

Instructions for use

AltoStar[®] CMV PCR Kit 1.5

08/2021 EN

AltoStar[®]

CMV PCR Kit 1.5

For use with

CFX96™ Deep Well Dx System (Bio-Rad)

CFX96™ Dx System (Bio-Rad)

ABI Prism[®] 7500 SDS (Applied Biosystems)

LightCycler[®] 480 Instrument II (Roche)

QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)

Rotor-Gene[®] Q5/6 plex Platform (QIAGEN)



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1. About these instructions for use

These instructions for use guide the user in utilizing the AltoStar® CMV PCR Kit 1.5. Chapters 1–6 and 9–14 contain general information and instructions which apply to each workflow used with the AltoStar® CMV PCR Kit 1.5. Chapter 7 gives instructions on how to use the product with the AltoStar® Automation System AM16 (Hamilton; in the following summarized as AltoStar® AM16) with the AltoStar® Connect software (Version 1.7.4 or higher, Hamilton) for automated PCR setup and on the CFX96™ Deep Well Dx System* (Bio-Rad, in the following summarized as CFX96™ DW Dx) with the CFX Manager™ Dx software (Version 3.1, Bio-Rad) for real-time PCR. Chapter 8 gives instructions regarding how to use the AltoStar® CMV PCR Kit 1.5 in conjunction with other nucleic acid extraction methods and real-time PCR instruments. For details on the use of the AltoStar® AM16, the AltoStar® Connect software, the AltoStar® Purification Kit 1.5, the AltoStar® Internal Control 1.5 and the CFX96™ DW Dx, refer to the respective instructions for use listed below:

- AltoStar® Automation System AM16 Operator's Manual IVD (Hamilton)
- AltoStar® Connect Software Manual IVD (Hamilton)
- Instructions for use AltoStar® Purification Kit 1.5
- Instructions for use AltoStar® Internal Control 1.5
- CFX96™ Dx and CFX96™ Deep Well Dx Systems Operation Manual (Bio-Rad)

* “CFX96™ Deep Well Dx System” is the new brand name for the IVD version of the CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad).

Throughout this manual, the terms CAUTION and NOTE have the following meanings:

CAUTION



Highlights operating instructions or procedures which, if not followed correctly, may result in personal injury or impact product performance. Contact altona Diagnostics technical support for assistance.

NOTE



Information is given to the user that is useful but not essential to the task at hand.

Read the instructions for use carefully before using the product.

2. Intended use

The AltoStar® CMV PCR Kit 1.5 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of cytomegalovirus (CMV) specific DNA in human plasma, whole blood and urine. It is intended to be used as an aid for diagnosis of CMV infection and for monitoring of the CMV load in individuals with CMV infection.

The results generated with the AltoStar® CMV PCR Kit 1.5 have to be interpreted in conjunction with other clinical and laboratory findings.

The AltoStar® CMV PCR Kit 1.5 is intended for use by professional users trained in molecular biological techniques and *in vitro* diagnostic procedures.

3. Kit content

The AltoStar® CMV PCR Kit 1.5 contains the following components:

Table 1: Kit components

Lid color	Component	Number of tubes	Nominal volume [µl/tube]
Blue	Master A ¹⁾	8	60 ²⁾
Purple	Master B ¹⁾	8	180 ³⁾
Red	QS1 ⁴⁾	2	250
Red	QS2 ⁴⁾	2	250
Red	QS3 ⁴⁾	2	250
Red	QS4 ⁴⁾	2	250
White	NTC ⁵⁾	2	250

¹⁾ Contains biological material of animal origin

²⁾ Contains an additional volume of 25 µl to compensate the dead volume of the AltoStar® AM16's liquid handling

³⁾ Contains an additional volume of 55 µl to compensate the dead volume of the AltoStar® AM16's liquid handling

⁴⁾ Quantification Standard (Positive Control)

⁵⁾ No Template Control (Negative Control)

CAUTION



Before first use check the product and its components for completeness with respect to number, type and filling. Do not use a defective or incomplete product, product performance could be compromised.

The AltoStar® CMV PCR Kit 1.5 contains enough reagents to perform 96 reactions in a maximum number of 8 runs.

The product is shipped on dry ice. Upon receipt and before first use, check the product and its components for:

- Integrity
- Completeness with respect to number, type and filling
- Correct labeling
- Expiration date
- Frozen state
- Clarity and absence of particles

If one or more product components are not frozen upon receipt, if tubes have been compromised during shipment or are missing, contact altona Diagnostics technical support for assistance (see chapter 12. Technical support).

4. Storage and handling

All reagents included in the AltoStar® CMV PCR Kit 1.5 are ready-to-use solutions.

4.1 Storage

All components of the AltoStar® CMV PCR Kit 1.5 must be stored at -25 °C to -15 °C upon arrival.

CAUTION



Improper storage conditions could compromise product performance.

CAUTION



Do not use products beyond the expiration date. The use of expired products could compromise product performance.

4.2 Handling

CAUTION



Do not exceed thaw-freeze-sequence and handling durations specified in these instructions for use, as this could compromise product performance.

CAUTION



Improper handling of product components and samples may cause contamination and could compromise product performance:

- Do not interchange vial or bottle caps.
- Store positive and/or potentially positive material separated from the kit components.
- Use separated working areas for sample preparation/reaction setup and amplification/detection activities.
- Always dispose gloves after handling positive and/or potentially positive material.
- Do not open the PCR plates and/or tubes post amplification.

CAUTION



Do not mix components from different kit lots. The use of different kit lots could compromise product performance.

4.2.1 Master A and Master B

After thawing, Master A and Master B are stable for 5 hours at up to +30 °C.

NOTE



If Master A and Master B were thawed but not used, they can be refrozen and thawed again once for later runs. If opened, discard the lids and use new lids to avoid contamination of the reagents.

4.2.2 Quantification Standards and No Template Control

1. After thawing, the Quantification Standards (QSs) and the No Template Control (NTC) are stable for 5 hours at up to +30 °C.
2. Discard the lids of the QSs and NTC tubes at each use and use new lids to avoid contamination of the reagents.
3. After use close the QSs and NTC tubes with new lids and freeze them immediately.
4. Do not exceed the following thaw-freeze-sequence for each QS and NTC tube:
Thaw 1 → Freeze 1 → Thaw 2 → Freeze 2 → Thaw 3 → Freeze 3 → Thaw 4

5. Product description

The AltoStar® CMV PCR Kit 1.5 is an *in vitro* diagnostic test for the detection and quantification of CMV specific DNA in human plasma, whole blood and urine.

It is based on real-time PCR technology, utilizing polymerase chain reaction (PCR) for the amplification of CMV specific target sequences and fluorescently labeled target specific probes for the detection of the amplified DNA.

In addition to the CMV DNA specific amplification and detection system the assay includes oligonucleotides for the amplification and detection of the internal control (in the following abbreviated as IC; AltoStar® Internal Control 1.5).

Probes specific for CMV DNA are labeled with the fluorophore FAM™. The probe specific for the IC is labeled with a fluorophore (JOE™) detectable in the e.g. VIC™ channel.

Using probes linked to distinguishable dyes enables the parallel detection of CMV specific DNA and the IC in corresponding detection channels of the real-time PCR instrument used.

5.1 Background

The human cytomegalovirus (CMV, human herpesvirus 5) is a member of the family *Herpesviridae* and belongs to the subfamily *Betaherpesvirinae*. It consists of an icosahedral capsid with a linear double-stranded DNA genome of approximately 230 kbp, a surrounding tegument and an outer envelope. CMV has a worldwide distribution and infects humans of all ages, with no seasonal or epidemic patterns of transmission. The seroprevalence of CMV increases with age in all populations and ranges from 40 to 100 %. Similar to infections with other herpesviruses, primary infection with CMV results in the establishment of a persistent or latent infection. Reactivation of the virus can occur in response to different stimuli, particularly immunosuppression. The vast majority of CMV infections are asymptomatic or subclinical, but congenital infections and infections in immunocompromised patients may be symptomatic and serious. In immunocompromised hosts, such as transplant recipients, HIV-infected or cancer patients, a CMV infection or reactivation may become a life-threatening disseminated disease [1,2,3].

5.2 Component description

5.2.1 Master A and Master B

Master A and Master B contain all components (PCR buffer, DNA polymerase, magnesium salt, primers and probes) to allow the PCR mediated amplification and target detection of CMV specific DNA and of the IC in one reaction setup.

5.2.2 Quantification Standards

The QSs contain standardized concentrations of CMV specific DNA (see table 2). They were calibrated against the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) [4]. The QSs are used to verify the functionality of the CMV DNA specific amplification and detection system as well as to generate a standard curve, which allows the quantification of CMV specific DNA in a sample.

Table 2: Quantification Standards

Quantification Standard	Concentration [IU/μl]
QS1	1.00E+04
QS2	1.00E+03
QS3	1.00E+02
QS4	1.00E+01

5.2.3 No Template Control

The NTC contains no CMV specific DNA but does contain the IC template. The NTC is used as negative control for the CMV DNA specific real-time PCR and indicates possible contamination of Master A and Master B.

5.3 Workflows

5.3.1 AltoStar® Workflow

The AltoStar® Workflow comprises the following IVD products:

- AltoStar® Automation System AM16 (Hamilton)
- AltoStar® Connect software version 1.7.4 or higher (Hamilton)
- CFX96™ Deep Well Dx System (Bio-Rad) with CFX Manager™ Dx software version 3.1 (Bio-Rad)

The workflow includes the following steps:

1. Programming an AltoStar® run.
2. Purification run on the AltoStar® AM16 using the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5.
3. PCR setup run on the AltoStar® AM16 using the AltoStar® CMV PCR Kit 1.5.
4. Real-time PCR run on a CFX96™ DW Dx.

For further details on steps 3 and 4 of the workflow, refer to chapter 7. Using the AltoStar® CMV PCR Kit 1.5 with the AltoStar® Workflow. All sample types and sample volumes specified for use with the AltoStar® Purification Kit 1.5 can be processed simultaneously on the AltoStar® AM16. Each sample can be analyzed with as many real-time PCR assays in parallel as the available eluate allows.

NOTE



Assays with differing PCR temperature profiles are automatically sorted to separate PCR plates.

5.3.2 Other workflows

The AltoStar® CMV PCR Kit 1.5 can be used with compatible workflows (manual or automated). Real-time PCR instruments that have been validated for use with the AltoStar® CMV PCR Kit 1.5 are listed in chapter 5.3.2.2 Real-time PCR instruments. Use of alternative extraction procedures has to be validated by the user.

5.3.2.1 Nucleic acid extraction

The AltoStar® CMV PCR Kit 1.5 can be used with nucleic acid extraction systems other than the AltoStar® AM16. Alternative procedures used for nucleic acid extraction have to be validated by the user. Refer to chapter 8.2.1 Sample preparation for instructions regarding how to use the AltoStar® Internal Control 1.5 in combination with other extraction methods than the AltoStar® Purification Kit 1.5.

5.3.2.2 Real-time PCR instruments

The AltoStar® CMV PCR Kit 1.5 has been developed and validated with the following real-time PCR instruments:

- CFX96™ Deep Well Dx System (Bio-Rad)
- CFX96™ Dx System (Bio-Rad)
- ABI Prism® 7500 SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)

Using one of the real-time PCR instruments listed above (except for the CFX96™ Deep Well Dx System), the PCR setup, the programming of the instrument as well as the data analysis have to be performed manually (for details see chapters 8.2.2 Master mix setup to 8.2.5 Data analysis).

5.4 Samples

5.4.1 Sample types

The following sample types are validated for use with the AltoStar® CMV PCR Kit 1.5:

- Human EDTA plasma
- Human citrate plasma
- Human EDTA whole blood
- Human citrate whole blood
- Human urine

CAUTION



Do not use other sample types! The use of other sample types could compromise product performance.

5.4.2 Sample collection and handling

Blood has to be collected with commercially available standard blood collection systems (e.g. Sarstedt, Becton Dickinson, Greiner or equivalent). Tube contents should be mixed directly after sample collection. The blood samples should be shipped cooled at +2 °C to +8 °C. Transport should occur following the local and national instructions for the transport of biological material. Before use blood should not be stored for more than 48 hours at room temperature (+20 °C to +25 °C), 3 days at +2 °C to +8 °C or 7 days at -25 °C to -15 °C.

For generation of EDTA plasma, whole blood should be centrifuged according to the instructions provided by the manufacturer of the collection system within 24 hours after collection. Before use EDTA plasma should not be stored for more than 48 hours at room temperature (+20 °C to +25 °C), 5 days at +2 °C to +8 °C or 2 months at -25 °C to -15 °C.

Urine samples must be collected in a sterile container. Urine samples should be stored at +2 °C to +8 °C for no longer than 24 hours.

CAUTION



Always treat samples as infectious and (bio-)hazardous material in accordance with safety and laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.

NOTE



Frozen storage of samples does not compromise kit performance. When working with frozen samples, make sure samples are completely thawed and properly mixed before use.

6. Warnings, precautions and limitations

- Before first use check the product and its components for completeness with respect to number, type and filling. Do not use a defective or incomplete product, product performance could be compromised.
- Improper storage conditions could compromise product performance.
- Do not use products beyond the expiration date. The use of expired products could compromise product performance.
- Do not exceed thaw-freeze-sequence and handling durations specified in these instructions for use, as this could compromise product performance.
- Improper handling of product components and samples may cause contamination and could compromise product performance:
 - Do not interchange vial or bottle caps.
 - Store positive and/or potentially positive material separated from the kit components.
 - Use separated working areas for sample preparation/reaction setup and amplification/detection activities.
 - Always dispose gloves after handling positive and/or potentially positive material.
 - Do not open the PCR plates and/or tubes post amplification.
- Do not mix components from different kit lots. The use of different kit lots could compromise product performance.
- Do not use other sample types! The use of other sample types could compromise product performance.
- Always treat samples as infectious and (bio-)hazardous material in accordance with safety and laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.
- Storage of eluates under wrong conditions may lead to degradation of the CMV target sequence and could compromise product performance.
- Do not use an assay protocol version other than the one indicated on the 2D barcode in these instructions for use. Using an incorrect assay protocol version could compromise product performance.
- A lack of centrifugation of the product components after thawing may cause contamination with reagent residues in the lids and could compromise product performance.

- Do not reuse tube caps to avoid contamination of the reagents, as this could compromise product performance.
- As with any diagnostic test, results shall be interpreted in consideration of all clinical and laboratory findings.
- The presence of PCR inhibitors (e.g. heparin) could cause false negative or invalid results.
- Do not use different volumes of Master A and Master B for master mix setup than specified in these instructions for use, as this could compromise product performance.
- Do not exceed the PCR mix storage time, as this could compromise product performance.
- Do not mix up samples or sample IDs during PCR setup or transfer to the PCR instrument. This could lead to false positive or false negative results due to incorrect assignment of the samples.
- Do not use other cycling conditions than specified in these instructions for use, as this could compromise product performance.
- Do not use other control settings for data analysis than specified in these instructions for use, as this could cause incorrect IVD examination results.
- In case the sample contains other pathogens than CMV, competition with the target amplification or cross-reactivities may occur, causing incorrect IVD examination results.
- Disposal of hazardous and biological waste shall comply with local and national regulations to avoid environmental contamination.
- Potential mutations within the target regions of the CMV genome covered by primers and/or probes used in the kit may result in underquantification and/or failure to detect the presence of the pathogen.

7. Using the AltoStar® CMV PCR Kit 1.5 with the AltoStar® Workflow

The following part of these instructions for use describes the use of the AltoStar® CMV PCR Kit 1.5 in conjunction with the AltoStar® Workflow. The AltoStar® Workflow comprises different IVD products (AltoStar® AM16, the AltoStar® Connect software, the AltoStar® Purification Kit 1.5, the AltoStar® Internal Control 1.5 and the CFX96™ DW Dx). Use of those products is described in detail in the respective instructions for use.

- AltoStar® Automation System AM16 Operator's Manual IVD (Hamilton)
- AltoStar® Connect Software Manual IVD (Hamilton)
- Instructions for use AltoStar® Purification Kit 1.5
- Instructions for use AltoStar® Internal Control 1.5
- CFX96™ Dx and CFX96™ Deep Well Dx Systems™ Operation Manual (Bio-Rad)

7.1 Sample volume

The AltoStar® CMV PCR Kit 1.5 is validated for nucleic acid purifications from a sample volume of 500 µl when using the AltoStar® AM16. Additional sample volume has to be provided to account for the dead volume of the sample tube used (see chapter 7.2 Sample tubes).

