | DNA I Blood_Cell High Performance | 100 µL |
| MagNA Pure 96 System<br>Roche Diagnostics | DNA and Viral NA Small Volume kit<br>Ref.: 05 467 497 001 | | Whole blood<br>Respiratory samples<br>Ocular samples | Viral NA Universal SV | 100 µL |
| NucliSENS® easyMAG®<br>bioMerieux ref. : 280110 | NucliSens® magnetic EasyMag reagents | | Respiratory samples | Generic | 50 µL |
| | | | Whole blood<br>New | The Manufacturer's "Whole Blood Viral Extraction" specific B protocol with 140 µL of silica (1) | 50 µL |
| | | | Whole blood | Specific B<br>+ 140 µL silica<br>+ 2 mL lysis buffer | 50 µL |
| | | | Stool | Specific A<br>+ 100 µL silica | 50 µL |
| BioRobot® M48<br>QIAGEN | Mag Attract DNA Mini M48 Kit (192)<br>Ref 953336 | | Respiratory samples | Bacteria DNA | 100 µL |
| QIAsymphony SP | QIAsymphony DNA Mini Kit | 300 µL of sample + 10 µL IC2(2) *(extraction 300 µL)* | Whole Blood | Virus blood DefaultIC V4 | 90 µL *(eluat 60 µL)* |
| m2000sp<br>Abbott | Sample Preparation System DNA PROMEGA (4x24 preps)<br>Ref : 06K12.24 | 800 µL of sample + 15 µL IC2(3) *(extraction 200 µL)* | Whole blood<br>Respiratory samples<br>Biopsies | DNA-Blood-LL-300-150 V081507 | 250 µL *(eluat 150 µL)* |
| Versant® kPCR Molecular System SP<br>Siemens ref. : 06635740 | Versant® Sample Preparation 1.0 Reagents | 400 µL of sample + 10 µL IC2(4) *(extraction 250 µL)* | Plasma<br>CSF | Sample Preparation Protocol 5 | 65 µL *(eluat 50 µL)* |

(1) A premix consisting of IC2, Lysis buffer and Silica must be prepared extemporaneously and added to the samples previously distributed in shuttles containing 2 mL of Lysis buffer. For n samples, mix 600µL Lysis buffer x (n+1) + 10µL IC2 x (n+1) + 140µL Silica x (n+1). Add 740µL of the mixture to each sample. For greater precision, see the detailed protocol "Worksheet easyMAG Viral Whole Blood extraction protocol" by bioMérieux

(2) If using the QIAsymphony SP automatic system, it is possible to prepare **a premix containing** IC2 (Argene-bioMérieux) and ATE buffer (QIASymphony) extemporaneously. For 24 samples, mix 1 414µL of ATE buffer + 266µL IC2.

(3) To extract samples of an initial volume higher than 800µL, add the following amounts of IC2 reagent:
For 1 initial sample amount, add 1/80th the amount of IC2 reagent (for example: to extract a 1mL sample, add 12.5µL of IC2 reagent).

(4) To extract samples of an initial volume higher than 400µL, add the following amounts of IC2 reagent:
For 1 initial sample amount, add 1/40th the amount of IC2 reagent (for example: to extract a 600µL sample, add 15µL of IC2 reagent).

ARGENE

# 11. Detection and Real-time quantification protocol

*Note: With a view to simplify the protocol, the device dedicated to holding the amplification reaction mix is referred to as a "**tube**".*

- **WARNING : The performances described have been validated and are guaranteed using the technical extraction/amplification unit combinations recommended in the technical information sheet.**
  **To monitor evolution of a patient's viral load test after test, it is vital for the successive sample analyses to take place using strictly the same protocol and with the same combinations of extraction/amplification units.**

- To determine the number of tubes, check if the experiment requires the creation of a standard curve (see section "The quantification standard (QS3)").
- Schedule:

  - 1 tube per tested sample;
  - 1 or 4 tube(s) for Adenovirus quantification standard curve imported/created
  - 1 tube for QS3 as positive control in the event of qualitative detection
  - 1 tube for adenovirus sensitivity control (**SC**)
  - 1 tube for reference extraction + inhibition control (**IC2W0**) also used as negative control for extraction and amplification.

Note:

- *Use the transparent plates (ref. : HSP9601) with the optical stoppers (ref.: TCS0803) for the Dx Real-Time System amplification device).*
- *When UNG is used, please refer to the protocols and programs described in the technical document of the product 65-001.*

### 11.1. Program :

- Regardless which real time PCR platform is used the amplification program remains the same. The amplification program is described in the table below.

**WARNING :** On Stratagene, Agilent or Versant® kPCR Molecular system AD, parameter to **20 sec.** the denaturation step.

| Steps | | Time | Temperature | Cycles | Fluorescence acquisition: | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | LC1 | LC2, LC480 | Applied Biosystems® | Rotor-Gene® | SC2 | Stratagene, Agilent or Versant® kPCR Molecular System AD | Dx Real-Time System |
| Taq Polymerase Activation | | 15 min. | 95°C | 1 | - | - | - | - | - | - | - |
| Amplification | Denaturation | 10 sec.<br>**20 sec.** for Stratagene | 95°C | 45 | - | - | - | - | - | - | - |
| | Hybridization Elongation | 40 sec. | 60°C | | **530** | **530 560** | **FAM VIC** | **Green Yellow** | **FAM Cy3** | **FAM - HEX** | |
| | | | | | End of the elongation | | | | | | |

*Note 1: Temperature transition rate/slope is pre-set at 20°C/sec or 100%.*

*Note 2: On LightCycler® instruments, add a cooling step : 30 sec / 40°C / 1 cycle at the end of the PCR.*

*Note 3: On LightCycler®, adjust the «SEEK TEMPERATURE» parameter to 60°C during programming.*

*Note 4:* On the LightCycler® 2, it is ESSENTIAL to use a colour compensation file to interpret the results.
Make sure that this is still valid (see appropriate technical information sheet) and has been created and recorded in the LightCycler( 2.0 management software, using the r-gene® Colour Compensation reagent (ref.:71-103)

*Note 5: On LightCycler® 480, there are two optical systems: only "System II" is compatible with the* ADENOVIRUS *R-gene® kit. "System II" includes automatic colour compensation in its software.*

*Note 6: On Applied Biosystems® select « NONE » in « PASSIVE REFERENCE » when programming.*

*Note 7: On Rotor-gene®, calibrate the signal by clicking on "GAIN OPTIMISATION".*

*Note 8: On Stratagene®, Agilent or Versant® kPCR Molecular System AD select "NONE" in "REFERENCE DYE".*

*Programming and analysis assistance sheets, per device type, downloadable at www.biomerieux.com/techlib*

ARGENE

### 11.2. Amplification preparation

**Amplification room**

- Before starting the experiment:
  - The reagents must be fully defrosted to room temperature before testing.
  - Mix each reagent (to a vortex for 2 seconds or through successive pipetting) and centrifuge briefly.
  - Homogenize thawed reagents by vortexing or by pipetting, then briefly centrifuge.
  - Make sure the cooling block was decontaminated by exposure under U.V. light for 30 min.
  - Make sure the cooling block was correctly pre-cooled at +2°C/ +8°C.

**WARNING:** **- In order to keep contamination to a minimum, close the tubes as you go along.**
**- Replace the amplification premix (R10),** , quantification standards (**QS**) and sensitivity test (SC) **at -18°C/-22°C immediately after use**
**- Each premix cannot undergo more than 7 freezing/defrosting cycles.**

- Collect 15 µL of the amplification premix (**R10**) by gently homogenizing with the pipet in order to distribute the same volume in all tubes.

