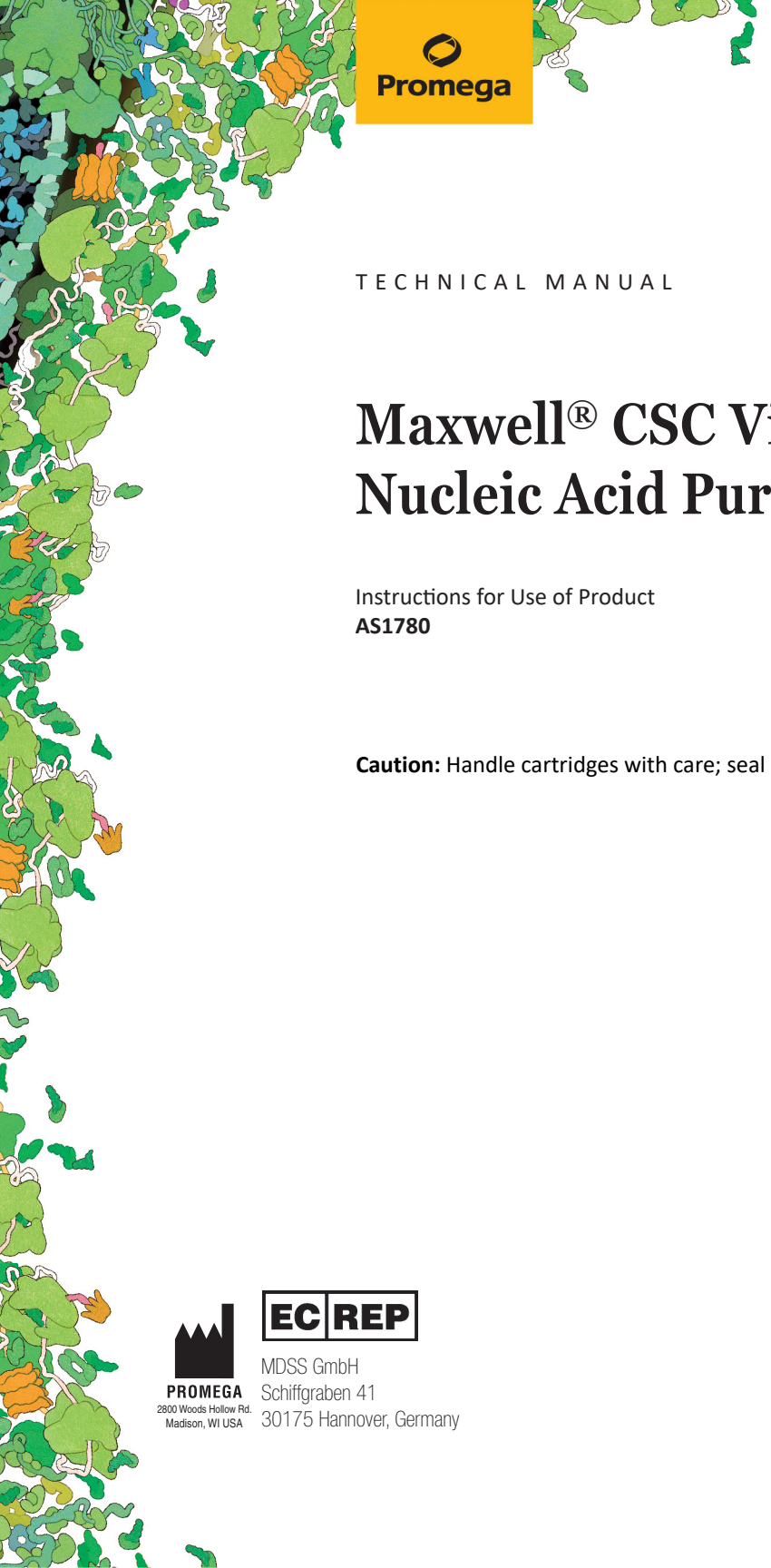

TECHNICAL MANUAL

Maxwell® CSC Viral Total Nucleic Acid Purification Kit

Instructions for Use of Product
AS1780

Caution: Handle cartridges with care; seal edges may be sharp.

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CE

INSTRUCTIONS FOR USE OF PRODUCT
AS1780

IVD

Revised 10/22
TM624

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Maxwell® CSC Viral Total Nucleic Acid Purification Kit

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

The Maxwell® CSC Viral Total Nucleic Acid Purification Kit is only available in certain countries.