7.2 Sample tubes

Sample tubes suitable for use on the AltoStar® AM16 can be purchased from Altona Diagnostics (7 ml tube with cap, 82 x 13 mm, VK000010). Other sample tubes can be tested for applicability by the user. For details, refer to the instructions for use of the AltoStar® Purification Kit 1.5.

7.3 Sample barcodes

For automated sample identification by the AltoStar® AM16 all sample tubes must be labeled with a suitable barcode. For details, refer to the instructions for use of the AltoStar® Purification Kit 1.5.

7.4 Material and devices required but not provided for the AltoStar® Workflow

The material and devices shown in table 3 must be ordered from altona Diagnostics.

Table 3: Required material and devices

Material	Description	Order No.
AltoStar® Molecular Diagnostic Workflow	Product bundle containing the AltoStar® Automation System AM16, the AltoStar® Connect software (Version 1.7.4 or higher) and IT hardware	AM16
AltoStar® Detection	Product bundle containing the CFX96™ Deep Well Dx System with CFX Manager™ Dx Software (Version 3.1), a barcode scanner and IT hardware	DT16
AltoStar® Purification Kit 1.5	Nucleic acid isolation and purification chemistry for use with the AltoStar® Automation System AM16	PK15-16/ PK15-46
AltoStar® Internal Control 1.5	Nucleic acid extraction and PCR amplification and detection control	IC15-16/ IC15-46
PCR Plate	Semi-skirted, barcoded 96 multi-well plate with white wells	VK000005
PCR Plate Sealing Foil	Sealing foil for the PCR plate	VK000006
1,000 µl CO-RE Tips	1,000 µl filter tips for use with the AltoStar® Automation System AM16	VK000007
300 µl CO-RE Tips	300 µl filter tips for use with the AltoStar® Automation System AM16	VK000008
Pooling Tube	Barcoded tube for pooling of PCR reagents	VK000002
Waste Bag	Autoclavable sterile bag for use with the AltoStar® Automation System AM16	VK000009
Screw Cap - red	Screw cap for QS1–QS4 tubes	VK000012
Screw Cap - blue	Screw cap for Master A tubes	VK000013
Screw Cap - purple	Screw cap for Master B tubes	VK000015

Material	Description	Order No.
Screw Cap - white	Screw cap for NTC tubes	VK000016

Table 4: Additional laboratory material and devices

Material	Description	Order No.
Plate Sealer	e.g. AltoStar® Plate Sealer	VK000023
	e.g. PX1 Plate Sealer (Bio-Rad)	VK000033

7.5 General material and devices

- Vortex mixer
- Powder-free gloves (disposable)
- Centrifuge for centrifugation of the AltoStar® CMV PCR Kit 1.5 components
- Centrifuge for centrifugation of PCR plates

7.6 Procedure

7.6.1 Overview of the AltoStar® Workflow

The steps of the complete AltoStar® Workflow are summarized in table 5. Information on specific settings for use with the AltoStar® CMV PCR Kit 1.5 are provided in chapter 7.6.2 Programming an AltoStar® run. For detailed instructions for steps 1–5, refer to the instructions for use of the AltoStar® Purification Kit 1.5, the AltoStar® Connect software and the AltoStar® AM16.

Steps 6–11 are described in more detail in chapters 7.6.3 Starting a PCR setup run to 7.6.10 Export of PCR results for manual result interpretation.

Table 5: Overview of the AltoStar® Workflow

Step	Action
1. Start the AltoStar® AM16	<ul style="list-style-type: none"> • Switch on the AltoStar® AM16. • Switch on the computer and the monitor. • Start the AltoStar® Connect software.
2. Perform maintenance	<ul style="list-style-type: none"> • In the menu bar click Application → Instrument Maintenance. <ul style="list-style-type: none"> ◦ If weekly maintenance is due, click Start Weekly Maintenance. ◦ If daily maintenance is due, click Start Daily Maintenance. • Follow the on-screen instructions for the maintenance process.
3. Program an AltoStar® run	<ul style="list-style-type: none"> • In the menu bar click Program Run → Program Run (AltoStar® Purification). Alternatively, go back to the Start screen and click the Program Run button. • Enter samples or import from the LIMS. • Select the following assay for the samples unless already imported from the LIMS: <ul style="list-style-type: none"> ◦ AltoStar® CMV PCR Kit 1.5 • Click the Create Run button in the tool bar to create the AltoStar® run.

Step	Action
<p>4. Start a purification run</p>	<ul style="list-style-type: none"> • In the menu bar click Purification → Start Purification. Alternatively, go back to the Start screen and click the Start Purification button. • Select the purification run to be started to display the samples included in the selected purification run. • Prepare the purification reagents: <ul style="list-style-type: none"> ◦ Ensure that the purification reagents to be used have the same loading number (except AltoStar® Internal Control 1.5) and are not expired. ◦ If precipitates are visible in the Lysis Buffer, heat it ($\leq +50\text{ }^{\circ}\text{C}$) until completely dissolved. ◦ Thaw the IC (AltoStar® Internal Control 1.5) and vortex for 5 seconds. ◦ Vortex the Magnetic Beads for 5 seconds without wetting the lid. • Prepare the samples for the purification run to be started as described in the instructions for use of the AltoStar® Purification Kit 1.5. • Click the Start Run button in the tool bar. • Follow the loading dialogs and load the instrument accordingly. • Confirm the Loading complete message with OK or wait 10 seconds. <p>The system will now perform the purification run automatically.</p>
<p>5. Finish the purification run</p>	<ul style="list-style-type: none"> • Make sure the loading tray is empty and confirm the Run finished dialog with OK. • Follow the instructions in the Maintenance dialog and confirm with OK. • Seal and store the components of the AltoStar® Purification Kit 1.5 that can be reused. <p>The eluates in the unsealed eluate plate are stable at room temperature (max. $+30\text{ }^{\circ}\text{C}$) for a total of 4 hours.</p> <ul style="list-style-type: none"> • If the associated PCR setup run is not started right away, seal the eluate plate with an Eluate Plate Sealing Foil and store at $+2\text{ }^{\circ}\text{C}$ to $+8\text{ }^{\circ}\text{C}$ for up to 24 hours. • View the purification run results to confirm successful processing of each sample.

Step	Action
<p>6. Start a PCR setup run</p>	<ul style="list-style-type: none"> • In the menu bar, click PCR Setup → Start PCR Setup. Alternatively, go back to the Start screen and click the Start PCR Setup button. • Select the PCR setup run to be started in order to display the eluate plate and reagents included in the selected PCR setup run. • Prepare the PCR reagents: <ul style="list-style-type: none"> ◦ Ensure that masters and controls to be used are from the same kit lot and are not expired. ◦ Thaw the required amount of master and control tubes, vortex briefly and spin down in a centrifuge. • If the eluate plate is sealed, briefly centrifuge the plate and unseal carefully. • Click the Start Run button in the tool bar. • Follow the Loading dialog and load the instrument accordingly. • Confirm the Loading complete message with OK or wait 10 seconds. <p>The system will now perform the PCR setup run automatically.</p>
<p>7. Finish the PCR setup run</p>	<ul style="list-style-type: none"> • Make sure the loading tray is empty and confirm the Run finished dialog with OK. • Follow the instructions in the Maintenance dialog and confirm with OK. • Close and store the components of the AltoStar® CMV PCR Kit 1.5 that can be reused. • View the PCR setup run results to confirm successful processing of each sample.
<p>8. Seal the PCR plate</p>	<ul style="list-style-type: none"> • Seal the PCR plate with a PCR Plate Sealing Foil.

Step	Action
9. Start the PCR run	<ul style="list-style-type: none"> • Switch on the CFX96™ DW Dx, the attached computer and the monitor. • Start the CFX Manager™ Dx software. • Open the CFX96™ DW Dx. • Centrifuge the PCR plate and insert it into the CFX96™ DW Dx. • Select File → Open → LIMS File... from the menu bar. • Scan the barcode of the PCR plate with the handheld barcode scanner. • Close the CFX96™ DW Dx. • Click the Start Run button to start the PCR run. Name and save the PCR run file. <p>The CFX96™ DW Dx will now perform the PCR run automatically.</p>
10. Separate assays for individual analysis	<ul style="list-style-type: none"> • Separate all assays in the PCR run into distinct well groups.
11. Analyze the data and interpret the PCR run results	<p>For each well group individually:</p> <ul style="list-style-type: none"> • Perform baseline correction in all wells for all detection channels used. • Exclude wells with irregular PCR signals. • Set the thresholds of all detection channels according to the controls. • Exclude wells containing invalid data. • Generate the LIMS result file for export of results to the LIMS. • Generate the result report for manual result interpretation.

CAUTION

Storage of eluates under wrong conditions may lead to degradation of the CMV target sequence and could compromise product performance.

7.6.2 Programming an AltoStar® run

For detailed information on starting an AltoStar® run, refer to the instructions for use of the AltoStar® Purification Kit 1.5, the AltoStar® Connect software and the AltoStar® AM16. Specific settings for use with the AltoStar® CMV PCR Kit 1.5 are listed below:

- For quantitative assay application QS1–4 and NTC are selected and for qualitative assay application QS4 and NTC are selected.
- The required sample volume is 500 µl plus the dead volume for the respective sample tube (see chapters 7.1 Sample volume and 7.2 Sample tubes).
- The required eluate volume for the AltoStar® CMV PCR Kit 1.5 is 10 µl.
- Make sure that the correct assay protocol version is used for the run. For information on the current protocol version see chapter 16. Assay protocol for the AltoStar® Connect software and information for LIMS integration. The respective assay protocol is encoded in the 2D barcode displayed there. For information on purification and assay protocol import into the AltoStar® Connect software, refer to the respective instructions for use.

CAUTION



Do not use an assay protocol version other than the one indicated on the 2D barcode in these instructions for use. Using an incorrect assay protocol version could compromise product performance.

7.6.3 Starting a PCR setup run

1. Select **PCR Setup** → **Start PCR Setup** in the menu bar. Alternatively, go back to the Start screen of the AltoStar® Connect software and select the **Start PCR Setup** button. The Start PCR Setup Run screen is displayed.

The pending PCR setup runs are displayed in the Programmed PCR Setup Runs table on the left side of the screen.

2. Select the PCR setup run to be started in the Programmed PCR Setup Runs table.
 - The samples included in the selected PCR setup run are displayed in the table on the top right side of the screen (Samples in selected PCR Setup Run).

- The Qs and controls required for the selected PCR setup run are displayed in the table on the middle right side of the screen (Controls in selected PCR Setup Run).
- The number of master tubes required for the selected PCR setup run is displayed in the table on the bottom right side of the screen (Required master tubes for the selected PCR Setup Run).

NOTE

The number of prioritized samples in a PCR setup run is displayed in the column **No. of prioritized Samples**. Conduct PCR setup runs with prioritized samples first to facilitate fastest processing of prioritized samples.

Before clicking the **Start Run** button in the tool bar, prepare the required reagents as described in chapter 7.6.3.1 Preparing reagents for a PCR setup run. If the eluate plate required for the selected PCR setup run has been sealed for storage prepare it as described in the instructions for use of the AltoStar® Purification Kit 1.5.

7.6.3.1 Preparing reagents for a PCR setup run

1. Thaw the required Qs, controls and the required number of master tubes completely at room temperature (max. +30 °C).
2. Mix the reagents by gentle vortexing.
3. Centrifuge the tubes briefly to remove drops from the lid.

CAUTION

A lack of centrifugation of the product components after thawing may cause contamination with reagent residues in the lids and could compromise product performance.

7.6.3.2 Loading the AltoStar® AM16 for a PCR setup run

For detailed information regarding the loading process, refer to the instructions for use of the AltoStar® Automation System AM16 and the AltoStar® Connect software.

1. Click the **Start Run** button in the tool bar of the Start PCR Setup Run screen to display the Loading dialog.

The Loading dialog consists of a visual representation of the AltoStar® AM16 deck at the top and a table specifying the carriers, the respective tracks on the AltoStar® AM16 deck for each carrier, the material to be loaded onto each carrier and comments with respect to the carrier loading.

NOTE

To visualize the position of an item on a carrier and the position of the carrier on the AltoStar® AM16 deck, select the respective row of the table in the Loading dialog.



The position of the item and its carrier is visualized:

- Highlighted in red in the visual representation of the instrument deck
- On the AltoStar® AM16 by flashing loading lights above the tracks where the selected carrier must be placed

2. Load the required material, the prepared eluate plate and the prepared reagents onto the suitable carriers.
 - Exchange only **completely empty** 1,000 µl tip racks for **completely full** 1,000 µl tip racks on the tip carrier.
 - Exchange only **completely empty** 300 µl tip racks for **completely full** 300 µl tip racks on the tip and plate carrier.

NOTE



Exchange of tip racks, which are not completely empty as well as handling of individual tips may interfere with the automatic tip management and cause run aborts.

- Place the required eluate plate with well A1 to the left of the black plate position.
- Place a PCR plate with well A1 to the left of the silver front plate position.
- Load a tube carrier 24 with one unused pooling tube for each assay in the PCR setup run.
- Gently push the tubes all the way to the bottom of the carrier and rotate the tubes until the tube barcodes are visible through the carrier window.

- Load the reagent tube carrier 32 with the assay components required for the PCR setup run.
- Gently push the tubes all the way to the bottom of the carrier and rotate the tubes until the tube barcodes are visible through the carrier window.

NOTE



The position of the individual tubes on the carriers is arbitrary.

NOTE



The volume of the loaded components is not checked by the system prior to processing. Insufficient component volume will prevent successful PCR setup for the affected assay.

NOTE



Starting a PCR setup run with lids still on the tubes may cause the run to abort during processing.

3. Load the carriers with the carrier barcode towards the rear facing right.
4. Insert populated carriers into the respective tracks between the front and rear slide blocks of the loading tray until they touch the stop hooks on the far side of the loading tray.

NOTE



Pushing the carriers past the stop hooks may damage the instrument and interfere with the loading process.

5. Check that the tip eject sheet and the tip waste container are in the correct position and a new waste bag is placed in the container.
6. Click **OK** in the Loading dialog to proceed with the loading process.

NOTE

By clicking **Cancel** the PCR setup run will be canceled, but it can be started again (see chapter 7.6.3 Starting a PCR setup run).

The AltoStar® AM16 draws the carriers into the instrument and performs barcode verification.

NOTE

The AltoStar® AM16 automatically verifies:

- Correct type and localization of the loaded carriers
- Correct identity and position of the items loaded on the carriers
- Lot congruence of the components of the individual AltoStar® assay kits
- Non-expiry of all loaded AltoStar® assay components
- Correct positioning of the tip eject sheet

If any of these checks fail, the user is prompted with a message dialog specifying the problem at hand and instructions to correct the issue accordingly. For further information regarding error handling, refer to the instructions for use of the AltoStar® Connect software.

NOTE

Altering positions of any loaded item after the carrier has been drawn into the instrument may result in the abort of the PCR setup run and/or damage to the instrument.

When all checks have passed the Loading complete dialog is displayed.

7. Confirm the Loading complete dialog by clicking **OK** or wait 10 seconds for the automatic start of the process.

NOTE

By clicking **Cancel** the PCR setup run will be canceled, but it can be started again (see chapter 7.6.3 Starting a PCR setup run).

The PCR setup run is started and will be conducted without user intervention.

7.6.3.3 During the PCR setup run

No further user interaction is required until the PCR setup run has finished. The Processing Status screen is displayed showing the status of the PCR setup run and the estimated time remaining.

NOTE

Pushing or pulling carriers or the door of the AltoStar® AM16 during a PCR setup run may abort the run.

7.6.4 End of the PCR setup run

At the end of the PCR setup run the Run finished dialog is displayed.

1. Make sure that the loading tray is empty.
2. Confirm the Run finished dialog by clicking **OK**.

The AltoStar® AM16 will unload the carriers. Make sure not to stand in the way of the unloading carriers.

After unloading the Maintenance dialog is displayed.

3. Follow the instructions of the Maintenance dialog.

The table of the dialog displays the number of reactions in the master tubes that were not used in the PCR setup run.

4. If another PCR setup run using the currently loaded eluate plate is to be started right away, the eluate plate can remain unsealed on the carrier position. If this is **not** the case, seal and store the eluate plate. For further information, refer to the instructions for use of the AltoStar® Purification Kit 1.5.

NOTE



The eluates in the eluate plate are stable at room temperature (max. +30 °C) for a total of 4 hours after completion of the purification run.