  **WARNING** : The order for adding samples/reagents as shown below must be followed :

- Add 10 µL of each extracted sample in the corresponding tubes.
- Add 10 µL of the sensitivity control (**SC**) in the corresponding tube (see chapter “Controls”).
- Add 10 µL of each standard (from **QS4** to **QS1**) in the corresponding tubes (see chapter “Controls”).
- Add 10 µL of extracted mix **IC2+W0** in the corresponding tube. This tube is the **IC2W0** control (see chapter “Controls”).
- Centrifuge the tubes with the corresponding device and transfer them to the thermocycler.

### 11.3. Running the ADENOVIRUS R-gene® program:

- Run the amplification program (stored according to the instructions described in section “Program”)
- Designate the samples and controls.

- For Adenovirus quantification enter the following values for the quantification standards in copies/mL or copies/PCR:

| | | **Quantification (whole blood, plasma, CSF, ocular samples, stool, respiratory samples)** | | | | |
|---|---|---|---|---|---|---|
| | Extraction 200µL Elution in 50 µL (copies/mL) | Extraction 200µL Elution in 100 µL (copies/mL) | Extraction 300 µL Elution in 150 µL (copies/mL) | Extraction 250 µL Elution in 65 µL (copies/mL) | Extraction 300 µL Elution in 90 µL (copies/mL) | Results in copies/PCR |
| **QS1** | 1 250 000 | 2 500 000 | 2 100 000 | 1 250 000 | 1 500 000 | 50 000 |
| **QS2** | 125 000 | 250 000 | 210 000 | 125 000 | 150 000 | 5 000 |
| **QS3** | 12 500 | 25 000 | 21 000 | 12 500 | 15 000 | 500 |
| **QS4** | 1 250 | 2 500 | 2 100 | 1 250 | 1 500 | 50 |

ARGENE

# 12. Data Analysis

*Programming and analysis assistance sheets, per device type, downloadable at www.biomerieux.com/techlib*

*Note : **530 nm**= the reading channel "FAM" or "Green" or other depending on the real-time PCR platforms. For reasons of simplification, only "530 nm" is used.*

***560 nm** = the reading channel "VIC", "Hex" or other according to the real-time PCR platforms. For reasons of simplification, only "**560 nm**" is used.*

### 12.1. With LightCycler® 1.0:

- Apply the FIT POINTS method in ARITHMETIC mode using 2 data points.
- Move the *threshold line (red horizontal cursor line)* to a position where it crosses the fluorescence curves of all the samples in their linear part, above the baseline noise.

*Note : If the position of the crossing line is not sufficient for crossing all the sample curves in their linear part, repeat the step as frequently as necessary to obtain the CP for each sample.*

- For each sample, a CP CROSSING POINT is calculated at 530 nm.
- To quantify the samples, use the "SECOND DERIVATIVE MAXIMUM" method in ARITHMETIC mode.
- The concentration calculated for Adenovirus appears in the CALCULATED column (COPIES/mL).

### 12.2. With LightCycler® 2.0:

- Analysis of the viral target is carried out in the ABSOLUTE QUANTIFICATION mode at 530 nm.
- Analysis of the extraction + inhibition control is carried out in the ABSOLUTE QUANTIFICATION mode at 560nm after activation of the colour compensation file (COLOUR COMPENSATION tab.) (**Colour Compensation r-gene®** ref: 71-103).
- Apply the FIT POINTS method.
- Move the *threshold line (red horizontal cursor line)* to a position where it crosses the fluorescence curves of all the samples in their linear part, above the baseline noise.

*Note : If the position of the crossing line is not sufficient for crossing all the sample curves in their linear part, repeat the step as frequently as necessary to obtain the CP for each sample.*

- For each sample, a CP CROSSING POINT is calculated at 530 nm.
- To quantify the samples, select AUTOMATED F'' MAX mode (second derivative method).
- The concentration calculated for Adenovirus appears in the CONC column (COPIES/mL).
- The extraction+inhibition controls are analysed by comparing the calculated CP value for each extraction+ inhibition control (**IC2sample**) to the CP value obtained with the reference extraction + inhibition test (**IC2W0**) at 560 nm.

### 12.3. With LightCycler® 480 (System II):

- Switch on the LC480 (System II) FAM – HEX automatic compensation
- The viral target is analysed in ABSOLUTE QUANTIFICATION mode at 530nm (FAM).
- The extraction + inhibition control is analysed in ABSOLUTE QUANTIFICATION mode at 560 nm (HEX).
- For each positive sample, a CROSSING POINT (CP) is calculated at 530 nm.
- The extraction+inhibition controls are analysed by comparing the calculated CP value for each extraction + inhibition control (**IC2sample**) to the CP value of the reference extraction + inhibition test (**IC2W0**) at 560 nm.

### 12.4. With SmartCycler® 2.0:

- The viral target is analysed in FAM mode at 530 nm.
- The extraction + inhibition test is analysed in CY3 mode at 560 nm.
- For each positive sample, a CROSSING POINT (CP) is calculated at 530 nm (FAM CT).
- The extraction + inhibition controls are analysed by comparing the calculated CT value for each extraction + inhibition controls (**IC2sample**) to the CT value of the reference extraction + inhibition test (**IC2W0**) at 560 nm (CY3 CT).
- The concentration calculated for each sample appears in the FAM STD/RES GREEN column.

### 12.5. With Applied Biosystems®:

- Make sure that NONE is selected in the PASSIVE REFERENCE field because the **R10** premix does not contain any passive reference fluorochrome.
- Analysis of the samples is carried out after having selected the FAM R-GENE detector in the DETECTOR field.
- Adjust the Manual Baseline (red horizontal cursor line) manually to a position where it crosses the fluorescence curves of all the samples in their linear part. This step is performed to identify the positive samples which correspond to a calculated CT value. Negative samples are defined as UNDETERMINED displayed in the CT column by the SDS software.
- Analysis of the extraction + inhibition controls (**IC2sample** and **IC2W0**) is carried out in the same way after having selected the VIC R-GENE detector in the DETECTOR field.
- Make sure that none is selected in the PASSIVE REFERENCE field because the Adenovirus R-gene® premix **R10** does not contain any passive reference fluorochrome.
- To quantify the samples, return to linear mode.
- The concentration calculated for Adenovirus appears in the report drafted and printed at the end of each experiment.
- The extraction+inhibition controls are analysed by comparing the calculated CT value for each extraction + inhibition control (**IC2sample**) to the CT value of the reference extraction + inhibition test (**IC2W0**) at 560 nm.

ARGENE

**12.6. With ROTOR-GENE®:**

- The viral target is analysed in CYCLING A GREEN mode at 530 nm.
- Analysis of the extraction + inhibition control is carried out in the CYCLING A YELLOW mode at 560 nm.
- The threshold line must be adjusted in LINEAR SCALE mode after selecting DYNAMIC TUBES and SLOPE CORRECT.
- The concentration calculated for each sample appears in the CALC CONC column (COPIES/ML) in the Quant. RESULTS CYCLING A GREEN window.
- The extraction+inhibition controls are analysed by comparing the calculated CT value for each extraction + inhibition control (**IC2sample**) to the CT value obtained with the reference extraction + inhibition test (**IC2W0**) at 560 nm.

**12.7. With STRATAGENE®, AGILENT, Versant® kPCR Molecular System AD**

- Make sure that NONE is selected in the REFERENCE DYE field because the ADENOVIRUS R-gene® R10 kit premix does not contain any passive reference fluorochrome.
- The viral target is analysed by deselecting the HEX button.
- The extraction + inhibition control is analysed by deselecting the FAM button.
- The threshold line must be adjusted in LINEAR SCALE mode.
- The concentration calculated for each sample appears in the QUANTITY column (copies) in the summary table QUANT window.
- The extraction+inhibition controls are analysed by comparing the calculated CT value for each extraction + inhibition control (**IC2sample**) with the CT value of the reference extraction + inhibition test (**IC2W0**) at 560 nm.