1. Description

The Maxwell® CSC Viral Total Nucleic Acid Purification Kit[a] is used with the Maxwell® Instruments specified in Table 1 to provide an easy method for efficient, automated sample preparation and purification of viral total nucleic acid. Maxwell® CSC Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. The Maxwell® method for the CSC Viral Total Nucleic Acid Kit can process from one to the maximum number of Maxwell® Instrument samples in approximately 30 minutes. The low elution volume of 50µl results in concentrated purified nucleic acid for downstream applications such as quantitative PCR (qPCR) or quantitative RT-PCR (qRT-PCR). After brief initial lysis, the sample is added to the Maxwell® CSC Viral Total Nucleic Acid Purification Cartridge, and the remaining processing is fully automated.

Table 1. Supported Instruments

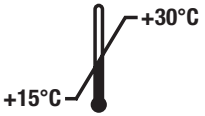
Instrument	Cat.#	Technical Manual
Maxwell® CSC	AS6000	TM457
Maxwell® CSC 48	AS8000	TM623

Principle of the Method: The Maxwell® CSC Viral Total Nucleic Acid Purification Kit purifies samples using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of nucleic acid. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind nucleic acids to the paramagnetic particle in the first well of a prefilled cartridge. The samples are processed through a series of washes before the total nucleic acid is eluted.

2. Product Components, Storage Conditions and Symbols Key

PRODUCT	SIZE	CAT.#
Maxwell® CSC Viral Total Nucleic Acid Purification Kit	**48 preps**	**AS1780**

For In Vitro Diagnostic Use. Professional use only. Sufficient for 48 isolations. Cartridges are for single use only.



Includes:

- 20ml Lysis Buffer
- 2 × 1ml Proteinase K (PK) Solution
- 50 CSC/RSC Plungers
- 48 Maxwell® CSC Cartridges (CSCA)
- 50 Elution Tubes (0.5ml)
- 25ml Nuclease-Free Water

Storage Conditions: Store components at room temperature (+15°C to +30°C).

Safety Information: The cartridges contain ethanol, isopropanol and guanidine hydrochloride. Ethanol and isopropanol should be considered flammable, harmful and irritants. Guanidine hydrochloride should be considered toxic, harmful and an irritant. Refer to the SDS for detailed safety information.

Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and safety glasses) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

Caution: Handle cartridges with care; edges may be sharp.

Additional Information: The Maxwell® CSC Viral Total Nucleic Acid Purification Kit components are qualified and quality control tested to work together. It is not recommended to mix kit components between different kit lots. Use only the components provided in the kit. Do not use cartridges if the seal on the cartridge is not intact on receipt. For additional safety information, see the Safety Data Sheet, available at: **www.promega.com**

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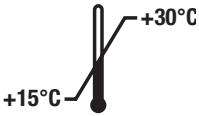
Symbols Key

Symbol	Explanation	Symbol	Explanation
	In Vitro Diagnostic Medical Device		Do not reuse
	Store at +15°C to +30°C.		Manufacturer
	Caution		Flammable
	Health hazard		Contains sufficient for "n" tests
	Warning. Pinch point hazard.		Warning. Biohazard.
	Lot number		Catalog number
	Conformité Européenne		Authorized Representative

3. Product Intended Purpose/Intended Use

The Maxwell® CSC Viral Total Nucleic Acid Purification Kit is intended for use, in combination with Maxwell® CSC Instruments and the Maxwell® CSC Viral Total Nucleic Acid purification method, as an in vitro diagnostic (IVD) medical device to perform automated isolation of total viral nucleic acid from human plasma, serum, viral transport medium or stabilized saliva samples. The purified nucleic acid is suitable for use in amplification-based in vitro diagnostic assays.

The Maxwell® CSC Viral Total Nucleic Acid Purification Kit is intended to be used at a temperature between 15°C and 30°C. Use outside of this temperature range may result in suboptimal results.

The Maxwell® CSC Viral Total Nucleic Acid Purification Kit is intended for professional use only. Diagnostic results obtained using the nucleic acid purified with this system must be interpreted in conjunction with other clinical or laboratory data.

4. Product Use Limitations

Performance of the Maxwell® CSC Viral Total Nucleic Acid Purification Kit was evaluated with serum, plasma, nasopharyngeal swabs in Universal Transport Medium for Virus (UTM) and stabilized saliva. The user is responsible for validating its use to extract viral nucleic acid from other sample types.

Appropriate controls must be included in any downstream diagnostic applications using nucleic acid purified using the Maxwell® CSC Viral Total Nucleic Acid Purification System. The user is responsible for validating the performance characteristics necessary for downstream diagnostic applications.

Users may choose to add exogenous internal controls (IC) to the sample or lysate. Certain nucleic acid internal controls smaller than 100bp may not be efficiently purified using the system.

5. Sample Preparation

Materials to Be Supplied By the User

- tubes for plasma, serum, UTM or stabilized saliva samples

Blood-borne pathogen precautions are recommended when handling any human-derived specimens.

For plasma samples, collect blood in EDTA- or ACD-anticoagulant Vacutainer® tubes. Avoid heparin as it may inhibit downstream amplifications.