5. Close reagent tubes with suitable unused tube caps.

CAUTION



Do not reuse tube caps to avoid contamination of the reagents, as this could compromise product performance.

6. Store reagents for reuse as described in chapter 4.2 Handling.
7. Dispose of the used materials (see chapter 10. Disposal).
8. Confirm the Maintenance dialog by clicking **OK**.

7.6.4.1 PCR setup run results

The PCR setup run results are saved in the AltoStar® Connect software.

1. Click **PCR Setup** → **PCR Setup Results** in the menu bar to access the Results screen.

The Results screen displays a table with all samples used in the latest PCR setup run and a column **Status** at the right showing if the PCR setup process for a given sample was conducted completely (see table 6).

Table 6: PCR setup run results

Status	PCR setup run result
Processed	<ul style="list-style-type: none"> The eluate was successfully processed in the PCR setup run. The resulting PCR mix is ready for use in a PCR run.
Error	<ul style="list-style-type: none"> The eluate was not processed successfully. The respective PCR mix will be automatically omitted in the following PCR analysis.

- To view the results of prior PCR setup runs, click the **Load** button in the menu bar, select the desired PCR setup run from the list in the opening Load Results dialog and click **OK**.

3 PCR setup run result files are automatically generated by the AltoStar® Connect software:

- A LIMS file (.xml) to pass detailed information about the PCR setup run including results back to the LIMS
- A report (.pdf) containing detailed information about the PCR setup run including results for documentation purposes
- A cycler file (.pln) for automatic programming of the CFX96™ DW Dx

These files are saved to the location specified in the system settings of the AltoStar® Connect software.

NOTE



PCR setup run result files can be generated again by loading the respective PCR setup run and clicking the **Create LIMS File** button to generate the LIMS file, the **Create Report** button to generate the report or the **Create Bio-Rad Cycler File** button to generate the cycler file.

7.6.5 Sealing of the PCR plate

After completion of the PCR setup run, the PCR plate must be sealed with a PCR Plate Sealing Foil. It is recommended to use the AltoStar® Plate Sealer [4s3™ Semi-Automatic Sheet Heat Sealer (4titude)] or the PX1 PCR Plate Sealer (Bio-Rad). The suitability of plate sealers other than the recommended plate sealers has to be evaluated by the user.

If one of the recommended plate sealers is used for sealing, proceed as follows:

1. Switch on the plate sealer and make sure that the plate adapter is not in the drawer.
2. Ensure that the settings of the plate sealer are as follows:

Table 7: Settings of the plate sealer

Plate sealer	Settings	
	Temperature [°C]	Time [s]
AltoStar® Plate Sealer [4s3™ Semi-Automatic Sheet Heat Sealer (4titude)]	170	2
PX1 PCR Plate Sealer (Bio-Rad)	175	3

3. Wait until the set temperature is reached. This may take several minutes.
4. Place the PCR plate on the plate adapter of the plate sealer.
5. Place one PCR Plate Sealing Foil on the PCR plate so that the print 'THIS SIDE UP' is readable. Make sure that all wells of the PCR plate are covered with foil and no well is obscured by the writing.




NOTE



Operating the plate sealer without the plate adapter placed in the drawer may render the sealer nonfunctional. In this case, contact altona Diagnostics technical support for assistance (see chapter 12. Technical support).

NOTE

If the PCR Plate Sealing Foil or the frame is placed incorrectly, the foil may stick to the heating plate within the plate sealer during sealing. This will render the sealer nonfunctional. In this case, or if the sealing step has been initiated without a PCR Plate Sealing Foil, let the plate sealer cool down to room temperature and contact Altona Diagnostics technical support for assistance (see chapter 12. Technical support).

6. Assemble the sealing frame on top to hold down the sealing foil.
7. Open the drawer by pressing the **Operate***/ ** button.
8. Place the assembly consisting of the plate adapter, the PCR plate, the PCR Plate Sealing Foil and the sealing frame into the plate sealer and press the **Operate***/ ** button.
9. The drawer closes automatically, seals for the set time and reopens automatically.
10. Take the sealed PCR plate and the plate adapter out of the plate sealer and close the plate sealer by pressing the **Close***/ ** button.

* AltoStar® Plate Sealer [4s3™ Semi-Automatic Sheet Heat Sealer (4titude)]

**PX1 PCR Plate Sealer (Bio-Rad)

7.6.5.1 PCR mix stability

After completion of the PCR setup run the PCR mix in the sealed PCR plate is stable at room temperature (max. +30 °C) for 30 minutes.

CAUTION

Do not exceed the PCR mix storage time, as this could compromise product performance.

7.6.6 Starting a PCR run

The PCR run is performed on a CFX96™ DW Dx under control of the CFX Manager™ Dx software.

1. Switch on the CFX96™ DW Dx, the attached computer and the monitor.
2. Start the CFX Manager™ Dx software.
3. In the menu bar of the CFX Manager™ Dx software select **File** → **Open** → **LIMS File...** to open the Open LIMS File dialog.
4. In the opening Open LIMS File dialog, make sure that the cursor is blinking in the **File name** field at the bottom. If it is not, click into the **File name** field.
5. Scan the PCR plate barcode with the handheld barcode scanner to automatically select and open the correct LIMS file. The Run Setup dialog is displayed.

NOTE



All parameters required for the start of the PCR run are transferred automatically from the AltoStar® Connect software to the CFX96™ DW Dx by means of the cyclers file.

6. Click the **Open Lid** button in the Run Setup dialog to open the lid of the CFX96 DW Dx.
7. Briefly centrifuge the sealed PCR plate to ensure all liquid is at the bottom of the wells.
8. Insert the sealed PCR plate into the heating block of the CFX96™ DW Dx with well A1 to the left side.
9. Close the CFX96™ DW Dx by clicking the **Close Lid** button in the Run Setup dialog.
10. Start the PCR run by clicking the **Start Run** button in the Run Setup dialog.

7.6.6.1 During the PCR run

No user interaction is required until the PCR run has finished. The Run Details dialog is displayed showing the status of the PCR run and the estimated time remaining.

NOTE



Opening the lid of the CFX96™ DW Dx during a PCR run by operating the button at the front of the lid or by clicking **Open Lid** in the Run Details dialog will abort the run and void all results.

At the end of the PCR run the Data Analysis window is displayed, which shows the amplification curves, the plate layout and the results.

7.6.6.2 Assigning assays to well groups

The AltoStar® Workflow processes one or several PCR assays simultaneously on one PCR plate. However, each assay must be analyzed separately by the user according to the instructions for use of the respective assay.

To this end, all assays on a PCR plate must be assigned to individual well groups in the CFX Manager™ Dx software by the user.

1. In the Data Analysis window click the **Plate Setup** button in the tool bar and select **View/Edit Plate**. The Plate Editor dialog is displayed (see figure 1).

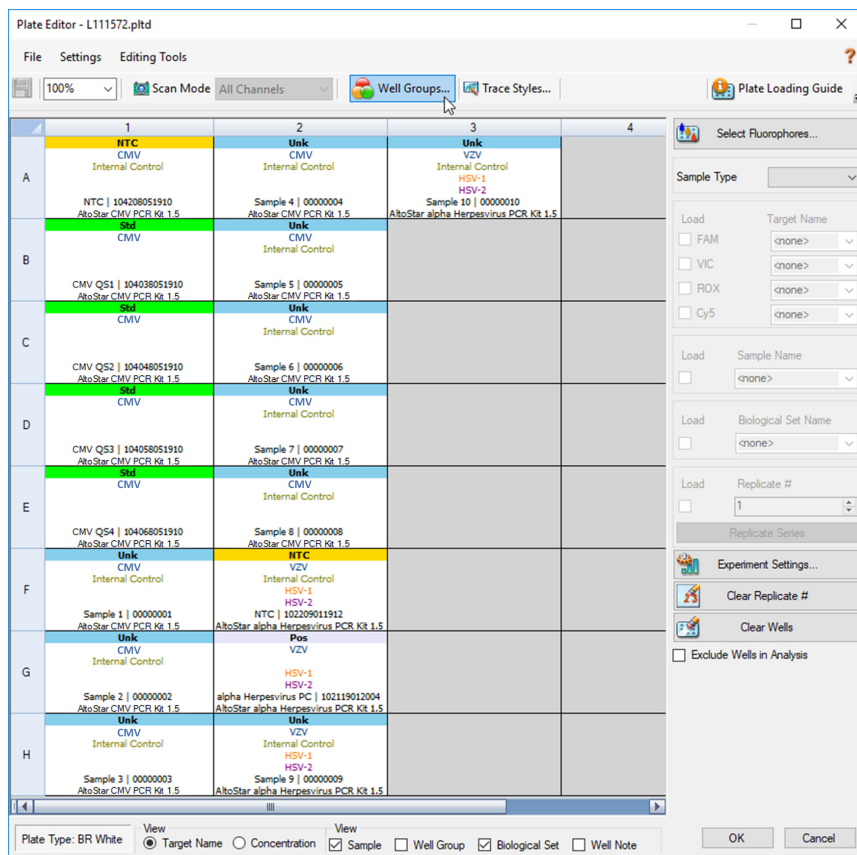


Figure 1: Plate Editor dialog

2. In the Plate Editor dialog click **Well Groups...** in the tool bar. The Well Groups Manager dialog is displayed (see figure 2).
3. Click the **Add** button.
4. Type the name of the first assay in the text box.

5. Select all wells in the PCR plate area that belong to the first assay (see figure 2). The wells belonging to an individual assay can be identified in the Plate Editor dialog by the entry in the **Biological Set** field.

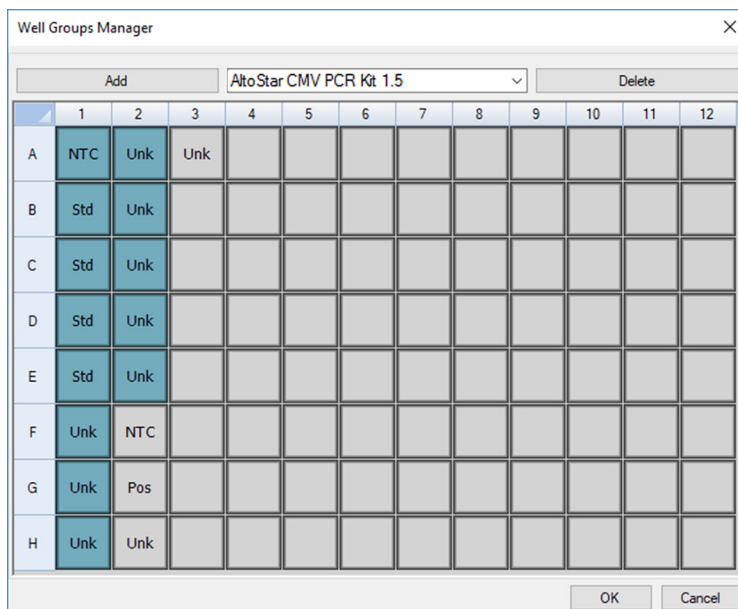


Figure 2: Well Groups Manager dialog

6. Repeat steps 3–5 for all assays on the PCR plate.
7. Confirm well group assignment by clicking **OK**. The Well Groups Manager dialog closes.
8. Close the Plate Editor dialog by clicking **OK**.
9. Confirm to apply the changes by clicking **Yes**.

7.6.7 PCR data analysis

The results of all assays (well groups) on the PCR plate have to be analyzed in the sequence depicted in figure 3.

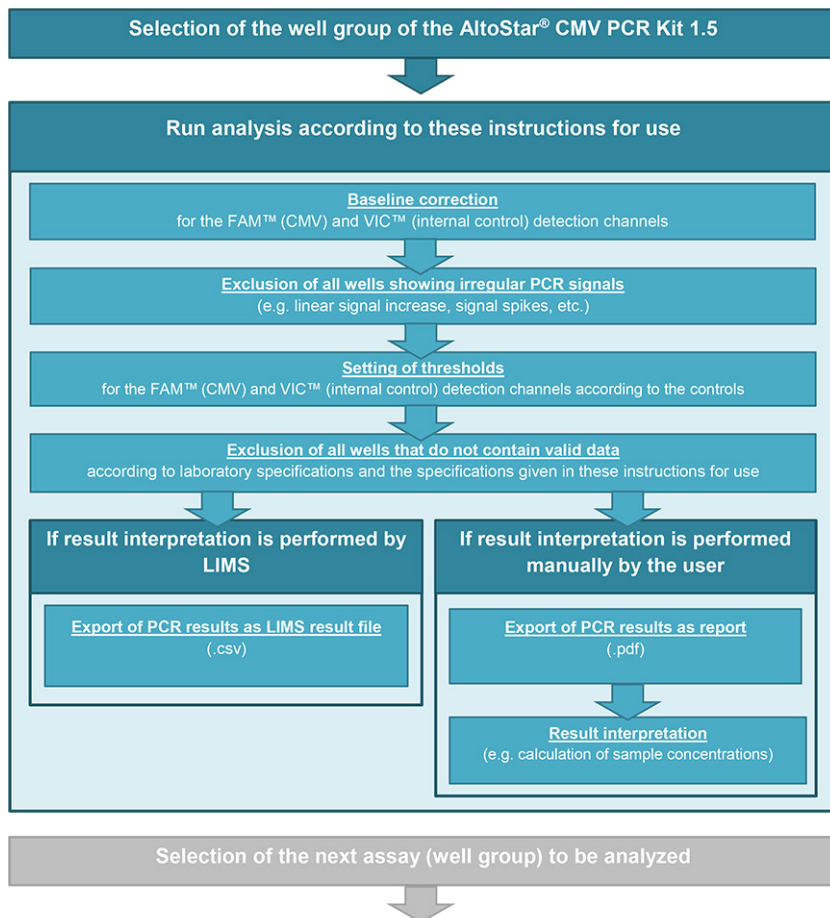


Figure 3: PCR Data Analysis process

In the Data Analysis window ensure to select the **Well Group** of the AltoStar® CMV PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar. Do not use the “All Wells” **Well Group**. The selection in figure 4 is used as a general example view.

Before analyzing the results, ensure that the well group of the AltoStar® CMV PCR Kit 1.5 contains all of the AltoStar® CMV PCR Kit 1.5 wells and no wells of other assays.

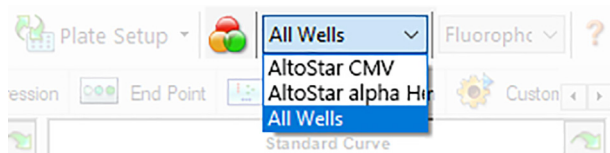


Figure 4: Well Group button and Well Group drop-down menu

NOTE



Combined analysis of more than one assay may lead to incorrect results.

CAUTION



As with any diagnostic test, results shall be interpreted in consideration of all clinical and laboratory findings.

7.6.7.1 Baseline correction

The baseline settings used by the CFX Manager™ Dx software may have to be corrected for individual wells of the assay (**Well Group**) under analysis.

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® CMV PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.
2. At the left side of the Data Analysis window tick only the **FAM** checkbox for the CMV target detection channel.
3. In the menu bar of the Data Analysis window click **Settings** → **Baseline Threshold...** to open the Baseline Threshold dialog (see figure 5).
4. Click the \diamond symbol in the **Baseline End** column header once to sort the table by ascending **Baseline End** values.

5. Select all lines that show a **Baseline End** value of 1–9 (see figure 5).

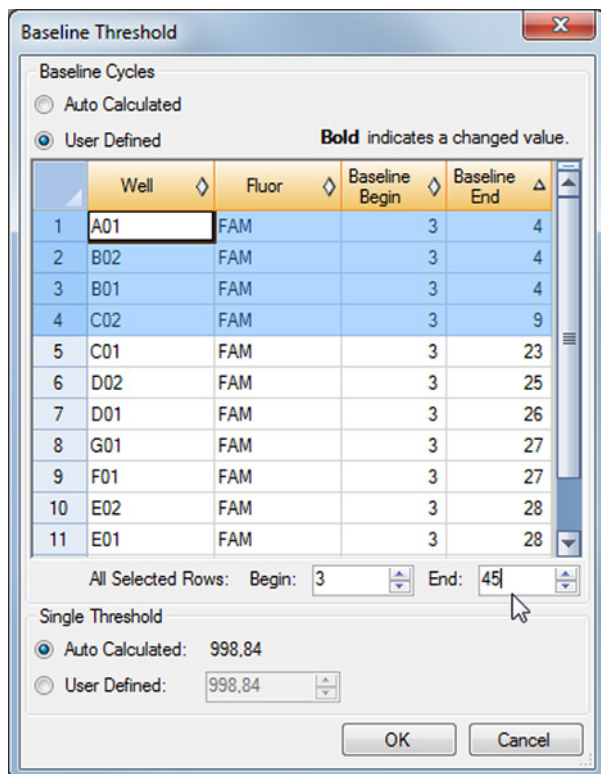


Figure 5: Baseline Threshold dialog

6. Set the value in the **End:** field to 45 for the selected lines (see figure 5).
7. Confirm the settings by clicking **OK**.
8. At the left side of the Data Analysis window untick the **FAM** checkbox and tick only the **VIC** checkbox for the IC target detection channel.
9. Repeat steps 3–7 for the VIC™ (IC) detection channel.