**12.8. With Dx Real-Time System.**

- Analysis of the viral target: select the QUANTITATION tab leaving the FAM button checked.
- If necessary, manually adjust the threshold line so as to cross each amplification curve at the end of the exponential phase. This step aims to identify positive samples for which a CT is calculated. Negative samples are indicated by N/A in the CT column. For each positive sample concentration calculated in column STARTING QUANTITY (SQ) tab QUANTITATION and QUANTITATION DATA.
- The analysis of the extraction + inhibition controls (**IC2sample** and **IC2W0**) are performed in the same way after selecting the HEX detector.

ARGENE

# 13. Validation and interpretation of results

*Note : For samples analyzed as positive, a further identification of the adenovirus subgroup can be carried out with the ADENOVIRUS CONSENSUS kit (ref.: 67-065). In this case, keep an aliquot of the corresponding sample.*

### 13.1. Test validation :

**WARNING: The test is only valid if all following conditions are fulfilled. If this is not the case, all samples and controls must be tested again.**

**1st CONDITION : IC2W0** should not give a detectable signal at **530 nm**.

**2nd CONDITION : IC2W0** must be equal or lower than **32** cycles at **560 nm**.

**3d CONDITION :** The **QS3** CT of the standard range should be between 29 and 33 cycles

**4th CONDITION :** The slope and/or efficiency required for the standard range should be between the values listed in the table below :

**QUANTIFICATION**
*Whole blood, plasma, CSF (Stools, BAL and respiratory samples)*

| Instruments | Valuable Slope/Efficiency | |
|---|---|---|
| | The standard curve is created with all 4 quantification standards for each experiment. | The standard curve is created with all 4 quantification standards for subsequent experiments. |
| **LightCycler® 1.0** | -3.917 < Slope<-3.103 | -3.587< Slope <-3.208 |
| **LightCycler® 2.0 / LightCycler® 480** | 1.8 < Efficacity < 2.1 | 1.9 < Efficacity < 2.05 |
| **SmartCycler® 2.0** | -0.322 < Slope < -0.255<br>-3. 917 < Slope* < -3.103 | -0.278< Slope < -0.311<br>-3.587< Slope* < -3.208 |
| **Rotor-Gene®** | 0.8 < Efficacity < 1.1 | 0.9 < Efficacity < 1.05 |
| **ABI®, ABI StepOne®, ABI Fast** | -3.917 < Slope < -3.103 | *Non applicable* |
| **Stratagene®, Agilent or Versant® kPCR Molecular System AD** | 0.8 < Efficacity < 1.1 | *Non applicable* |
| **Dx Real-Time System** | 0.8 < E < 1.1 | *Non applicable* |

* With SmartCycler®, the slope of the regression line Ct = f(Log (Concentration) is obtained by right-clicking on the graph and by selecting "TRANSPOSE X AND Y AXES".

**QUALITATIVE DETECTION**
*Recommended for stools, BAL, biopsies, nasal samples*

*Not applicable*

**=> If all conditions are fulfilled, the results obtained with the samples can be validated.**

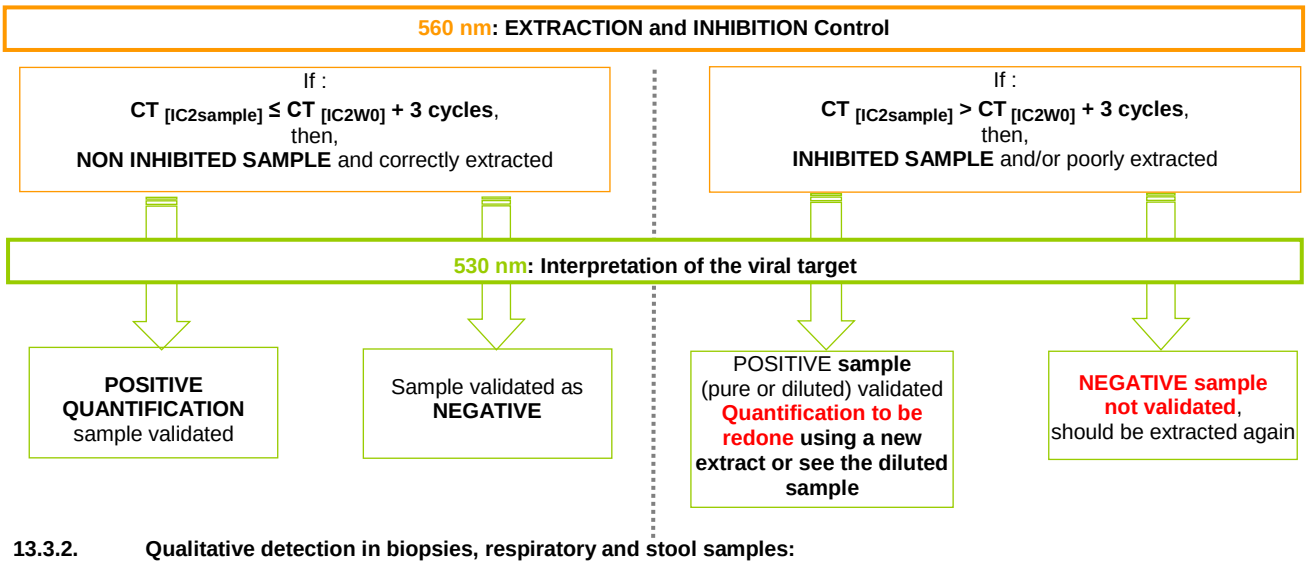
### 13.2. Sample reading:

- Each sample must be analysed one by one.
- There is a **530 nm** CT for all positive samples.
- The absence of CT at **530 nm** corresponds to a negative or incorrectly extracted sample.
- However, in all cases, it is necessary to ensure there is no inhibition and that the extraction stage at **560 nm** was carried out correctly according to the factors described in paragraph 13.3. "Interpretation of results".

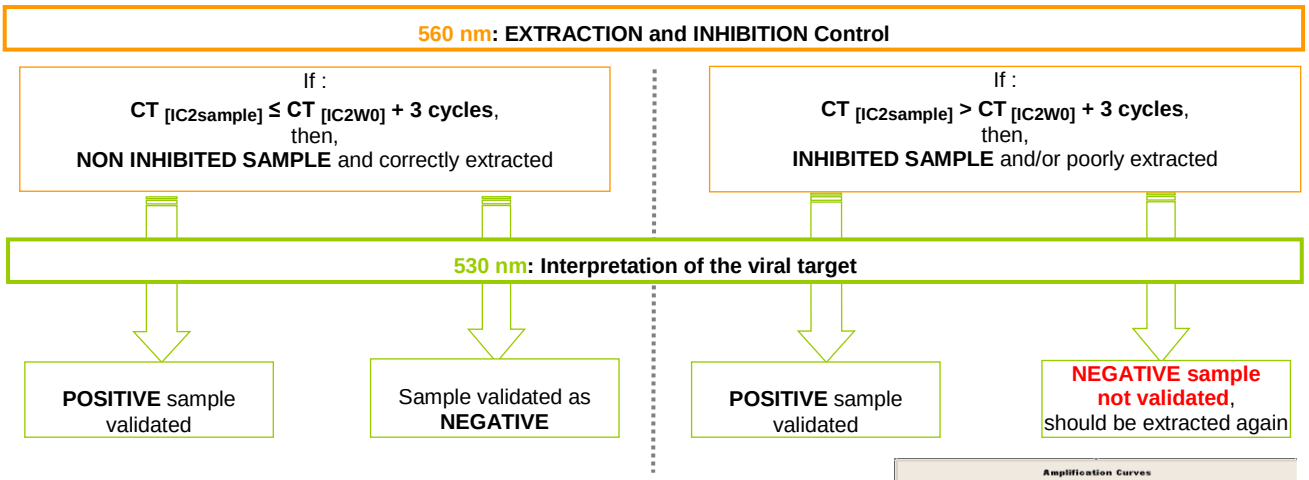
ARGENE

**13.3. Interpretation of results :**

**13.3.1. Quantitative detection in whole blood, CSF and plasma samples, ocular samples (in copies/mL) and in stool samples and respiratory specimens (in copies/PCR):**

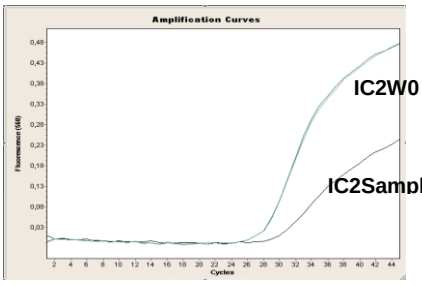



**13.3.2. Qualitative detection in biopsies, respiratory and stool samples:**




**13.4. Exceptional cases:**

**WARNING:** In case of a negative sample:
If the slope of the curve generates a fall of final fluorescence (≥ 50%) compared to the final fluorescence of **IC2W0** (see figure opposite) a weak inhibition is possible. We suggest to extract and to test the sample again.