7.6.7.2 Exclusion of irregular PCR signals

Valid results can only be derived from PCR signals that are free of signal artefacts, which may be caused e.g. by impurities or bubbles in the PCR mix. PCR signals that contain signal artefacts have to be excluded by the user.

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® CMV PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.

2. Identify wells with irregular PCR signals (linear signal increase, signal spikes, etc.) in any of the FAM™ (CMV target) and the VIC™ (IC) detection channels (see figure 6).

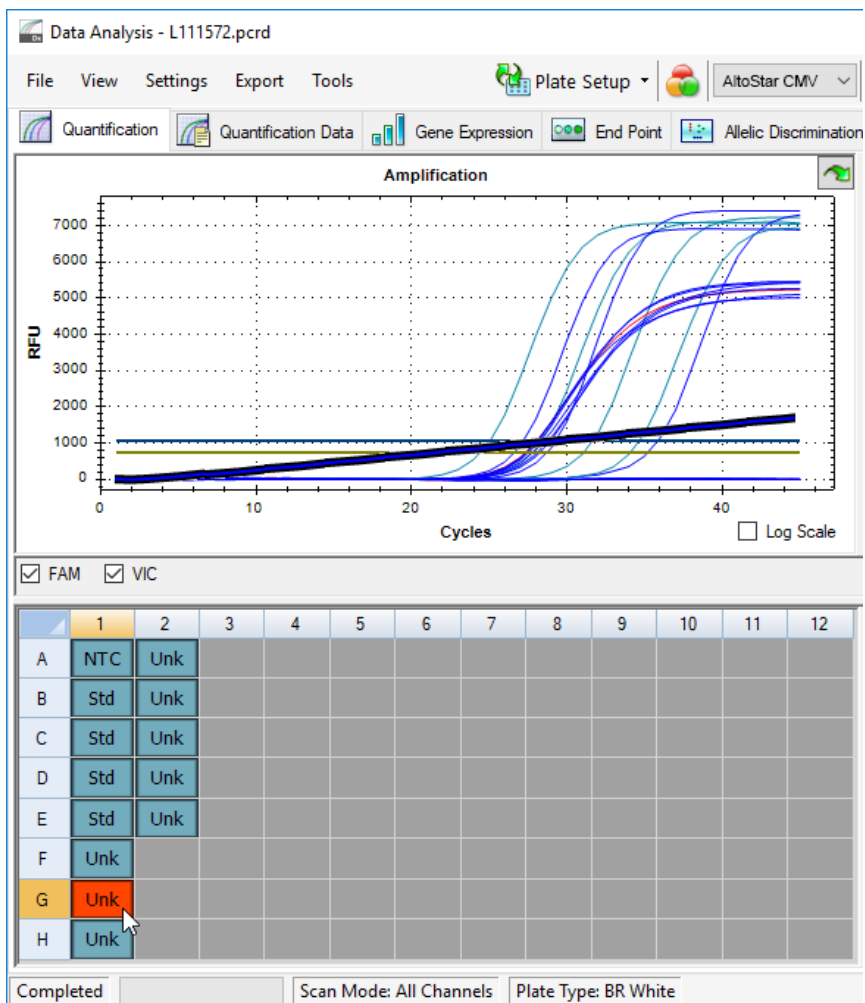


Figure 6: Data Analysis window: irregular PCR signal

- Click each affected well with the right mouse button and select **Well... → Exclude from Analysis** (see figure 7).

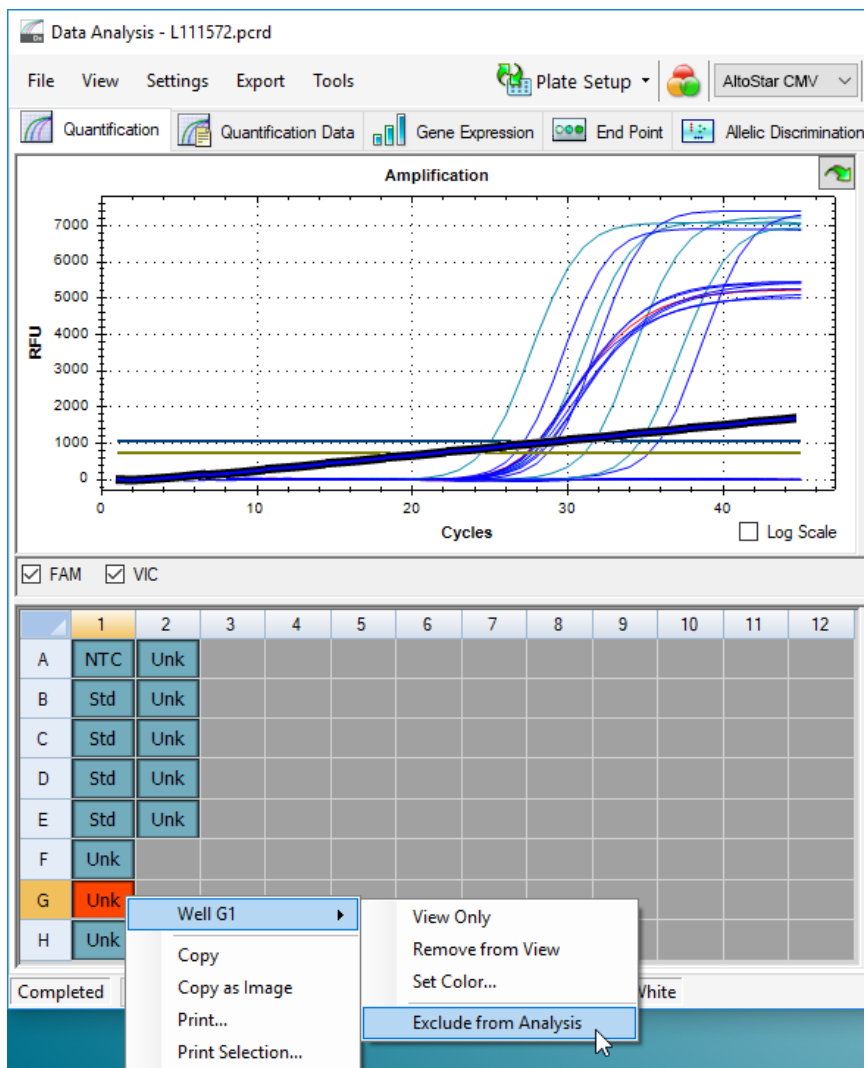


Figure 7: Data Analysis window: exclude well from analysis

4. The selected well is excluded from the analysis. No results will be generated for this well (see figure 8).

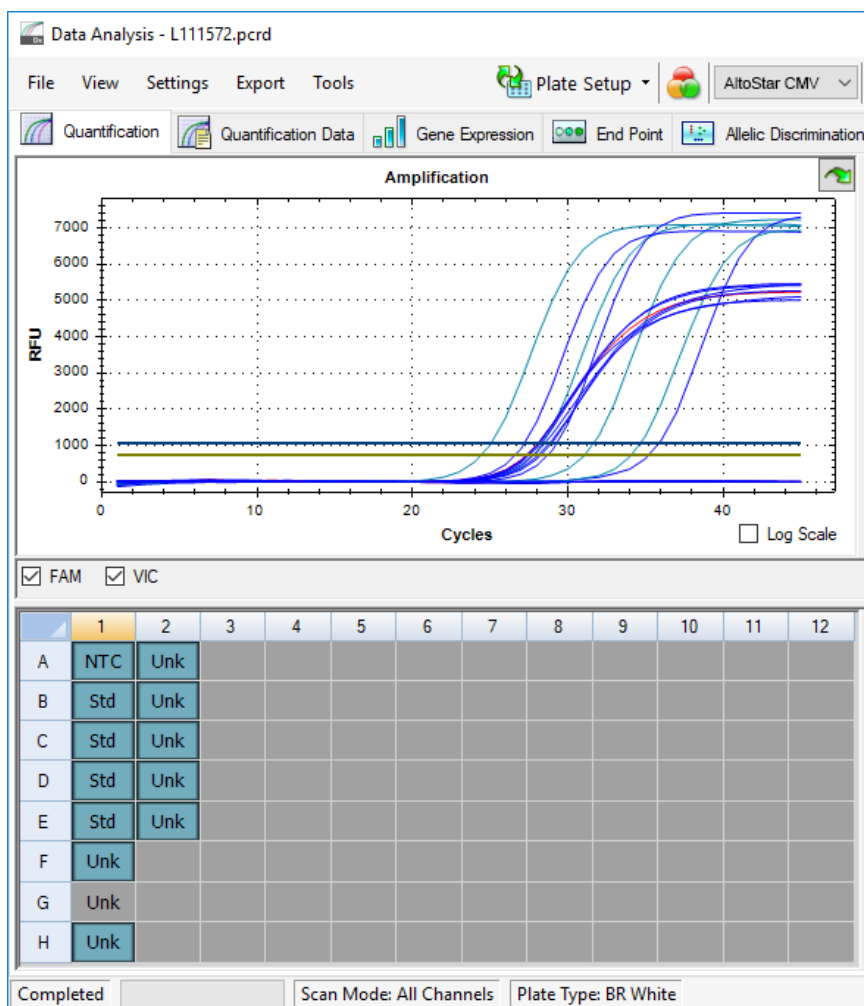


Figure 8: Data Analysis window: excluded well

7.6.7.3 Setting of thresholds

The thresholds for the FAM™ (CMV target) and the VIC™ (IC) detection channels have to be set manually by the user according to the signals of the controls.

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® CMV PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.

2. At the left side of the Data Analysis window tick only the **VIC** checkbox for the detection channel of the IC (see figure 9).

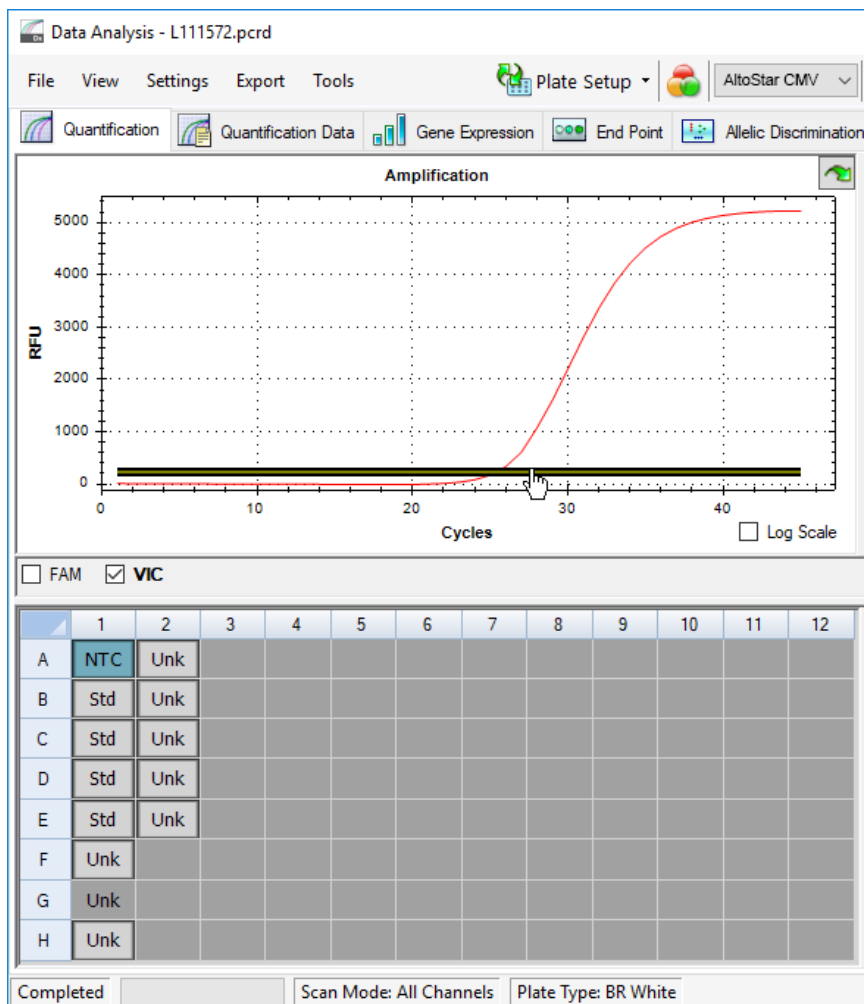


Figure 9: Data Analysis window: setting the VIC™ threshold

3. Select only the NTC well in the plate view of the Data Analysis window (see figure 9).
4. Drag the threshold into the exponential area of the NTC signal (see figure 9).

NOTE



The NTC contains the IC template, which leads to an IC signal in a valid NTC well.

5. At the left side of the Data Analysis window untick the **VIC** checkbox and tick the **FAM** checkbox for the detection channel of the CMV target (see figure 10).

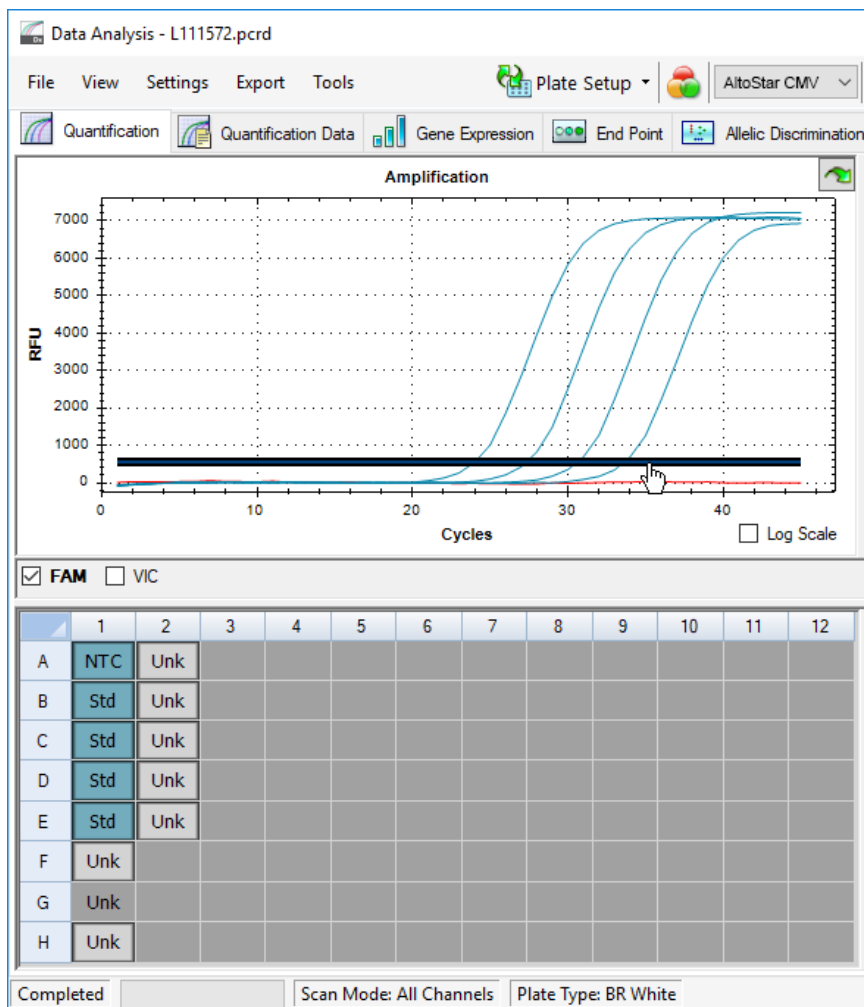


Figure 10: Data Analysis window: setting the FAM™ threshold

6. Select only the wells containing the NTC and the Qs in the plate view of the Data Analysis window (see figure 10).
7. Drag the threshold well above the signal of the NTC into the exponential area of the Qs signals (see figure 10).