IMPORTANT NOTES:
- It is highly recommended to use the log10 reporting format ($log_{10}$).
- Two quantification results are considered as different if the difference between both values is at least higher than 0.5 $log_{10}$, taking into account these results were obtained by using the same instruments and the same methods for extraction and amplification.
- It is absolutely necessary to compare results obtained with the Adenovirus R-gene® kit to other diagnostic investigation methods in order to define patient viral status.

ARGENE

# 14. Troubleshooting

### 14.1. No signal or underestimated quantification in positive samples.

| POSSIBLES CAUSES | RECOMMENDATIONS |
|---|---|
| The amplification premix has been defrosted too many times.<br>The amplification premix has remained at room temperature for too long or has been defrosted at too high a temperature. | • Please refer to the "Content of the kit and storage" section. The premixes must not be defrosted more than 7 times.<br>• Check that the amplification premixes, the quantification standards and the sensitivity control have been returned to -18/-22°C immediately after use.<br>• Check that the amplification premixes, the quantification standards and the sensitivity control have been defrosted at room temperature.<br>• Use a cooling block when preparing and distributing the premixes. |
| Incorrect blood collection, transport and storage in the laboratory. | • See chapter "Sample treatment and transport" that defines the optimal conditions (temperature, time) for transport and storage.<br>• Check the delay between the sample collection and its analysis.<br>• Collect blood in EDTA tubes. |
| Transport and storage conditions were not met. | • Follow instructions in section "Content of the kit and storage" regarding the storage of ADENOVIRUS R-gene® kit 69-010B at -18°C/-22°C and preferably in the dark. |
| Problem in extraction step. | • Check if you carefully homogenized the samples before performing extraction.<br>• Perform all washing steps and respect the incubation time when using the DNA Extraction kit ref. 67-000. (See section "With DNA EXTRACTION KIT").<br>• Check if material and protocol used to extract sample correspond to material and protocol recommended for analysis with the ADENOVIRUS R-gene® kit 69-010B (See section "Sample extraction protocol").<br>• Always perform preventive maintenance of workstations for automated extraction, and centrifuge systems, according to the manufacturer's recommendations. |
| Pipetting error. | • Check the calibration of your pipets.<br>• Check the distributed volume of reagents and samples.<br>• Carefully homogenize reagents and samples before their distribution in amplification tubes. |
| Programming error. | • Check all programming data (detection channel, mode, number of cycles, temperature and time).<br>• Check all the steps regarding the entry of the samples.<br>• Check the concentrations of the stored standards.<br>• If you imported an external standard curve, check the validity of this importation. |
| Problem in amplification step. | • Check the performances of the real time PCR platform as recommended by the manufacturer.<br>• Always perform preventive maintenance of real time PCR platform, and centrifuge, systems according to the manufacturer's recommendations.<br>• Check the attachment of the locking ring of the Rotor-Gene® carrousel. |
| Error in data analysis. | • Check the threshold line adjustment.<br>• Check that the imported range is valid (see chapter "Internal quantification standards and controls"). |
| Error in interpretating results | • Check the validity of the results obtained in the experiments (check all the validation conditions as described in section "Validation and interpretation of results").<br>• With Applied Biosystems® : check if NONE is selected in PASSIVE REFERENCE FIELD.<br>• With LightCycler® 2.0: Make sure the results obtained were corrected using a valid colour compensation file created in advance using kit ref.: 71-103<br>• Compare the result of the extraction+inhibition control (**IC2sample**) of the suspected sample to the result of the reference extraction + inhibition control (**IC2W0**) (see chapter "Sample reading"). Dilute the extracted sample if necessary. |

ARGENE

### 14.2. Fluorescent signal on negative samples or overestimated quantification of clinical sample.

| POSSIBLES CAUSES | RECOMMENDATIONS |
|---|---|
| Contamination during experiment. | • Follow all recommendations in section “Warnings and precautions”.<br>• Decontaminate the cooling block with U.V. light.<br>• Repect the manufacturer’s recommendations for the decontamination of automated extraction workstation and real time PCR instrument.<br>• Only a trained staff must handle the Adenovirus R-gene® kit.<br>• Use the **R0** reagent provided in the kit, in parallel with the samples extracted, to identify the contaminated stage. |
| Pipetting error. | • Check the calibration of your pipettes.<br>• Check the distributed volumes of reagents and samples.<br>• Carefully homogenize reagents and samples before their distribution in amplification tubes. |
| Programming error. | • Check all programming data (detection channel, mode, number of cycles, temperature and time).<br>• Check all the steps regarding the entry of the samples.<br>• Check the concentrations of the stored standards.<br>• If you imported an external standard curve, check the validity of this importation. |
| Error in data analysis. | • Check the threshold line adjustment.<br>• Check that the imported range is valid (see chapter “Internal quantification standards and controls”). |
| Error in results interpretation. | • Check the validity of the results obtained in the experiments (check all the validation conditions described in section “Validation and interpretation of results”).<br>• With Applied Biosystems® : check if NONE is selected in PASSIVE REFERENCE FIELD.<br>• With LightCycler® 2.0: Make sure the results obtained were corrected using a valid colour compensation file created in advance using kit ref.: 71-103.<br>• Compare the result of the extraction+inhibition control (**IC2sample**) of the suspected sample to the result of the reference extraction + inhibition control (**IC2W0**) (see chapter “Sample reading”). Dilute the extracted sample if necessary. |

### 14.3. The samples all seem inhibited.

| POSSIBLES CAUSES | RECOMMENDATIONS |
|---|---|
| Inadequate extraction stage. | • Check that the samples have been properly homogenised before being extracted.<br>• In the case of manual extraction using the DNA EXTRACTION KIT R-gene®. ref. : 67-000, perform the number of washes and incubation time stated in the “DNA Extraction Kit” section.<br>• Check the materials and protocols used for extracting samples.<br>• Kit performances are only validated for the extractions described in the “Sample extraction protocol” section.<br>• Carefully monitor to ensure that the extraction devices are maintained in line with the manufacturer’s recommendations.<br>• For coloured extracts and inhibited samples obtained on the NucliSENS easyMAG for the whole blood matrix, use the "**Whole Blood Viral Extraction**" protocol by preference |
| The IC2W0 does not result from the same run extraction | • Make sure that every sample tested includes the same batch of IC2 as IC2W0.<br>• Each extraction run should have its own IC2W0. |

ARGENE

# 15. Performance of the assay

- **<u>WARNING</u>: The described performances of the HSV1 HSV2 VZV R-gene® kit can only be guaranteed for use on the use recommended extraction systems and PCR instruments..**

### 15.1. Intra-assay and inter-assay reproducibility of ADENOVIRUS R-gene® kit ref.: 69-010

Accuracy studies were conducted on the ADENOVIRUS R-gene® kit by means of a repeatability study (intra-assay variation) and a reproducibility study (inter-assay variation) on different samples analysed 10 times using the ABI Prism® 7500 Fast (Applied Biosystems) instrument; after extraction with the MagNA Pure Compact (Roche).

#### 15.1.1. Intra-assay reproducibility

One serotype each from A to F species was chosen with a concentration which enables us to obtain CTs within a range between 25 and 33 cycles.

The table below shows the average CT obtained for each sample. The standard deviation and coefficient of variation were determined. The coefficient of variation is between 0.49% and 4.54%. These values show that the ADENOVIRUS R-gene® kit provides a good repeatability level.

| | | Mean CT (cycles) | Standard deviation | Coefficient of variation (%) |
|---|---|---|---|---|
| **Adenovirus** (Cell culture ) | AdV A (12) | 30,49 | 1,37 | 4,50 |
| | AdV B1 (3) | 27,53 | 1,25 | 4,54 |
| | AdV B2 (11) | 28,47 | 0,87 | 3,07 |
| | AdV C (5) | 25,84 | 0,31 | 1,20 |
| | AdV D (8) | 29,19 | 0,14 | 0,49 |
| | AdV E (4) | 31,47 | 0,20 | 0,64 |
| | AdV F (40) | 33,05 | 0,22 | 0,67 |
| Reference extraction+inhibition control | | 29,60 | 0,11 | 0,37 |

Intra-assay reproducibility of ADENOVIRUS R-gene® kit *ref. : 69-010*

#### 15.1.2. Inter-assay reproducibility

Inter-trial reproducibility was carried out on culture samples of Adenovirus serotype 5, species C, for the 4 quantificationstandards (QS) and the sensitivity control (SC)

| Target | 530 nm | Mean CT *(cycles)* | Standard Deviation | Coefficient of variation *(%)* |
|---|---|---|---|---|
| AdV C (5) - *Dilution 1* | | 28,70 | 0,39 | 1,35 |
| AdV C (5) - *Dilution 2* | | 32,15 | 0,54 | 1,67 |
| AdV C (5) - *Dilution 3* | | 36,22 | 1,58 | 4,37 |
| QS1 | | 24,84 | 0,08 | 0,32 |
| QS2 | | 28,39 | 0,05 | 0,19 |
| QS3 | | 31,96 | 0,04 | 0,13 |
| QS4 | | 35,07 | 0,38 | 1,09 |
| SC | | 37,73 | 0,99 | 2,62 |

Inter-assay reproducibility of samples, QS and sensitivity control (SC)

The average CT (cycles), the standard deviation and the coefficient of variation (%) were determined using the 3 Adenovirus dilutions tested, the quantification standards (QS1 to QS4) and the sensitivity control (SC). The coefficient of variation for the 3 samples is between 1.35% and 4.37% according to the number of copies of the Adenovirus in the sample. The coefficient of variation is between 0.13% and 1.09% for the quantification standards. For the sensitivity control, at 1 copy/µL, the coefficient of variation is 2.62%. The average effectiveness of the standard range was 96.10%.

This study enables us to demonstrate the high level of reproducibility of the ADENOVIRUS R-gene® 69-010B kit.

The 3 Adenovirus dilutions (AdV5) were quantified with the standard range with regard to each experiment. The concentrations obtained were averaged and then raised to the logarithm.

| Adenovirus | Mean Concentration | | Standard Deviation | Coefficient of variation *(%)* |
|---|---|---|---|---|
| | *copies/mL* | *Log copies/mL* | | |
| AdV C (5) - *Dilution 1* | 197 879 | 5,30 | 0,11 | 2,13 |
| AdV C (5) - *Dilution 2* | 19 308 | 4,29 | 0,18 | 4,10 |
| AdV C (5) - *Dilution 3* | 1 228 | 3,09 | 0,54 | 17,34 |

Inter-assay reproducibility of Adenovirus quantification (AdV 5) at 3 viral load levels

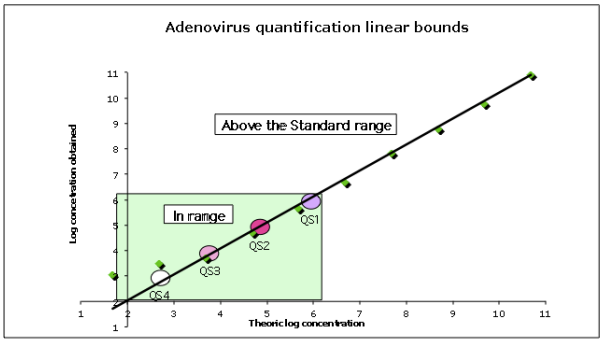
The coefficients of variation obtained for the 3 dilutions were between 2.13% and 17.34%, depending on the number of copies present in the sample.
This study demonstrates high quantification reproducibility by the ADENOVIRUS R-gene® kit within the range of significant quantification within the context of Adenovirus infections.

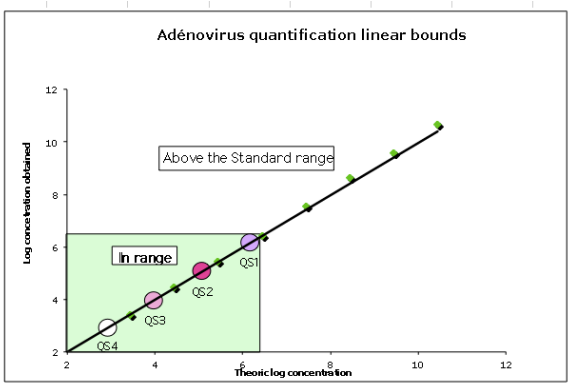
### 15.2. Study of the range of linearity of the ADENOVIRUS R-gene kit® - Ref. : 69-010

One of the problems which can arise with adenovirus diagnosis is the large difference in viral loads shown by the samples.
The linearity range of the Adenovirus R-gene® kit was determined on a range of dilutions of adenovirus type 3 culture (AdV B1) previously titrated using the Reed-Muench $TCID_{50}$ method (parent solution at $3.16.10^5$ $TCID_{50}$).
The series of dilutions were produced from nasal secretions or whole blood samples, previously validated as negative for this virus. The dilution range of 10 extended from $1/10^1$ to $1/10^{10}$.
Each dilution was extracted either with the MagNA Pure Compact (Roche) automatic unit (protocol DNA Blood 100_400), or the easyMAG (bioMérieux) automatic unit (protocol Specific B.2.0.1 (200 µL of sample, 50 µL elution), then amplified with the Adenovirus R-gene® on the ABI 7500 Fast Real-Time PCR (Applied Biosystems).
The nasopharyngeal samples underwent a pretreatment with proteinase K (10µL at 20 mg/mL for 15 minutes at 56°C).
The following graph shows the kit's linearity range :

**Linearity range on nasopharyngeal samples:**

The quantification of adenovirus serotype 3 (dilutions made from the nasopharyngeal sample) was perfectly linear from $5.19.10^3$ copies/mL to $8.25.10^{10}$ copies/mL.




**Linearity range on whole blood sample:**

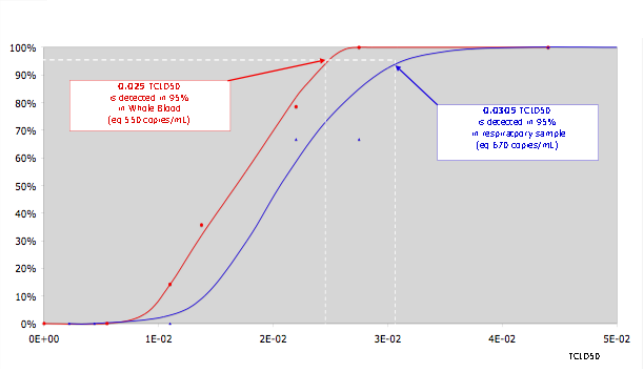
The quantification of Adenovirus serotype 3 (dilutions made in a whole blood matrix) was perfectly linear from $2.58.10^3$ copies/mL to $4.65.10^{10}$ copies/mL.

The linearity range was homogeneous for any matrix used (nasopharyngeal or whole blood sample).