7.6.8 Validity of PCR results

7.6.8.1 Exclusion of wells containing invalid data

Wells that do not contain valid data have to be excluded from result generation by the user.

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® CMV PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.
2. Identify all wells containing invalid data. A well is invalid if any of the following conditions apply:
 - a) The complete run is invalid [see chapters 7.6.8.2 Validity of a diagnostic PCR run (qualitative) and 7.6.8.3 Validity of a diagnostic PCR run (quantitative)].
 - b) The well data does not meet the control conditions for a valid result (see chapter 7.6.8.4 Validity of results for a sample).

- Click each well containing invalid data according to chapters 7.6.8.2 Validity of a diagnostic PCR run (qualitative) to 7.6.8.4 Validity of results for a sample with the right mouse button and select **Well...** → **Exclude from Analysis** (see figures 11 and 12).

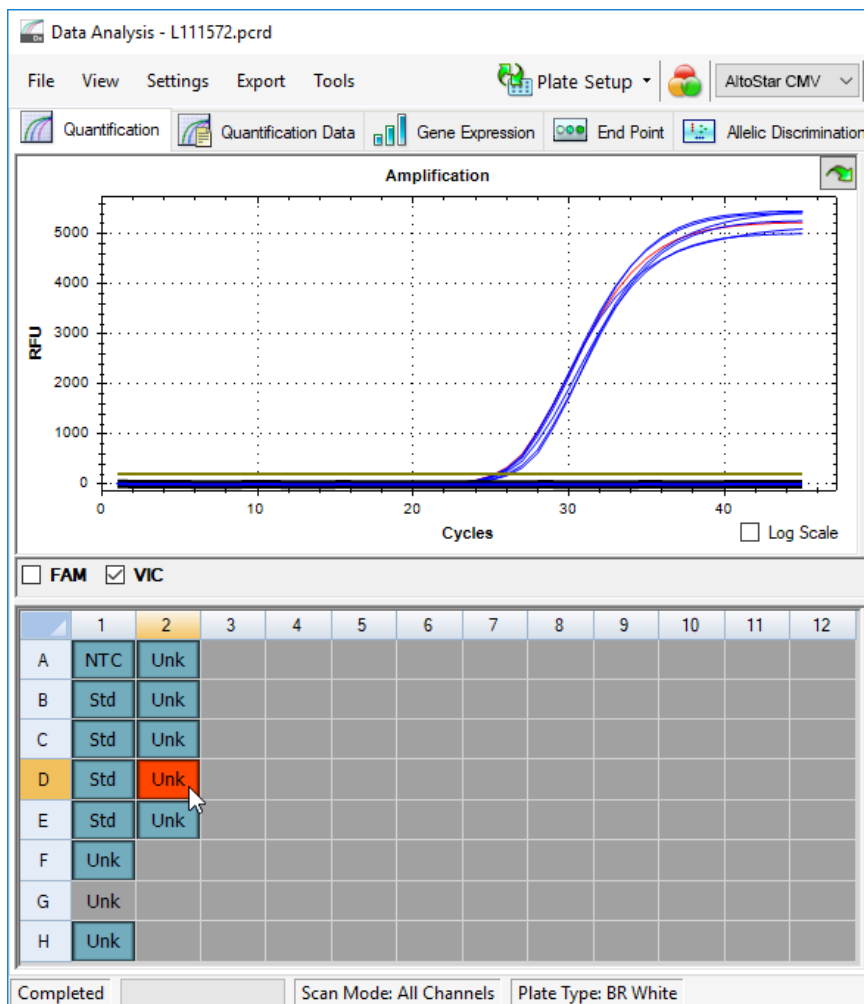


Figure 11: Data Analysis window: invalid well

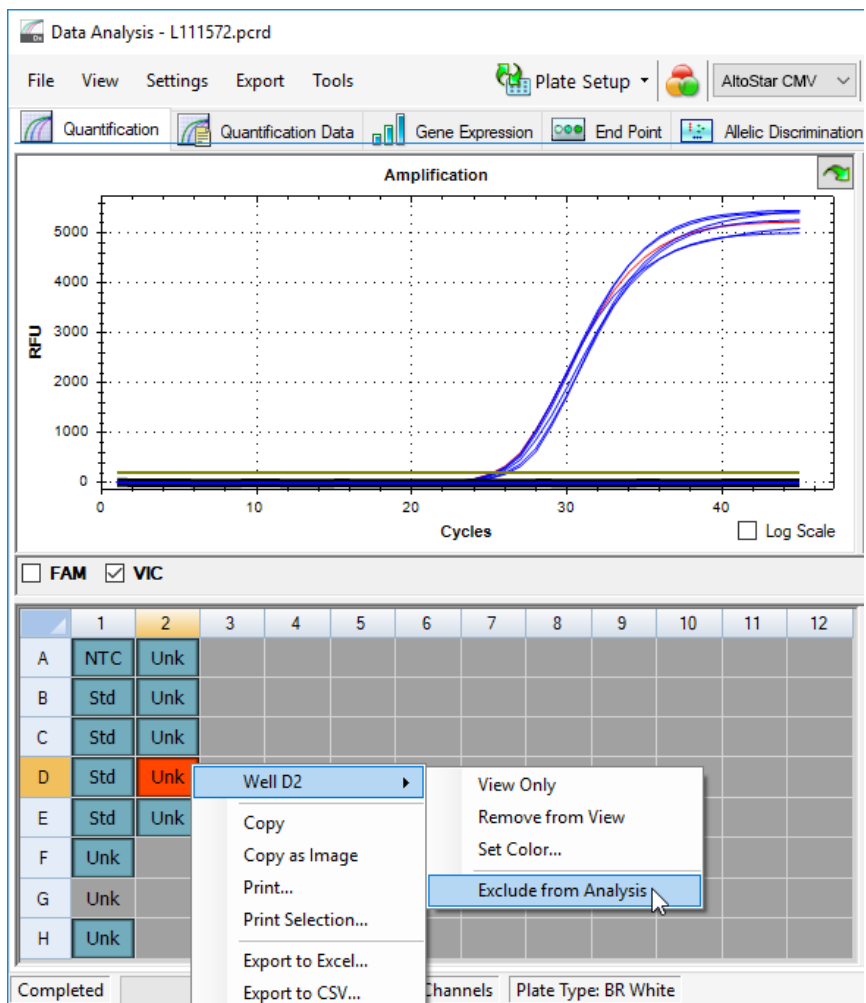


Figure 12: Data Analysis window: exclude invalid well from analysis

The selected well is excluded from the analysis. No results will be generated for this well.

7.6.8.2 Validity of a diagnostic PCR run (qualitative)

A qualitative diagnostic PCR run is **valid**, if the following control conditions are met:

Table 8: Control conditions for a valid PCR run (qualitative)

Control	Detection channel	
	FAM™ (CMV target)	VIC™ (IC)
QS	+	Not applicable
NTC	-	+

A qualitative diagnostic PCR run is **invalid**, if:

- The run has not been completed.
- Any of the control conditions for a valid qualitative diagnostic PCR run are not met.

In case of an invalid diagnostic PCR run, exclude all wells from the analysis and repeat the AltoStar® run starting from the original samples.

7.6.8.3 Validity of a diagnostic PCR run (quantitative)

A quantitative diagnostic PCR run is **valid**, if the following conditions are met:

- All control conditions for a valid qualitative diagnostic PCR run are met [see chapter 7.6.8.2 Validity of a diagnostic PCR run (qualitative)].
- The generated standard curve reaches the following control parameter value:

Table 9: Standard curve control parameter

Control parameter	Valid value
R square (R ²)	≥ 0.98

The standard curve's control parameter is displayed below the Standard curve graph in the Data Analysis window (see figure 13).

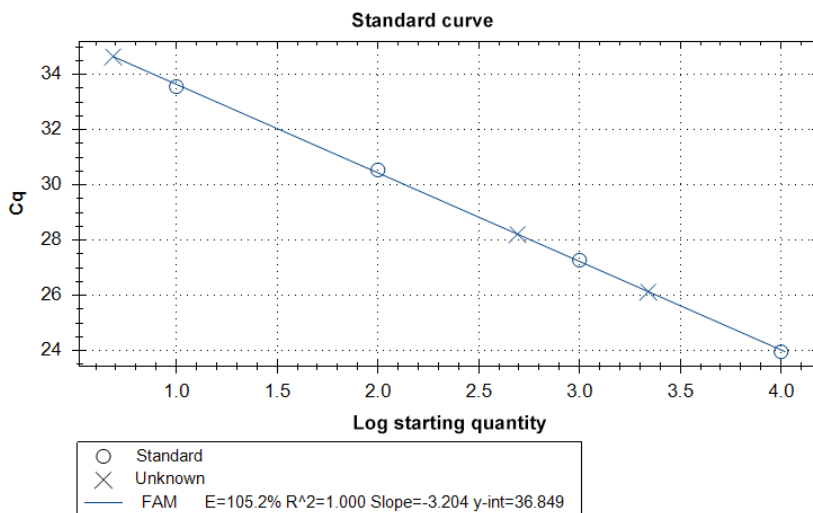


Figure 13: Standard curve data

A quantitative diagnostic PCR run is **invalid**, if:

- The run has not been completed.
- Any of the control conditions for a valid quantitative diagnostic PCR run are not met.

In case of an invalid diagnostic PCR run, exclude all wells from the analysis and repeat the AltoStar® run starting from the original samples.

7.6.8.4 Validity of results for a sample

The result for an individual sample is **invalid**, if the signals in both the VIC™ (IC) detection channel and the FAM™ (CMV target) detection channel are negative (see table 10). In case of an invalid result for a sample, exclude the well from the analysis and repeat testing from the original sample or collect and test a new sample.

Table 10: Result validity

Detection channel		Result validity
FAM™ (CMV target)	VIC™ (IC)	
+	+	Valid result
+	-	Valid result*
-	+	Valid result
-	-	Invalid result

* Detection of the IC is not required when the CMV target is detected. A high CMV DNA load in the sample can lead to a reduced or absent IC signal.

7.6.9 Export of PCR results for automated result interpretation

To make the PCR run results available to a connected LIMS for automated result interpretation they need to be exported in the form of a LIMS result file (.csv).

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® CMV PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.
2. Ensure that all steps of the analysis process (see chapters 7.6.7.1 Baseline correction to 7.6.8.1 Exclusion of wells containing invalid data) have been completed for the well group of the AltoStar® CMV PCR Kit 1.5.
3. In the menu bar of the Data Analysis window click **Export** → **Export All Data Sheets** to open the Browse For Folder dialog.
4. In the Browse For Folder dialog specify the location for the LIMS result files to be generated and click **OK**.

NOTE



The LIMS integration has to be implemented according to the specifications of altona Diagnostics. For information regarding LIMS integration, see chapter 16. Assay protocol for the AltoStar® Connect software and information for LIMS integration and/or contact altona Diagnostics technical support (see chapter 12. Technical support).

NOTE

Saving results of more than one assay (well group) from a PCR run in the same folder leads to replacement of the LIMS result files of the first assay (well group) with the LIMS result files of the second assay (well group). In this case the LIMS result files of the first assay (well group) can be exported again.

7.6.10 Export of PCR results for manual result interpretation

If the results are not passed to a LIMS for automatic result interpretation, the result interpretation has to be performed manually by the user. To this end the analysis results of each assay (well group) need to be exported in the form of a report.

1. In the Data Analysis window ensure to select the **Well Group** of the AltoStar® CMV PCR Kit 1.5. Therefore, click on the **Well Group** drop-down menu right next to the **Well Group** button (see figure 4) of the toolbar.
2. At the left side of the Data Analysis window tick the **VIC** checkbox as well as the **FAM** checkbox.
3. Ensure that all steps of the analysis process (see chapters 7.6.7.1 Baseline correction to 7.6.8.1 Exclusion of wells containing invalid data) have been completed for the well group of the AltoStar® CMV PCR Kit 1.5.
4. In the menu bar of the Data Analysis window click **Tools** → **Reports...** to open the Report dialog.

5. Ensure that at least the following content is selected for report generation in the upper left part of the Report dialog (see figure 14):

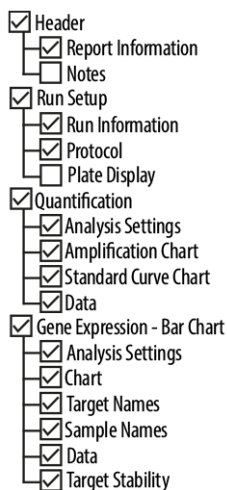


Figure 14: Report dialog

6. Select or deselect additional content of the report by ticking the respective check boxes as required.
7. In the menu bar of the Report dialog click **File** → **Save As...** to open the Save Report dialog.
8. In the Save Report dialog specify the name and location for the report file to be generated and click **Save**.

7.6.10.1 Manual interpretation of results

1. Open the report file generated for the well group of the AltoStar® CMV PCR Kit 1.5 (see chapter 7.6.10 Export of PCR results for manual result interpretation).
2. Refer to the Quantification Data table in the report (see figure 15). The table comprises 2 rows for each **Sample** – one for the **Target CMV** and one for the **Target Internal Control**.

Quantification Data

Well	Floor	Target	Content	Sample	Biological Set Name	Cq	Cq Mean	Cq Std. Dev	Starting Quantity (SQ)	Log Starting Quantity	SQ Mean	SQ Std. Dev	Well Note
A01	FAM	CMV	NTC	NTC 104208051910	AltoStar CMV PCR Kit 1.5	N/A	0.00	0.000	N/A	N/A	0.00E+00	0.00E+00	
A02	FAM	CMV	Unkn	Sample 4 00000004	AltoStar CMV PCR Kit 1.5	N/A	0.00	0.000	N/A	N/A	0.00E+00	0.00E+00	Concentration factor: 160.00 IU/ml
B01	FAM	CMV	Std	CMV QS1 104038051910	AltoStar CMV PCR Kit 1.5	23.97	23.97	0.000	1.000E+04	4.000	1.00E+04	0.00E+00	
B02	FAM	CMV	Unkn	Sample 5 00000005	AltoStar CMV PCR Kit 1.5	34.65	34.65	0.000	4.855E+00	0.686	4.85E+00	0.00E+00	Concentration factor: 160.00 IU/ml
C01	FAM	CMV	Std	CMV QS2 104048051910	AltoStar CMV PCR Kit 1.5	27.28	27.28	0.000	1.000E+03	3.000	1.00E+03	0.00E+00	
C02	FAM	CMV	Unkn	Sample 6 00000006	AltoStar CMV PCR Kit 1.5	N/A	0.00	0.000	N/A	N/A	0.00E+00	0.00E+00	Concentration factor: 160.00 IU/ml
D01	FAM	CMV	Std	CMV QS3 104058051910	AltoStar CMV PCR Kit 1.5	30.54	30.54	0.000	1.000E+02	2.000	1.00E+02	0.00E+00	
E01	FAM	CMV	Std	CMV QS4 104068051910	AltoStar CMV PCR Kit 1.5	33.56	33.56	0.000	1.000E+01	1.000	1.00E+01	0.00E+00	
E02	FAM	CMV	Unkn	Sample 8 00000008	AltoStar CMV PCR Kit 1.5	28.22	28.22	0.000	4.924E+02	2.692	4.92E+02	0.00E+00	Concentration factor: 160.00 IU/ml
F01	FAM	CMV	Unkn	Sample 1 00000001	AltoStar CMV PCR Kit 1.5	26.14	26.14	0.000	2.195E+03	3.341	2.20E+03	0.00E+00	Concentration factor: 160.00 IU/ml
H01	FAM	CMV	Unkn	Sample 3 00000003	AltoStar CMV PCR Kit 1.5	N/A	0.00	0.000	N/A	N/A	0.00E+00	0.00E+00	Concentration factor: 160.00 IU/ml
A01	VIC	Internal Control	NTC	NTC 104208051910	AltoStar CMV PCR Kit 1.5	25.27	25.27	0.000	N/A	N/A	N/A	0.00E+00	
A02	VIC	Internal Control	Unkn	Sample 4 00000004	AltoStar CMV PCR Kit 1.5	26.08	26.08	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 160.00 IU/ml
B02	VIC	Internal Control	Unkn	Sample 5 00000005	AltoStar CMV PCR Kit 1.5	25.46	25.46	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 160.00 IU/ml
C02	VIC	Internal Control	Unkn	Sample 6 00000006	AltoStar CMV PCR Kit 1.5	25.33	25.33	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 160.00 IU/ml
E02	VIC	Internal Control	Unkn	Sample 8 00000008	AltoStar CMV PCR Kit 1.5	26.25	26.25	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 160.00 IU/ml
F01	VIC	Internal Control	Unkn	Sample 1 00000001	AltoStar CMV PCR Kit 1.5	25.45	25.45	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 160.00 IU/ml
H01	VIC	Internal Control	Unkn	Sample 3 00000003	AltoStar CMV PCR Kit 1.5	25.70	25.70	0.000	N/A	N/A	N/A	0.00E+00	Concentration factor: 160.00 IU/ml

Figure 15: Report: Quantification Data

Qualitative results would be marked by the term *qualitative* in the **Well Note** column of the Quantification Data table.