ARGENE

### 15.3. Analytical sensitivity of ADENOVIRUS R-gene® kit - Ref. : 69-010

The analytical sensitivity of the kit has been determined on a dilution range of Adenovirus Type-5 cultures (AdV C) previously titrated by means of the Reed-Muench $TCID_{50}$ method.
Serial dilutions were carried out on either nasal secretions or whole blood samples previously confirmed as negative.
Each dilution was extracted 15 times using the MagNA Pure Compact (ROCHE) extraction instrument, and then amplified with the ADENOVIRUS R-gene® kit on the ABI 7500 Fast Real-Time PCR instrument (Applied Biosystems).
The opposite curve represents the probability analysis.




We found a 95% probability to detect an adenovirus 5 present at 0.0305 TCID50, equal to 670 copies/mL, i.e. 13 copies/PCR, in nasal secretions quantified with the ADENOVIRUS R-gene® standard range.

We found a 95% probability to detect an adenovirus 5 present at 0.025 TCID50, equal to 550 copies/mL, i.e. 11 copies/PCR, in whole blood quantified with the ADENOVIRUS R-gene® standard range.

We found a 5 % probability to detect an adenovirus present at 0,012 TCID50, equal to 261 copies/mL, i.e. 10 copies/PCR in nasal secretions.
We found a 5 % probability to detect an adenovirus present at 0.009 TCID50, equal to 200 copies/mL, i.e. 8 copies/PCR in whole blood.

### 15.4. Analytical specificity of Adenovirus R-gene® kit - Ref. : 69-010

The specificity of the Adenovirus primers and probes was tested by means of sequence analysis (of virus, bacteria and human sequences) in the data banks and has been validated on the following viruses:
- Human Herpesvirus : infected cells with HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7, HHV-8;
- Respiratory viruses: Influenza A/B, VRS A/B, hMPV A/B, Bocavirus

® None of these viruses were amplified with the ADENOVIRUS R-gene® kit, thus proving the specificity of the assay.

*Note : In order to prove the ADENOVIRUS R-gene® assay does not amplify human sequences, additional tests have been performed on adenovirus negative blood samples and human specimens.*

### 15.5. Serotypes validated with the ADENOVIRUS R-gene® kit - Ref. : 69-010

The 52 serotypes belonging to 7 species, form A to G, have been tested with the ADENOVIRUS R-gene® kit. The samples were either patient samples, reference strains, or viral cultures of MRC5 or 293 cells.

**Adenovirus serotypes validated with Adenovirus R-gene™kit**

| Species | | Serotypes | Validation | Species | Serotypes | Validation |
|---|---|---|---|---|---|---|
| Adenovirus A | | AdV 12 | yes | Adenovirus D | AdV 24 | yes |
| | | AdV 18 | yes | | AdV 25 | yes |
| | | AdV 31 | yes | | AdV 26 | yes |
| Adenovirus B | B1 | AdV 3 | yes | | AdV 27 | yes |
| | | AdV 7 | yes | | AdV 28 | yes |
| | | AdV 16 | yes | | AdV 29 | yes |
| | | AdV 21 | yes | | AdV 30 | yes |
| | | AdV 50 | yes | | AdV 32 | yes |
| | B2 | AdV 11 | yes | | AdV 33 | yes |
| | | AdV 14* | yes | | AdV 36 | yes |
| | | AdV 34 | yes | | AdV 37 | yes |
| | | AdV 35 | yes | | AdV 38 | yes |
| Adenovirus C | | AdV 1 | yes | | AdV 39 | yes |
| | | AdV 2 | yes | | AdV 42 | yes |
| | | AdV 5 | yes | | AdV 43 | yes |
| | | AdV 6 | yes | | AdV 44 | yes |
| Adenovirus D | | AdV 8 | yes | | AdV 45 | yes |
| | | AdV 9 | yes | | AdV 46 | yes |
| | | AdV 10 | yes | | AdV 47 | yes |
| | | AdV 13 | yes | | AdV 48 | yes |
| | | AdV 15 | yes | | AdV 49 | yes |
| | | AdV 17 | yes | | AdV 51 | yes |
| | | AdV 19 | yes | Adenovirus E | AdV 4 | yes |
| | | AdV 20 | yes | Adenovirus F | AdV 40 | yes |
| | | AdV 22 | yes | | AdV 41 | yes |
| | | AdV 23 | yes | Adenovirus G | AdV 52 | yes |

* Including the AdV 14 strain responsible for outbreaks of severe respiratory disease at the US Air Force training facility.

ARGENE

### 15.6. Test report from QCMD panel 2010

During the European Proficiency Testing of Adenoviruses, launched by the QCMD in 2010, 8 samples were blindly tested using the ADENOVIRUS R-gene® kit.
200µL of each sample was extracted with the NucliSENS easyMAG or the MagNA Pure Compact then amplified on ABI Prism® 7500 Fast with a specific amplification premix (**R10**) included in the Adenovirus R-gene kit.®
⇒ 100% (8/8) of the samples tested are in line with the expected results.

| | Sample Content | QCMD Results | | | Adenovirus R-gene® Results (Log) |
|---|---|---|---|---|---|
| | | cp/mL | Log | Sample Type | |
| ADV10-01 | ADV Type 1 | 447 | 2.65 | | 3.13 |
| ADV10-02 | ADV Type 41 | 113 | 2.05 | | 2.31 |
| ADV10-03 | ADV Type 1 | 64 121 | 4.8 | Core | 4.99 |
| ADV10-04 | ADV Type 4 | 767 | 2.88 | Core | 3.55 |
| ADV10-05 | ADV Type 1 | 4 613 | 3.66 | Core | 4.34 |
| ADV10-06 | ADV Type 1 | 4 055 | 3.60 | Core | 4.01 |
| ADV10-07 | Negative | - | - | Core | - |
| ADV10-08 | ADV Type 34 | 1 225 | 3.08 | | 3.37 |

With the Adenovirus 2010 panel, 7 samples of 8 were Adenovirus positive.

The negative sample is confirmed negative by the kit.

The detection of samples of low viral load (ADV10-02 à 113 copies/mL) attests to the high sensitivity of the kit.

In terms of quantification, one observes an excellent correlation with the QCMD results.

The results obtained on the “Core” samples, selected by the QCMD due to the clinical relevance they represent, are 100% concordant with the expected results.

### 15.7. Clinical studies carried out on the ADENOVIRUS R-gene® kit

#### 15.7.1. Clinical studies on respiratory samples - Virology Laboratory in CHU Clémenceau [Teaching Hospital] (Caen - France)

A retrospective clinical trial on 186 pre-classified nasal aspirate and 2 bronchoalveloar lavage (BAL) samples by using the routine laboratory techniques was carried out in the virology laboratory of CHU Clémenceau in Caen (France). The samples were derived from pediatric patients within the context of respiratory infections.
Among these samples, 10 positive samples for Influenza A, 10 positive for Rhinovirus and 10 positive for RSV were tested.
The study consisted of a comparison between the ADENOVIRUS R-gene® kit, DAKO immunofluorescence technique (IMAGEN® ADENOVIRUS -Ref.: K6101) and cell culture. The in-house adenovirus PCR of the virology laboratory and the conventional Adenovirus consensus PCR kit (Ref.: 67-065), were used to analyse the discordant results. The Real-Time PCR was carried out on SmartCycler 2.0 (Cepheid) after BioRobot® M48 extraction (QIAGEN).

ADENOVIRUS R-gene® / Immunofluorescence DAKO

Analysis of the results between DAKO Immunofluorescence and ADENOVIRUS R-gene® Real-Time PCR shows a 79.46% concordance between both techniques.

| | | ADENOVIRUS R-gene® + | ADENOVIRUS R-gene® - | |
|---|---|---|---|---|
| DAKO IF | + | 48 | 5 | 53 |
| | - | 33 | 99 | 132 |
| | | 81 | 104 | 185 |

Out of the 185 tested samples, 147 samples showed concordant results.
In total 38 samples gave discordant results: 5 samples were positive in DAKO Immunofluorescence which were negative with the ADENOVIRUS R-gene® PCR and 33 samples were positive in ADENOVIRUS R-gene® which were negative in DAKO Immunofluorescence.
The five positive discordant samples in DAKO Immunofluorescence were also negative in cell culture, as well as in the in-house adenovirus PCR and the conventional Adenovirus consensus PCR kit which would lead us to assume these 5 positive results in DAKO Immunofluorescence are false positives.
Out of the 33 positive discordant samples in ADENOVIRUS R-gene® 25 samples were confirmed positive by cell culture.
Out of the remaining 8 discordant results (also negative in cell culture) 2 were confirmed positive by the in-house adenovirus PCR.
The 6 remaining discordant results had low CT values between 36.91 and 41.27 cycles for 5 samples and 32.05 for the last discordant sample.

ARGENE

ADENOVIRUS R-gene® / Cell Culture

Analysis of results between cell culture and the ADENOVIRUS R-gene® Real-Time PCR shows a 91.44% concordance between both techniques.

| | | ADENOVIRUS R-gene® + | ADENOVIRUS R-gene® - | |
|---|---|---|---|---|
| Culture | + | 66 | 1 | 67 |
| | - | 15 | 105 | 120 |
| | | 81 | 106 | 187 |

Out of the 187 tested samples, 171 showed concordant results.
In total 16 samples gave discordant results: 1 sample was positive in cell culture which was negative with the ADENOVIRUS R-gene® PCR and 15 samples were positive in ADENOVIRUS R-gene® which were negative in cell culture. These discordant results were analysed again.
The discordant sample found to be negative with ADENOVIRUS R-gene® was confirmed negative in both the in-house adenovirus PCR and Adenovirus Consensus PCR (Ref. : 67-065).
Out of the 15 discordant positive samples in ADENOVIRUS R-gene® 8 samples were confirmed positive by DAKO Immunofluorescence and 2 samples were confirmed positive by the in-house adenovirus PCR.
Out of the remaining 7 discordant samples (also negative in DAKO Immunofluorescence) 2 were confirmed positive by both the in-house adenovirus PCR and Adenovirus Consensus PCR ( Ref.: 67-065).
The 5 remaining discordant samples had low CT values between 36.91 and 41.27 cycles for 4 samples and 31.23 for the last discordant sample.

These results show a high correlation between the different techniques. In addition Real-Time PCR proved to be more sensitive than DAKO Immunofluorescence.

ADENOVIRUS R-gene® / All reference techniques

Analysis of the total of all the reference techniques (DAKO, Cell Culture, in-house PCR and Adenovirus Consensus PCR) with the ADENOVIRUS R-gene® Real-Time PCR shows a concordance of 93.61%.
Out of 188 samples the resolved sensitivity shows 176 concordant results.
Out of the 12 identified discordant results, 5 were positive with ADENOVIRUS R-gene® and negative with the other techniques. These 5 discordant samples had consistently low CT values between 36.91 and 41.27 cycles for 4 samples and 32.05 for the remaining discordant sample.

| | | ADENOVIRUS R-gene® + | ADENOVIRUS R-gene® - | |
|---|---|---|---|---|
| All reference techniques | + | 76 | 7 | 83 |
| | - | 5 | 100 | 105 |
| | | 81 | 107 | 188 |

Coefficient of concordance : 93.61%

Out of the 7 samples found to be positive with one of the reference techniques and negative with ADENOVIRUS R-gene®, only 5 were positive with DAKO Immunofluorescence and only 1 was positive with the in-house PCR. The remaining discordant samples underwent specific treatment prior to extraction.

We recommend a qualitative analysis of adenoviruses in respiratory samples since the exact amount of cells in a sample can not be determined. Moreover, the sample volume is variable, depending on how the sample was obtained.

In addition, high viral loads are frequently found in this sample type (74% of samples tested positive with ADENOVIRUS R-gene® showed a CT value below 22 cycles) leading to inhibition of ampification due to an excess of materials (only 25% of positive samples did not present signs of inhibition after analysis with the ADENOVIRUS R-gene® inhibition control).

**15.7.2. Clinical study on Plasma, Whole Blood and BAL– Virology Laboratory of CHU Necker Enfants Malades [Teaching Hospital for Sick Children] (Paris - France)**

A retrospective clinical study was carried out in the virology laboratory of the CHU Necker Enfants Malades (Paris) on 31 whole blood samples, 200 plasma samples and 16 BAL samples. The samples were derived from pediatric patients within the context of monitoring bone-marrow transplant patients.
These samples had been previously classified with routine laboratory techniques on ABI Prism® 7300 (Applied Biosystems) using the laboratory reference Real-Time PCR technique after MagNA Pure LC extraction (ROCHE).

Qualitative analysis:

Analysis of the results between the reference Real-Time technique and the ADENOVIRUS R-gene® Real-Time PCR shows a 91.1% concordance between both techniques.

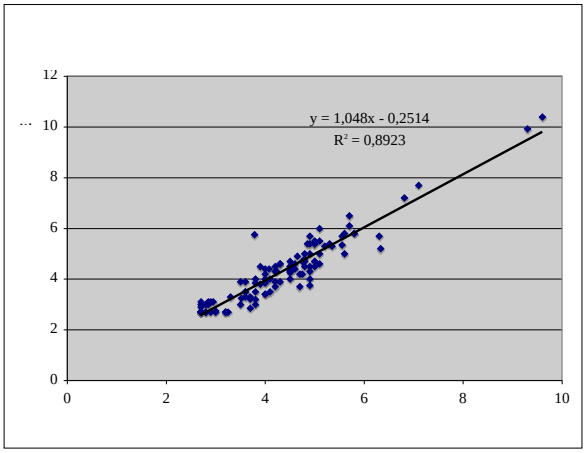
| | | ADENOVIRUS R-gene® + | ADENOVIRUS R-gene® - | |
|---|---|---|---|---|
| PCR Adenovirus Necker | + | 120 | 19 | 139 |
| | - | 3 | 105 | 108 |
| | | 123 | 124 | 247 |

Coefficient of concordance = 91.1%

Out of the 247 tested samples, 225 samples showed concordant results.
Analysis of the 22 discordant samples detected as positive with only one of the 2 techniques demonstrated that 16 samples showed a viral load lower than the threshold of 500 copies/mL with the reference method.
The other 6 discordant samples presented viral loads between 1200 and 4800 copies/mL (low viral loads) and can be explained by the fact that the 2 techniques were not carried out on the same aliquots.
These samples could not be re-tested simultaneously on the same aliquot due to a shortage of material.

ARGENE

Quantitative Analysis:
Quantitative analysis of the results between the reference Real-Time PCR technique and the ADENOVIRUS R-gene® Real-Time PCR was carried out on confirmed positive samples. This analysis showed a correlation coefficient of 0.89 between both techniques.
The slope of the regression line (1.048) shows high correlation levels between the quantification of the 2 techniques




**15.7.3. Clinical trial on CSF, biopsies, whole blood and stool – Virology Laboratory of the CHU Nancy (Nancy – France)**
A retrospective clinical trial was carried out on 22 CSF samples, 24 digestive biopsies, 78 whole blood samples and 22 stool samples in the virology laboratory of the CHU Nancy.
The samples were collected from patients as part of routine laboratory activity and characterised in advance using the laboratory's standard Real-Time PCR technique, a technique described by Heim et al.
These samples were analysed on the ABI Prism® 7000 (Applied Biosystems) instrument using:
- MagNA Pure LC (ROCHE) automatic extraction for whole blood and stool samples
- QIAamp DNA Blood Mini Kit (QIAGEN) manual extraction for CSF and biopsies and QIAamp DNA Stool Mini Kit (QIAGEN) for stool samples.