3. Identify each row with the **Target CMV** and the term *qualitative* in the **Well Note** column.
4. In these rows, refer to the **C_q** column for the result of the respective **Sample**.

5. Refer to table 11 for interpretation of qualitative results.

Table 11: Qualitative results: result interpretation

Threshold cycle (C_q) of the CMV target	Result interpretation
1–45	CMV specific DNA detected.
N/A	No CMV specific DNA detected. Sample does not contain detectable amounts of CMV specific DNA.

Quantitative results are marked by a *Concentration factor* in the **Well Note** column of the Quantification Data table (see figure 15).

6. Identify each row with the **Target CMV** and a *Concentration factor* in the **Well Note** column.
7. In these rows, refer to the **Starting Quantity (SQ)** column for the concentration of the CMV target measured in the eluate of the respective **Sample**. To calculate the result for the original patient sample, the **Starting Quantity (SQ)** value has to be multiplied by the respective *Concentration factor* (including the unit) by the user.
8. Refer to table 12 for interpretation of quantitative results.

Table 12: Quantitative results: result interpretation

Starting Quantity (SQ) of the CMV target	Result interpretation
> 0	CMV specific DNA detected. Multiply the Starting Quantity (SQ) value by the <i>Concentration factor</i> in the Well Note column (including the unit) to calculate the concentration of the original patient sample.
N/A	No CMV specific DNA detected. Sample does not contain detectable amounts of CMV specific DNA.

8. Using the AltoStar® CMV PCR Kit 1.5 with other real-time PCR instruments than the CFX96™ Deep Well Dx System

In addition to the CFX96™ DW Dx the AltoStar® CMV PCR Kit 1.5 has been validated with other real-time PCR instruments (see chapter 5.3.2.2 Real-time PCR instruments). The following chapters 8.1 Material and devices required but not provided and 8.2 Procedure describe how to use the AltoStar® CMV PCR Kit 1.5 with these instruments.

8.1 Material and devices required but not provided

The following instruments and materials are required:

- General materials and devices (see chapter 7.5 General material and devices)
- CFX96™ Deep Well Dx System (Bio-Rad)
 - 96-well PCR plates and sealing foil (details see table 3)
- CFX96™ Dx System (Bio-Rad)
 - 96-well PCR plates and sealing foil (details see table 3)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
 - 0.1 ml strip tubes and lids [STRIP Tubes 0.1 ml for Rotor-Gene® cyclers (LTF Labortechnik) or equivalent]
- QuantStudio™ 5 Real-Time PCR System and ABI Prism® 7500 SDS (Applied Biosystems)
 - 96-well PCR plates and sealing foil [MicroAmp™ Optical 96-Well Reaction Plate and MicroAmp™ Optical Adhesive Film (Applied Biosystems) or equivalent]
- LightCycler® 480 Instrument II (Roche)
 - 96-well PCR plates and sealing foil [LightCycler® 480 Multiwell Plate 96, white and LightCycler® 480 Sealing Foil (Roche) or equivalent]

NOTE

It is not recommended to use other materials or devices than specified in these instructions for use.

8.2 Procedure

8.2.1 Sample preparation

The AltoStar® CMV PCR Kit 1.5 was validated using the AltoStar® AM16 in combination with the AltoStar® Purification Kit 1.5.

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with the AltoStar® CMV PCR Kit 1.5 has to be validated by the user.

The AltoStar® CMV PCR Kit 1.5 has to be used with a heterologous IC (AltoStar® Internal Control 1.5), which allows to control the sample preparation procedure (nucleic acid extraction) and the following PCR.

- When using other methods for nucleic acid extraction than the AltoStar® AM16 in combination with the AltoStar® Purification Kit 1.5 the IC has to be added during the lysis step of the nucleic acid extraction procedure.
- The IC should always be added to the sample/lysis buffer mixture.
- The volume of the IC which has to be added, always and only depends on the elution volume. It represents 50 % of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 30 µl of IC per sample must be added into the sample/lysis buffer mixture.

CAUTION

Storage of eluates under wrong conditions may lead to degradation of the CMV target sequence and could compromise product performance.

8.2.2 Master mix setup

All components of the AltoStar® CMV PCR Kit 1.5 and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use. Set up the master mix according to the following pipetting scheme:

Table 13: Pipetting scheme (master mix setup)

Number of reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume master mix	20 µl	240 µl

CAUTION



Do not use different volumes of Master A and Master B for master mix setup than specified in these instructions for use, as this could compromise product performance.

CAUTION



A lack of centrifugation of the product components after thawing may cause contamination with reagent residues in the lids and could compromise product performance.

8.2.3 Reaction setup

1. Pipette 20 µl of the master mix into each required well of an appropriate optical 96-well PCR plate or an appropriate optical reaction tube.
2. Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (QS1–4 or NTC).

Table 14: Pipetting scheme (reaction setup)

Reaction setup	
Master mix	20 µl
Sample or control	10 µl
Total volume	30 µl

3. Make sure that for quantitative analysis QS1–4 and one NTC are used. For qualitative analysis make sure that at least QS4 and one NTC are used.
4. Thoroughly mix the samples and controls with the master mix by pipetting up and down.
5. Close the 96-well PCR plate with a PCR Plate Sealing Foil and the reaction tubes with appropriate lids (see chapter 8.1 Material and devices required but not provided).
6. Centrifuge the 96-well PCR plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1,000 x g (~ 3,000 rpm).

After completion of the PCR setup, the PCR mix is stable at room temperature (max. +30 °C) for 30 minutes.

CAUTION

Do not exceed the PCR mix storage time, as this could compromise product performance.

CAUTION

Do not mix up samples or sample IDs during PCR setup or transfer to the PCR instrument. This could lead to false positive or false negative results due to incorrect assignment of the samples.

8.2.4 PCR run

8.2.4.1 Programming the real-time PCR instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, refer to the instructions for use of the respective instrument.

For detailed programming instructions regarding the use of the AltoStar® CMV PCR Kit 1.5 on specific real-time PCR instruments, contact altona Diagnostics technical support (see chapter 12. Technical support).

8.2.4.2 Run settings

Define the following basic settings:

Table 15: Run settings

Settings	
Reaction volume	30 µl
Ramp rate	Default
Passive reference*	ROX™

*If applicable

Define the following fluorescence detectors (dyes):

Table 16: Fluorescence detectors

Target	Detector name	Reporter	Quencher
CMV	CMV	FAM™	(None)
Internal control	IC	JOE™	(None)

Define the following temperature profile and dye acquisition:

Table 17: Temperature profile and dye acquisition

	Stage	Cycle repeats	Acquisition	Temperature [°C]	Time [min:sec]
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			Yes	58	00:45
			-	72	00:15

CAUTION



Do not use other cycling conditions than specified in these instructions for use, as this could compromise product performance.

8.2.5 Data analysis

For basic information regarding data analysis on specific real-time PCR instruments, refer to the instructions for use of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the AltoStar® CMV PCR Kit 1.5 on different real-time PCR instruments, contact Altona Diagnostics technical support (see chapter 12. Technical support).

Criteria for validity of diagnostic PCR runs and result interpretation regardless of the real-time PCR instrument are described in chapters 7.6.8.2 Validity of a diagnostic PCR run (qualitative) to 7.6.8.4 Validity of results for a sample, chapter 7.6.10.1 Manual interpretation of results and tables 11 and 12.

NOTE



The concentration of the "sample" is displayed in IU/μl and refers to the concentration in the eluate.

To determine the **viral load of the original sample**, the following formula has to be applied:

$$\text{Viral load (Sample) [IU/ml]} = \frac{\text{Volume (Eluate) [\mu l]} \cdot \text{Viral load (Eluate) [IU/\mu l]}}{\text{Sample input [ml]}}$$

CAUTION



Do not use other control settings for data analysis than specified in these instructions for use, as this could cause incorrect IVD examination results.

CAUTION



As with any diagnostic test, results shall be interpreted in consideration of all clinical and laboratory findings.

9. Performance data

The performance of the AltoStar® CMV PCR Kit 1.5 was evaluated using the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) and CMV standard material calibrated against the WHO International Standard.

9.1 Plasma

9.1.1 Analytical sensitivity

For the determination of the limit of detection (LoD) a dilution series of the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) in plasma ranging from 1.00E+03 to 1.00E+01 IU/ml was generated.

Each dilution was tested in 8 replicates in 3 different runs (total n = 24 per dilution) using combinations of:

- 3 AltoStar® CMV PCR Kit 1.5 lots
- 3 AltoStar® Purification Kit 1.5 lots
- 3 AltoStar® Internal Control 1.5 lots
- 3 AltoStar® AM16 instruments
- 3 CFX96™ DW Dx instruments

Data from all runs were combined and a probit analysis was performed to determine the 95 % LoD value.

Table 18: PCR results used for the calculation of the analytical sensitivity of the AltoStar® CMV PCR Kit 1.5

Concentration [IU/ml]	N [total]	N [positive]	Hit rate [%]
1.00E+03	24	24	100
5.00E+02	24	24	100
2.50E+02	24	23	96
2.00E+02	24	23	96
1.50E+02	24	23	96
1.00E+02	24	16	67
5.00E+01	24	10	42
1.00E+01	24	4	17

The LoD of the AltoStar® CMV PCR Kit 1.5 for the detection of CMV in plasma is 215 IU/ml (95 % confidence interval: 163–330 IU/ml).

9.1.2 Analytical specificity

The analytical specificity of the AltoStar® CMV PCR Kit 1.5 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant CMV genotypes will be detected.

For the verification of the analytical specificity of the AltoStar® CMV PCR Kit 1.5 the following experiments were performed (see chapters 9.1.2.1 Negative samples to 9.1.2.3 Cross-reactivity).

9.1.2.1 Negative samples

35 CMV negative plasma samples from individual donors were tested with the AltoStar® CMV PCR Kit 1.5. All (35 out of 35) samples were tested negative for CMV specific DNA and positive for the IC. The analytical specificity of the AltoStar® CMV PCR Kit 1.5 for plasma samples is $\geq 95\%$.

9.1.2.2 Interfering substances

To evaluate the influence of potentially interfering endogenous and exogenous substances on the performance of the AltoStar® CMV PCR Kit 1.5 selected substances were spiked in plasma samples. These plasma samples contained CMV in a concentration of the 3 x LoD ($6.45E+02$ IU/ml), $1.00E+04$ IU/ml and no CMV, respectively.

Results obtained for samples containing potentially interfering substances were compared to results generated for plasma samples containing no spiked interferent. Each sample was processed in 3 replicates.

No interference was observed for samples containing elevated levels of:

- Endogenous substances
 - Bilirubin
 - Hemoglobin
 - Human genomic DNA
 - Human serum albumin
 - Triglycerides
- Exogenous substances
 - Azathioprine
 - Cyclosporine
 - Foscarnet
 - Ganciclovir

CAUTION



The presence of PCR inhibitors (e.g. heparin) could cause false negative or invalid results.

9.1.2.3 Cross-reactivity

The analytical specificity of the AltoStar® CMV PCR Kit 1.5 with respect to cross-reactivity with other pathogens than CMV was evaluated by testing:

- Viruses related to CMV
- Viruses causing similar symptoms as an infection with CMV
- Viruses likely to be present in patients suffering from a CMV infection

The AltoStar® CMV PCR Kit 1.5 did not cross-react with any of the following pathogens:

- Adenovirus (ADV)
- BK virus (BKV)
- Epstein-Barr virus (EBV)
- Hepatitis A virus (HAV)
- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV)
- Herpes simplex virus 1 (HSV-1)
- Herpes simplex virus 2 (HSV-2)
- Human herpesvirus 6A (HHV-6A)
- Human herpesvirus 6B (HHV-6B)
- Human immunodeficiency virus 1 (HIV-1)
- JC virus (JCV)
- Parvovirus B19
- Varicella-Zoster virus (VZV)

CAUTION



In case the sample contains other pathogens than CMV, competition with the target amplification or cross-reactivities may occur, causing incorrect IVD examination results.

9.1.3 Linear range

For the determination of the linear range of the AltoStar® CMV PCR Kit 1.5 a dilution series of CMV in plasma ranging from 1.00E+08 to 2.00E+02 IU/ml was tested:

- Dilutions with a concentration between 1.00E+08 and 1.00E+06 IU/ml were tested in 4 replicates.
- Dilutions with a concentration between 1.00E+05 and 2.00E+02 IU/ml were tested in 8 replicates.

Analysis was performed based on a polynomial regression.

The linear range of the AltoStar® CMV PCR Kit 1.5 for the quantification of CMV in plasma is 2.50E+02–1.00E+08 IU/ml. A graphical representation of the data is shown in figure 16.

log₁₀ estimated concentration vs. log₁₀ nominal concentration AltoStar® CMV PCR Kit 1.5

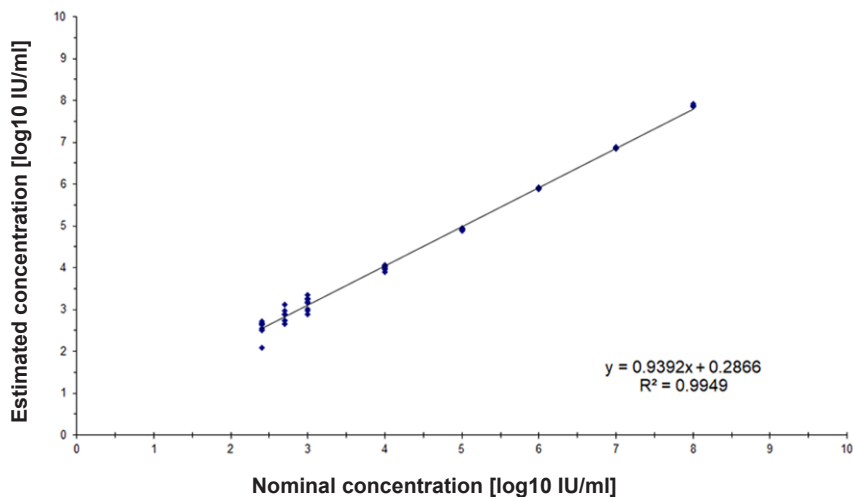


Figure 16: Linear regression analysis of the AltoStar® CMV PCR Kit 1.5 with plasma samples

9.1.4 Precision

Precision of the AltoStar® CMV PCR Kit 1.5 was evaluated using a panel consisting of:

- 1 CMV high positive (1.00E+04 IU/ml) plasma sample
- 1 CMV low positive [1.25E+03 IU/ml (5 x lower limit of quantitation (LLoQ))] plasma sample
- 1 CMV negative plasma sample

Each panel member was tested in at least 4 replicates per run.

5 runs were performed on 5 different days using combinations of:

- 3 AltoStar® CMV PCR Kit 1.5 lots
- 3 AltoStar® Purification Kit 1.5 lots
- 3 AltoStar® Internal Control 1.5 lots
- 3 AltoStar® AM16 instruments
- 3 CFX96™ DW Dx instruments

Repeatability (intra-run variability), inter-lot variability and reproducibility (total variability) were determined based on:

- Quantification values for the CMV high and low positive samples (see table 19)
- Threshold cycle (C_q^*) values for the IC in the CMV negative samples (see table 20)

* Please note that the chosen term C_q is equivalent to the designation of C_t , which might be used by other cyclers than the CFX96™ Deep Well Dx System (Bio-Rad).

Table 19: Precision data (CV % log10 quantification data) for CMV high and low positive plasma samples

	CMV high positive sample (1.00E+04 IU/ml)	CMV low positive sample (1.25E+03 IU/ml)
Intra-run variability	0.84–2.29	2.57–4.57
Inter-lot variability	2.65	4.64
Total variability	2.41	4.19

Table 20: Precision data (CV % C_q values) for the IC in CMV negative plasma samples

	IC
Intra-run variability	0.12–0.37
Inter-lot variability	0.22
Total variability	0.42

9.1.5 Total failure rate

The robustness of the the AltoStar® CMV PCR Kit 1.5 was assessed by testing 30 CMV negative plasma samples from individual donors spiked with CMV to a final concentration of the 3 x LoD (6.45E+02 IU/ml). All (30 out of 30) samples were tested positive in the CMV specific fluorescence detection channel (FAM™).

9.1.6 Carry over

Carry over is mostly a workflow dependent risk and independent of the PCR assay used. For the AltoStar® Workflow the AltoStar® Parvovirus B19 PCR Kit 1.5 was used as exemplary model. Potential cross-contamination through carry over from high positive samples was evaluated by testing alternating parvovirus B19 high positive (1.00E+07 IU/ml) and negative samples (n = 44 each per run; 2 runs) with the AltoStar® Parvovirus B19 PCR Kit 1.5. No carry over was observed, i.e. all parvovirus B19 negative samples were tested negative.

9.1.7 Clinical performance

The AltoStar® CMV PCR Kit 1.5 was evaluated in a comparative study with the CE-marked kPCR PLX® Cytomegalovirus (CMV) DNA Assay. Retrospectively, 142 plasma samples from routine CMV monitoring were tested in parallel with the kPCR PLX® Cytomegalovirus (CMV) DNA Assay and the AltoStar® CMV PCR Kit 1.5.

The kPCR PLX® Cytomegalovirus (CMV) DNA Assay was used in combination with the VERSANT® kPCR Molecular System (Siemens).

The AltoStar® CMV PCR Kit 1.5 was used in combination with the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5 on the AltoStar® AM16 and the CFX96™ DW Dx.

For the qualitative analysis all samples with an invalid result for one or both assays as well as samples with a quantitative result below the LoD of one or both assays were excluded.

Results for the remaining 125 samples are shown in table 21.

Table 21: Results of the evaluation of the diagnostic sensitivity and specificity for CMV in plasma samples

		kPCR PLX® Cytomegalovirus (CMV) DNA Assay	
		POSITIVE	NEGATIVE
AltoStar® CMV PCR Kit 1.5	POSITIVE	81	0
	NEGATIVE	0	44

The diagnostic sensitivity and specificity of the AltoStar® CMV PCR Kit 1.5 compared to the kPCR PLX® Cytomegalovirus (CMV) DNA Assay were 100 %.

For the quantitative correlation samples tested negative with one or both assays and samples with a quantitative result below the LLoQ of one or both assays were excluded.

The results of the remaining 77 samples were used for the quantitative correlation by linear regression analysis (see figure 17).

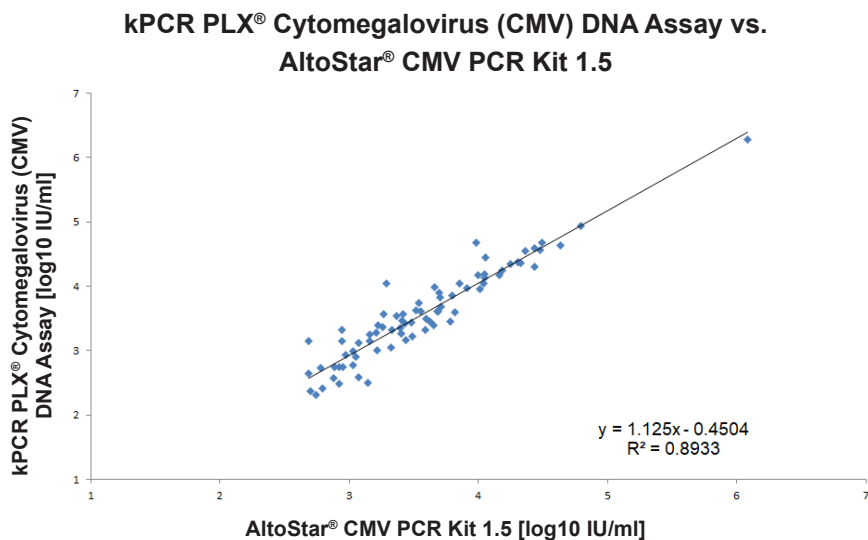


Figure 17: Linear regression analysis of the results obtained with the kPCR PLX® Cytomegalovirus (CMV) DNA Assay (reference) and the AltoStar® CMV PCR Kit 1.5

There was very good correlation between the quantitative results obtained with the AltoStar® CMV PCR Kit 1.5 and the kPCR PLX® Cytomegalovirus (CMV) DNA Assay [correlation coefficient $R = 0.95$ ($R^2 = 0.89$)].

9.2 Whole blood

9.2.1 Analytical sensitivity

For the determination of the LoD a dilution series of the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) in whole blood ranging from 1.00E+04 to 1.00E+01 IU/ml was generated.

Each dilution was tested in 8 replicates in 3 different runs (total n = 24 per dilution) using combinations of:

- 3 AltoStar® CMV PCR Kit 1.5 lots
- 3 AltoStar® Purification Kit 1.5 lots
- 3 AltoStar® Internal Control 1.5 lots
- 3 AltoStar® AM16 instruments
- 3 CFX96™ DW Dx instruments

Data from all runs were combined and a probit analysis was performed to determine the 95 % LoD value.

Table 22: PCR results used for the calculation of the analytical sensitivity of the AltoStar® CMV PCR Kit 1.5

Concentration [IU/ml]	N [total]	N [positive]	Hit rate [%]
1.00E+04	24	24	100
1.00E+03	24	24	100
7.50E+02	24	24	100
5.00E+02	24	24	100
3.50E+02	24	24	100
2.00E+02	24	21	88
1.00E+02	24	17	71
5.00E+01	24	5	21
2.00E+01	24	2	8
1.00E+01	24	3	13

The LoD of the AltoStar® CMV PCR Kit 1.5 for the detection of CMV in whole blood is 305 IU/ml (95 % confidence interval: 128–495 IU/ml).

9.2.2 Analytical specificity

The analytical specificity of the AltoStar® CMV PCR Kit 1.5 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant CMV genotypes will be detected.

For the verification of the analytical specificity of the AltoStar® CMV PCR Kit 1.5 the following experiments were performed (see chapters 9.2.2.1 Negative samples to 9.2.2.3 Cross-reactivity).

9.2.2.1 Negative samples

31 CMV negative whole blood samples from individual donors were tested with the AltoStar® CMV PCR Kit 1.5. All (31 out of 31) samples were tested negative for CMV specific DNA and positive for the IC. The analytical specificity of the AltoStar® CMV PCR Kit 1.5 for whole blood samples is $\geq 95\%$.

9.2.2.2 Interfering substances

To evaluate the influence of potentially interfering endogenous and exogenous substances on the performance of the AltoStar® CMV PCR Kit 1.5 selected substances were spiked in whole blood samples. These whole blood samples contained CMV in a concentration of the $3 \times \text{LoD}$ ($9.15\text{E}+02$ IU/ml), $1.00\text{E}+04$ IU/ml and no CMV, respectively.

Results obtained for samples containing potentially interfering substances were compared to results generated for whole blood samples containing no spiked interferent. Each sample was processed in 3 replicates.

No interference was observed for samples containing elevated levels of:

- Endogenous substances
 - Bilirubin
 - Hemoglobin
 - Human genomic DNA
 - Human serum albumin
 - Triglycerides
- Exogenous substances
 - Azathioprine
 - Cyclosporine
 - Foscarnet
 - Ganciclovir

CAUTION

The presence of PCR inhibitors (e.g. heparin) could cause false negative or invalid results.

9.2.2.3 Cross-reactivity

Since the same pathogens are likely to be found in plasma and whole blood, no additional pathogens were tested for whole blood. For the evaluation of cross-reactivity see chapter 9.1.2.3 Cross-reactivity.

CAUTION

In case the sample contains other pathogens than CMV, competition with the target amplification or cross-reactivities may occur, causing incorrect IVD examination results.

9.2.3 Linear range

For the determination of the linear range of the AltoStar® CMV PCR Kit 1.5 a dilution series of CMV in whole blood ranging from 1.00E+08 to 5.00E+02 IU/ml was tested:

- Dilutions with a concentration between 1.00E+08 and 1.00E+06 IU/ml were tested in 4 replicates.
- Dilutions with a concentration between 1.00E+05 and 5.00E+02 IU/ml were tested in 8 replicates.

Analysis was performed based on a polynomial regression.

The linear range of the AltoStar® CMV PCR Kit 1.5 for the quantification of CMV in whole blood is 5.00E+02–1.00E+08 IU/ml. A graphical representation of the data is shown in figure 18.

log₁₀ estimated concentration vs. log₁₀ nominal concentration AltoStar® CMV PCR Kit 1.5

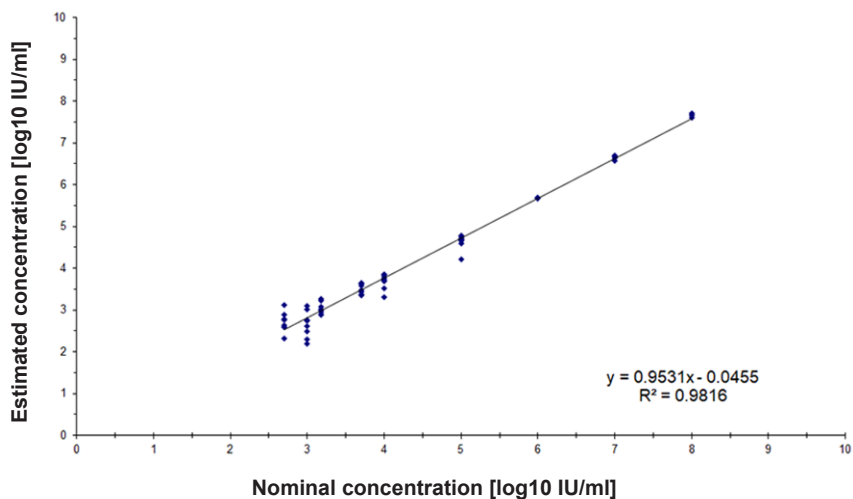


Figure 18: Linear regression analysis of the AltoStar® CMV PCR Kit 1.5 with whole blood samples

9.2.4 Precision

Precision of the AltoStar® CMV PCR Kit 1.5 was evaluated using a panel consisting of:

- 1 CMV high positive (1.00E+04 IU/ml) whole blood sample
- 1 CMV low positive [2.50E+03 IU/ml (5 x LLoQ)] whole blood sample
- 1 CMV negative whole blood sample

Each panel member was tested in at least 4 replicates per run.

5 runs were performed on 5 different days using combinations of:

- 3 AltoStar® CMV PCR Kit 1.5 lots
- 3 AltoStar® Purification Kit 1.5 lots
- 3 AltoStar® Internal Control 1.5 lots
- 3 AltoStar® AM16 instruments
- 3 CFX96™ DW Dx instruments

Repeatability (intra-run variability), inter-lot variability and reproducibility (total variability) were determined based on:

- Quantification values for the CMV high and low positive samples (see table 23)
- Threshold cycle (C_q^*) values for the IC in the CMV negative samples (see table 24)

* Please note that the chosen term C_q is equivalent to the designation of C_t , which might be used by other cyclers than the CFX96™ Deep Well Dx System (Bio-Rad).

Table 23: Precision data (CV % log10 quantification data) for CMV high and low positive whole blood samples

	CMV high positive sample (1.00E+04 IU/ml)	CMV low positive sample (2.50E+03 IU/ml)
Intra-run variability	1.17–2.66	1.65–4.11
Inter-lot variability	3.22	4.33
Total variability	4.55	6.13

Table 24: Precision data (CV % C_q values) for the IC in CMV negative whole blood samples

	IC
Intra-run variability	0.09–0.67
Inter-lot variability	1.06
Total variability	1.08

9.2.5 Total failure rate

The robustness of the the AltoStar® CMV PCR Kit 1.5 was assessed by testing 30 CMV negative whole blood samples from individual donors spiked with CMV to a final concentration of the 3 x LoD (9.15E+02 IU/ml). All (30 out of 30) samples were tested positive in the CMV specific fluorescence detection channel (FAM™).

9.2.6 Carry over

Refer to chapter 9.1.6 Carry over.

9.2.7 Clinical performance

The AltoStar® CMV PCR Kit 1.5 was evaluated in a comparative study with the CE-marked kPCR PLX® Cytomegalovirus (CMV) DNA Assay. Retrospectively, 79 whole blood samples from routine CMV monitoring were tested in parallel with the kPCR PLX® Cytomegalovirus (CMV) DNA Assay and the AltoStar® CMV PCR Kit 1.5.

The kPCR PLX® Cytomegalovirus (CMV) DNA Assay was used in combination with the VERSANT® kPCR Molecular System (Siemens).

The AltoStar® CMV PCR Kit 1.5 was used in combination with the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5 on the AltoStar® AM16 and the CFX96™ DW Dx.

For the qualitative analysis all samples with an invalid result for one or both assays as well as samples with a quantitative result below the LoD of one or both assays were excluded.

Results for the remaining 74 samples are shown in table 25.

Table 25: Results of the evaluation of the diagnostic sensitivity and specificity for CMV in whole blood samples

		kPCR PLX® Cytomegalovirus (CMV) DNA Assay	
		POSITIVE	NEGATIVE
AltoStar® CMV PCR Kit 1.5	POSITIVE	54	0
	NEGATIVE	0	20

The diagnostic sensitivity and specificity of the AltoStar® CMV PCR Kit 1.5 compared to the kPCR PLX® Cytomegalovirus (CMV) DNA Assay were 100 %.

For the quantitative correlation samples tested negative with one or both assays and samples with a quantitative result below the LLoQ of one or both assays were excluded.

The results of the remaining 53 samples were used for the quantitative correlation by linear regression analysis (see figure 19).

kPCR PLX® Cytomegalovirus (CMV) DNA Assay vs. AltoStar® CMV PCR Kit 1.5

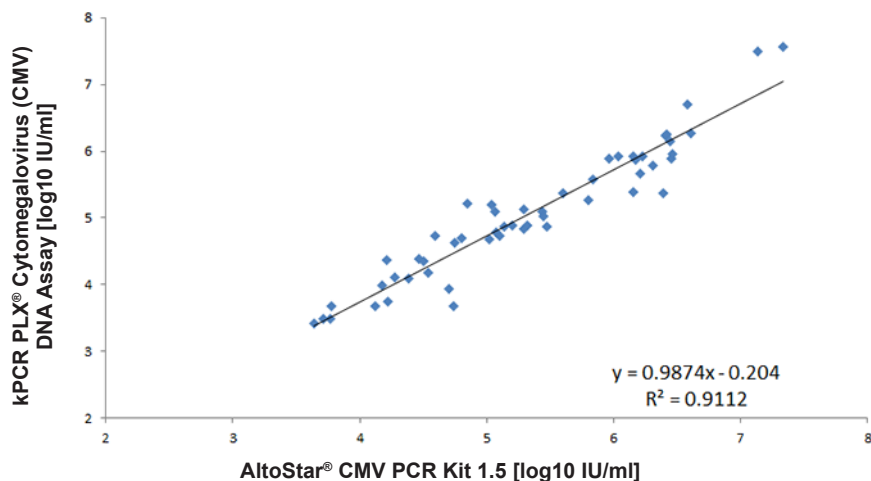


Figure 19: Linear regression analysis of the results obtained with the kPCR PLX® Cytomegalovirus (CMV) DNA Assay (reference) and the AltoStar® CMV PCR Kit 1.5

There was very good correlation between the quantitative results obtained with the AltoStar® CMV PCR Kit 1.5 and the kPCR PLX® Cytomegalovirus (CMV) DNA Assay [correlation coefficient $R = 0.95$ ($R^2 = 0.91$)].

9.3 Urine

9.3.1 Analytical sensitivity

For the determination of the LoD a dilution series of the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) in urine ranging from $5.00E+03$ to $1.00E+01$ IU/ml was generated.

Each dilution was tested in 8 replicates in 3 different runs (total n = 24 per dilution) using combinations of:

- 3 AltoStar® CMV PCR Kit 1.5 lots
- 3 AltoStar® Purification Kit 1.5 lots
- 3 AltoStar® Internal Control 1.5 lots
- 3 AltoStar® AM16 instruments
- 3 CFX96™ DW Dx instruments

Data from all runs were combined and a probit analysis was performed to determine the 95 % LoD value.