Qualitative results:
The results are presented according to the type of sample. All the samples are merged in the table below.

| CSF | | Heim et al. + | Heim et al. - |
|---|---|---|---|
| ADENOVIRUS R-gene® | + | 1 | 1 |
| | - | 0 | 20 |

| Digestive biopsies | | Heim et al. + | Heim et al. - |
|---|---|---|---|
| ADENOVIRUS R-gene® | + | 11 | 0 |
| | - | 2 | 11 |

| Whole blood | | Heim et al. + | Heim et al. - |
|---|---|---|---|
| ADENOVIRUS R-gene® | + | 55 | 2 |
| | - | 2 | 19 |

| Stool | | Heim et al. + | Heim et al. - |
|---|---|---|---|
| ADENOVIRUS R-gene® | + | 17 | 2 |
| | - | 0 | 3 |

| All specimen | | Heim et al. + | Heim et al. - |
|---|---|---|---|
| ADENOVIRUS R-gene® | + | 86 | 5 |
| | - | 4 | 55 |

Coefficient of concordance = 94,0%

Analysis of discordant results

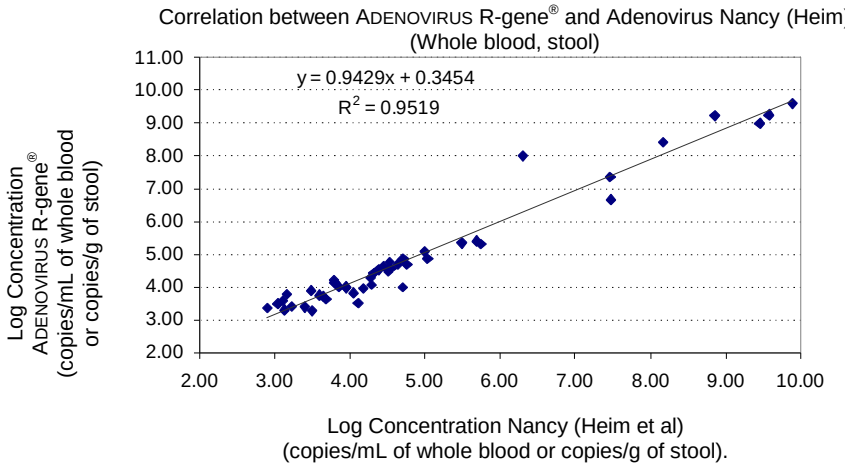
All 9 discordant samples are low positives, of which the two highest were recorded at 3.46 log and 3.13 log.

| Discrepant | Specimen type | Heim et al. | Adenovirus R-gene™ |
|---|---|---|---|
| 1 | CSF | Neg | 3.46 Log |
| 2 | Biopsy | "Detectable" | Neg |
| 3 | Biopsy | "Detectable" | Neg |
| 4 | Whole blood | "Detectable" | Neg |
| 5 | Whole blood | 3.13 Log | Neg |
| 6 | Whole blood | Neg | "Detectable" |
| 7 | Whole blood | Neg | "Detectable" |
| 8 | Stool | Neg | "Detectable" |
| 9 | Stool | Neg | "Detectable" |

ARGENE

Quantitative results:

Quantitative analysis of the results between the laboratory's Real-Time PCR technique (Heim et al) and the ADENOVIRUS R-gene® Real-Time PCR was carried out on the whole blood and stool samples quantified by the 2 techniques. This analysis gives a coefficient of correlation of 0.95 between the 2 techniques.
The slope of the regression line (0.94) shows a high level of correlation between quantification using the 2 techniques.



ARGENE

# 16. References

*Programming and analysis assistance sheets, per device type, downloadable at www.biomerieux.com/techlib*

*Detailed protocol "Worksheet easyMAG Viral Whole Blood extraction protocol" from bioMérieux.*

### Publications :

1) H. JEULIN, A. SALMON, P.BORDIGONI and V. VENARD
Comparison of In-House Real-Time Quantitative PCR to the Adenovirus R-Gene Kit for Determination of Adenovirus Load in Clinical Samples
Journal of Clinical Microbiology, Sept. 2010, p. 3132–3137
2) TATE JE, BUNNING ML, LOTT L, LU X, SU J, METZGAR D, BROSCH L, PANOZZO CA, MARCONI VC, FAIX DJ, PRILL M, JOHNSON B, ERDMAN DD, FONSECA V, ANDERSON LJ, WIDDOWSON MA
Outbreak of Severe Respiratory Disease Associated with Emergent Human Adenovirus Serotype 14 at a US Air Force Training Facility in 2007.
J Infect Dis. 15;199(10):1419-1426, May 2009.
3) ECHAVARRIA M.
Adenoviruses in Immuno compromised Host.
Clinical Microbiology Reviews, Oct. 2008, p.704-715.
4) VABRET A., GOUARIN S., JOANNES M., BARRANGER C., PETITJEAN J., CORBET S., BROUARD J., DUHAMEL JF., GUILLOIS B. and FREYMUTH F.
Development of a PCR and hybridization-based assay (PCR ADENOVIRUS CONSENSUS) for the detection and the species identification of adenoviruses in respiratory specimens.
J. Clin virology 31(2):116-22, Oct 2004.
5) HEIM A., EBNET C., HARSTE G., PRING-AKERBLON P.
Rapid and Quantitative Detection of Human Adenovirus DNA by Real-Time PCR.
Journal of Medical Virology 70 : 228-239, 2003.

### Posters :

1) MAGRO S., ECHAVARRIA M., BERRIOT A., MARECHAL P., BARRANGER C. and JOANNES M.
Development of a new diagnostic tool for the quantification of ADENOVIRUSES by real time PCR.
Poster CVS 2010, Buenos Aires.
2) JEULIN H., SALMON A., BENSOUSSAN D., BORDIGONI P., VENARD V.
Prognostic value of qPCR for Adenovirus detection in stool samples compared with antigen detection and cell culture in hematopoietic cell transplant recipients and eveluation of adenovirus R-gene® kit.
Poster, CVS 2010, Buenos aires.
3) MAGRO S., ECHAVARRIA M., RESA C., MARECHAL P., BARRANGER C., JOANNES M.
Development of a new diagnostic tool for the quantification of Adenoviruses by real time PCR.
CVS, USA, April 2009.
4) ECHAVARRIA M., GALANTE M., HERRERA F., VIDELA C., RICARTE C., TEMPORITI E., CARBALLAL G., BONVEHI P.
Outbreak of adenoviruses keratoconjuntivitis in health care workers : coinfection with different adenoviruses species.
CVS, USA, April 2009.
5) MAGRO S., ECHAVARRIA M., RESA C., MARECHAL P., BARRANGER C., JOANNES M.
Development of a new diagnostic tool for the quantification of Adenoviruses by real time PCR.
ESCV 2009, Istambul.
6) H. JEULIN, S. MAGRO, M. JOANNES, M. DIDIER, S. PERNET, V. VENARD
Comparison of Adenovirus R-gene®Kit vs In-House Real Time PCR for Determination of Adenovirus Viral Load
Poster ESCV 2009, Istambul.

# 17. Related products

- DICO Extra r-gene® ref.: 71-101
- Colour compensation r-gene® ref.: 71-103
- CELL Control r-gene® ref.: 71-106
- ADENOVIRUS CONSENSUS ref.: 67-065

ARGENE

## 18. Index of symbols

| Symbol | Meaning |
|---|---|
| REF | Catalogue number |
| IVD | In Vitro Diagnostic Medical Device |
|  | Manufacturer |
|  | Temperature limitation |
|  | Use by |
| LOT | Batch code |
|  | Consult Instructions for Use |
|  | Contains sufficient for <n> tests |
|  | Protect from light |
|  | Keep dry |
| CE 0086 | Identification of notified body |



BIOMÉRIEUX

**bioMérieux SA**
Chemin de l'Orme
69280 Marcy-l'Etoile - France

RCS LYON 673 620 399
Tel. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com