Table 26: PCR results used for the calculation of the analytical sensitivity of the AltoStar® CMV PCR Kit 1.5

Concentration [IU/ml]	N [total]	N [positive]	Hit rate [%]
5.00E+03	24	24	100
2.50E+03	24	24	100
1.00E+03	24	24	100
5.00E+02	24	22	92
2.50E+02	24	21	88
2.00E+02	24	19	79
1.00E+02	24	13	54
5.00E+01	24	13	54
1.00E+01	24	6	25

The LoD of the AltoStar® CMV PCR Kit 1.5 for the detection of CMV in urine is 711 IU/ml (95 % confidence interval: 420–1,625 IU/ml).

9.3.2 Analytical specificity

The analytical specificity of the AltoStar® CMV PCR Kit 1.5 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant CMV genotypes will be detected.

For the verification of the analytical specificity of the AltoStar® CMV PCR Kit 1.5 the following experiments were performed (see chapters 9.3.2.1 Negative samples to 9.3.2.3 Cross-reactivity).

9.3.2.1 Negative samples

35 CMV negative urine samples from individual donors were tested with the AltoStar® CMV PCR Kit 1.5. All (35 out of 35) samples were tested negative for CMV specific DNA and positive for the IC. The analytical specificity of the AltoStar® CMV PCR Kit 1.5 for urine samples is $\geq 95\%$.

9.3.2.2 Interfering substances

To evaluate the influence of potentially interfering endogenous and exogenous substances on the performance of the AltoStar® CMV PCR Kit 1.5 selected substances were spiked in urine samples. These urine samples contained CMV in a concentration of the 3 x LoD (2.13E+03 IU/ml), 5.00E+04 IU/ml and no CMV, respectively.

Results obtained for samples containing potentially interfering substances were compared to results generated for urine samples containing no spiked interferent. Each sample was processed in 3 replicates.

No interference was observed for samples containing elevated levels of:

- Endogenous substances
 - Bilirubin
 - Glucose
 - Human serum albumin

- Human whole blood
- Exogenous substances
 - Azathioprine
 - Cyclosporine
 - Foscarnet
 - Ganciclovir

CAUTION



The presence of PCR inhibitors (e.g. heparin) could cause false negative or invalid results.

9.3.2.3 Cross-reactivity

The analytical specificity of the AltoStar® CMV PCR Kit 1.5 with respect to cross-reactivity with other pathogens than CMV was evaluated. Additionally to the pathogens tested for plasma and whole blood samples (see chapters 9.1.2.3 Cross-reactivity and 9.2.2.3 Cross-reactivity), pathogens potentially to be found in the sample matrix urine (e.g. pathogens associated to urinary tract infections and sexually transmitted diseases) were tested.

The AltoStar® CMV PCR Kit 1.5 did not cross-react with any of the following pathogens:

- *Chlamydia trachomatis*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Mycoplasma hominis*
- *Proteus mirabilis*
- *Ureaplasma urealyticum*
- *Ureaplasma parvum*

CAUTION



In case the sample contains other pathogens than CMV, competition with the target amplification or cross-reactivities may occur, causing incorrect IVD examination results.

9.3.3 Linear range

For the determination of the linear range of the AltoStar® CMV PCR Kit 1.5 a dilution series of CMV in urine ranging from 1.00E+08 to 2.00E+02 IU/ml was tested:

- Dilutions with a concentration between 1.00E+08 and 1.00E+06 IU/ml were tested in 4 replicates.
- Dilutions with a concentration between 1.00E+05 and 2.00E+02 IU/ml were tested in 8 replicates.

Analysis was performed based on a polynomial regression.

The linear range of the AltoStar® CMV PCR Kit 1.5 for the quantification of CMV in urine is 1.00E+03–1.00E+08 IU/ml. A graphical representation of the data is shown in figure 20.

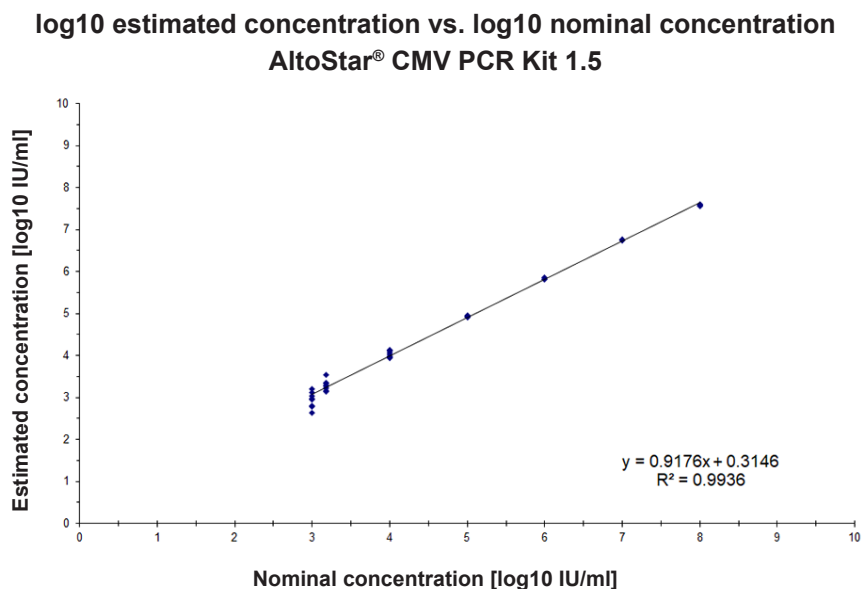


Figure 20: Linear regression analysis of the AltoStar® CMV PCR Kit 1.5 with urine samples

9.3.4 Precision

Precision of the AltoStar® CMV PCR Kit 1.5 was evaluated using a panel consisting of:

- 1 CMV high positive (5.00E+04 IU/ml) urine sample
- 1 CMV low positive [5.00E+03 IU/ml (5 x LLoQ)] urine sample
- 1 CMV negative urine sample

Each panel member was tested in at least 4 replicates per run.

5 runs were performed on 5 different days using combinations of:

- 3 AltoStar® CMV PCR Kit 1.5 lots
- 3 AltoStar® Purification Kit 1.5 lots
- 3 AltoStar® Internal Control 1.5 lots
- 3 AltoStar® AM16 instruments
- 3 CFX96™ DW Dx instruments

Repeatability (intra-run variability), inter-lot variability and reproducibility (total variability) were determined based on:

- Quantification values for the CMV high and low positive samples (see table 27)
- Threshold cycle (C_q^*) values for the IC in the CMV negative samples (see table 28)

* Please note that the chosen term C_q is equivalent to the designation of C_t , which might be used by other cyclers than the CFX96™ Deep Well Dx System (Bio-Rad).

Table 27: Precision data (CV % log10 quantification data) for CMV high and low positive urine samples

	CMV high positive sample (5.00E+04 IU/ml)	CMV low positive sample (5.00E+03 IU/ml)
Intra-run variability	0.09–0.67	0.47–0.85
Inter-lot variability	2.89	3.69
Total variability	2.29	2.90

Table 28: Precision data (CV % C_q values) for the IC in CMV negative urine samples

	IC
Intra-run variability	0.20–0.36
Inter-lot variability	0.40
Total variability	1.00

9.3.5 Total failure rate

The robustness of the the AltoStar® CMV PCR Kit 1.5 was assessed by testing 30 CMV negative urine samples from individual donors spiked with CMV to a final concentration of the 3 x LoD (2.13E+03 IU/ml). All (30 out of 30) samples were tested positive in the CMV specific fluorescence detection channel (FAM™).

9.3.6 Carry over

Refer to chapter 9.1.6 Carry over.

9.3.7 Clinical performance

The AltoStar® CMV PCR Kit 1.5 was evaluated in a comparative study with the CE-marked CMV R-gene® kit (bioMérieux). Retrospectively, 87 urine samples from routine CMV monitoring were tested in parallel with the CMV R-gene® kit (bioMérieux) and the AltoStar® CMV PCR Kit 1.5.

The CMV R-gene® kit (bioMérieux) was used in combination with the MagNA Pure® 96 DNA and Viral Nucleic Acid Small Volume Kit (Roche) and the MagNA Pure® 96 Instrument (Roche).

The AltoStar® CMV PCR Kit 1.5 was used in combination with the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5 on the AltoStar® AM16 and the CFX96™ DW Dx.

For the qualitative analysis all samples with an invalid result for one or both assays as well as samples with a quantitative result below the LoD of one or both assays were excluded.

Results for the remaining 80 samples are shown in table 29.

Table 29: Results of the evaluation of the diagnostic sensitivity and specificity for CMV in urine samples

		CMV R-gene® kit (bioMérieux)	
		POSITIVE	NEGATIVE
AltoStar® CMV PCR Kit 1.5	POSITIVE	54	0
	NEGATIVE	1	25

The diagnostic sensitivity and specificity of the AltoStar® CMV PCR Kit 1.5 compared to the CMV R-gene® kit (bioMérieux) were 98 % and 100 %, respectively.

For the quantitative correlation samples tested negative with one or both assays and samples with a quantitative result below the LLoQ of one or both assays were excluded.

The results of the remaining 45 samples were used for the quantitative correlation by linear regression analysis (see figure 21).

CMV R-gene® kit (bioMérieux) vs. AltoStar® CMV PCR Kit 1.5

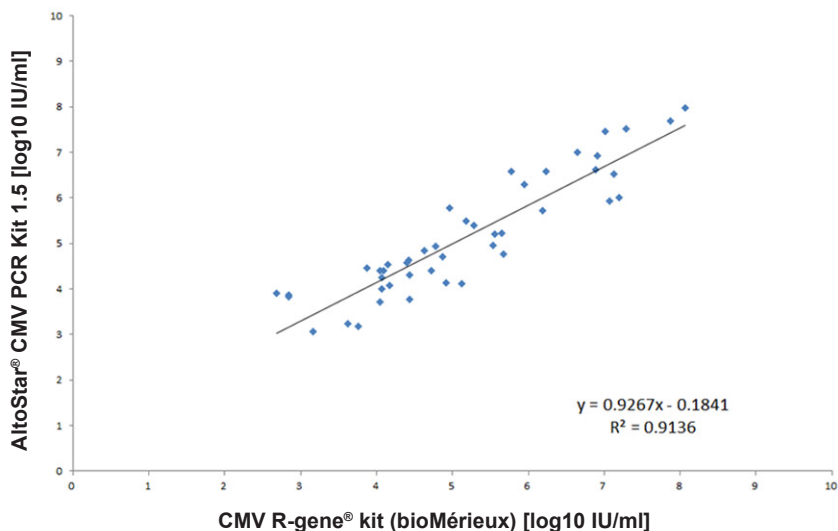


Figure 21: Linear regression analysis of the results obtained with the CMV R-gene® kit (bioMérieux) (reference) and the AltoStar® CMV PCR Kit 1.5

There was very good correlation between the quantitative results obtained with the AltoStar® CMV PCR Kit 1.5 and the CMV R-gene® kit (bioMérieux) [correlation coefficient $R = 0.96$ ($R^2 = 0.91$)].

10. Disposal

Dispose of hazardous and biological waste in compliance with local and national regulations. Leftover product components and waste should not be allowed to enter sewage, water courses or the soil.

CAUTION



Always treat samples as infectious and (bio-)hazardous material in accordance with safety and laboratory procedures. For sample material spills promptly use an appropriate disinfectant. Handle contaminated materials as biohazardous.

CAUTION



Disposal of hazardous and biological waste shall comply with local and national regulations to avoid environmental contamination.

NOTE



The PCR plate must be disposed of in a sealed state since the PCR Plate Sealing Foil cannot be removed.

11. Quality control

In accordance with the Altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of AltoStar® CMV PCR Kit 1.5 is tested against predetermined specifications to ensure consistent product quality.

12. Technical support

For customer support, contact altona Diagnostics technical support:

e-mail: support@altona-diagnostics.com

phone: +49-(0)40-5480676-0

NOTE



Any serious incident that has occurred in relation to this product shall be reported to altona Diagnostics and the competent authority of your country.

13. Literature

- [1] Hodinka RL. Human Cytomegalovirus. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, eds. *Manual of Clinical Microbiology*, 10th ed., American Society for Microbiology, Washington. 2011:1558-1574.
- [2] Mocarski, Jr ES, Shenk T, Griffiths PD, Pass RF. Cytomegaloviruses. In: Knipe DM, Howley PM, et al., eds. *Fields Virology*, 6th ed., Lippincott Williams & Wilkins, Philadelphia. 2013:1961-2014.
- [3] Pellett PE, Roizman B. Herpesviridae. In: Knipe DM, Howley PM, et al., eds. *Fields Virology*, 6th ed., Lippincott Williams & Wilkins, Philadelphia. 2013:1803-1822.
- [4] Fryer JF, Heath AB, Anderson R, Minor PD and the Collaborative Study Group. 2010 Collaborative Study to Evaluate the Proposed 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification (NAT)-Based Assays. WHO ECBS Report WHO/BS/10.2138.

14. Trademarks and disclaimers

4s3™ (4titude); AltoStar®, kPCR PLX® (altona Diagnostics); ABI Prism®, QuantStudio™ (Applied Biosystems); R-gene® (bioMérieux); CFX96™, CFX Manager™ (Bio-Rad); Rotor-Gene® (QIAGEN); LOINC® (Regenstrief Institute, Inc.); LightCycler®, MagNA Pure® (Roche); FAM™, JOE™, MicroAmp™, VIC™ (Thermo Fisher Scientific).

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The AltoStar® CMV PCR Kit 1.5 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

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15. Symbols

Symbol	Explanation
	<i>In vitro</i> diagnostic medical device
	Global Trade Item Number
	Batch code
	Content
	Cap color
	Catalogue number
	Number
	Component
	Consult instructions for use
	Contains sufficient for "n" tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Material number
	Version
	Note

Symbol	Explanation
--------	-------------



Contains biological material of animal origin

16. Assay protocol for the AltoStar® Connect software and information for LIMS integration

The 2D barcode in figure 22 is to be used for installing the latest assay protocol for use of the AltoStar® CMV PCR Kit 1.5 on the AltoStar® AM16. The barcode can only be scanned in printed form. You can scan the barcode directly from the manual or print it on a separate sheet. Please note that the size of the print affects the scannability of the barcode. Make sure to scale the size to 100 %. For scanning, aim the scanner to the red line on the barcode. For details on management of assay protocols, please refer to the respective chapter in the instructions for use of the AltoStar® Connect software. For information for LIMS integration, see table 31.

AltoStar® CMV PCR Kit 1.5

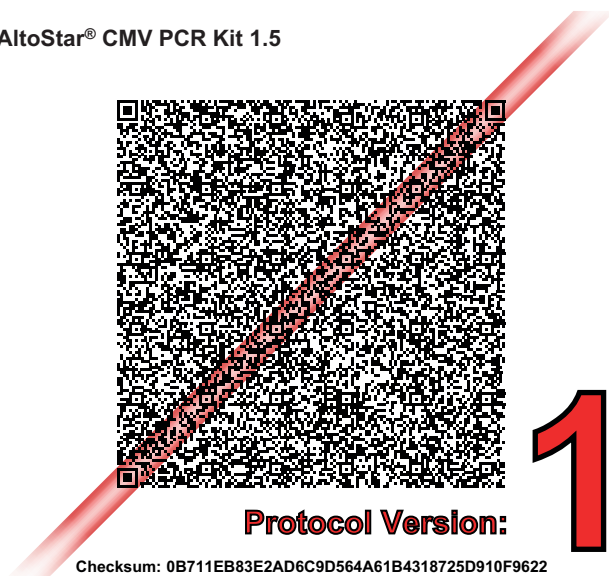


Figure 22: Assay protocol barcode for the AltoStar® CMV PCR Kit 1.5

Table 30: Changelog for the assay protocol

Protocol version	Release updates
1	Initial version

Table 31: Information for LIMS integration

Usage	Data
Test order (LIMS → AltoStar® AM16)	AltoStar® CMV PCR Kit 1.5
Test result (CFX96™ DW Dx → LIMS) unit	IU/ml
Test result (CFX96™ DW Dx → LIMS) channel 1	CMV
Test result (CFX96™ DW Dx → LIMS) channel 2	Internal Control

For LOINC® (Logical Observation Identifiers Names and Codes) please refer to the Altona Diagnostics GmbH website (www.altona-diagnostics.com) or contact Altona Diagnostics technical support (see chapter 12. Technical support).

17. Revision history

Table 32: Revision history

Identifier	Date of issue [month/year]	Modifications
MAN-AS0021540- EN-S01	08/2021	Initial release

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always a drop ahead.

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