The following general recommendations are for preparing and storing samples (1,2):

1. Separate plasma from cells within 1 hour of drawing blood by centrifuging at 1,500 × *g* for 20 minutes at 25°C, and then transfer plasma layer into a clean tube.
2. Separate serum from clotted blood by centrifuging at 1,000 × *g* for 10 minutes at 25°C, and then decant into a clean tube.
3. For swabs in UTM, use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 2–3ml of viral transport medium.

Store plasma and serum samples at 2–8°C for up to 24 hours, or freeze samples that are not processed within 24 hours at –20°C for up to 5 days. Store UTM and stabilized saliva samples at 2–8°C for up to 72 hours, or freeze samples at –70°C. Avoid repeated freeze-thaw cycles, and do not store samples in a frost-free freezer. Specific collection and storage conditions may vary, depending on the virus isolated.

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6. Before You Begin

Materials to Be Supplied by the User

- 1.5–2.0ml tubes for incubation of samples (e.g., ClickFit Microtube, 1.5ml [Cat.# V4741]; recommended to prevent the cap from opening during heating)
- 15ml or 50ml conical tube for preparation of Lysis Solution
- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- heating block or water bath set to 56°C

6.A. Lysis Solution Preparation

If the Lysis Buffer is cloudy or contains precipitate, heat at 37–56°C until the Lysis Buffer clears.

Note: Prepare fresh Lysis Solution for each batch of samples as described in Table 2. Invert tube to mix.

Table 2. Preparing Lysis Solution.

For 100µl and 200µl of plasma or serum samples, or 200µl of UTM or stabilized saliva samples:

Reagent	Amount/Reactions	Reactions (Number to be run + 2)	Total
Lysis Buffer[1]	200µl	n + 2	200µl × (n + 2)
Proteinase K Solution	20µl	n + 2	20µl × (n + 2)

For 300µl of plasma or serum samples:

Reagent	Amount/Reactions	Reactions (Number to be run + 2)	Total
Lysis Buffer[1]	300µl	n + 2	300µl × (n + 2)
Proteinase K Solution	30µl	n + 2	30µl × (n + 2)

[1]If an internal control is used, it may be added to the Lysis Solution. Internal controls are not provided in this kit.

Note: Some respiratory viruses from sample types such as nasophyrangeal swabs may not require the use of Proteinase K.

6.B. Sample Preparation for Maxwell® Viral Total Nucleic Acid Purification Cartridges

Samples may be fresh or frozen. Thaw frozen specimens at room temperature or on ice, and mix by vortexing for 10 seconds before use.

1. Pipet each plasma or serum sample or 200µl of UTM or stabilized saliva into a 1.5ml or 2ml microcentrifuge tube with a cap.
2. Add Lysis Solution prepared in Section 6.A.
   a. For sample volumes of 100µl or 200µl, add 220µl of Lysis Solution.
   b. For sample volume of 300µl, add 330µl of Lysis Solution.
3. Close tubes, and vortex for 10 seconds.
4. For serum samples, incubate at room temperature (15–30°C) for 10 minutes, and then proceed to Step 5.
5. Incubate at 56°C in a heat block or water bath for 10 minutes. During this incubation, proceed to Section 6.C to prepare the cartridges.

   Note: Some viruses, such as hepatitis B virus, may require incubation at 80°C for optimal nucleic acid recovery. due to secondary structure of the viral genome.

6.C. Maxwell® CSC Viral Total Nucleic Acid Purification Cartridge Preparation

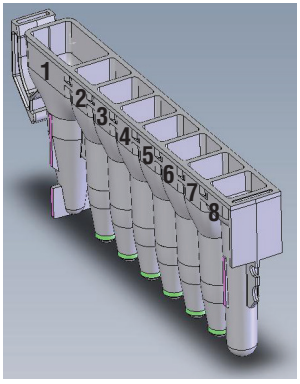
1. Change gloves before handling Cartridges, Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.
2. Place one plunger into well #8 of each cartridge.
3. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s).

6.C. Maxwell® CSC Viral Total Nucleic Acid Purification Cartridge Preparation (continued)

4. Add 50µl of Nuclease-Free Water to the bottom of each elution tube.
5. Pulse samples in a microcentrifuge to collect liquid at the bottom of the tube. Transfer sample lysate to well #1 (the largest well) of the cartridge.
6. Proceed to Section 7, Maxwell® Instrument Setup and Run.

Notes:

1. Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bactericidal spray or wipe and then water. Do not use bleach on instrument parts.
2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.



User Adds to Wells
1. Sample lysates
8. CSC/RSC Plunger

Figure 1. Maxwell® Viral Total Nucleic Acid Purification Cartridge. Preprocessed sample is added to well #1, and a plunger is added to well #8.

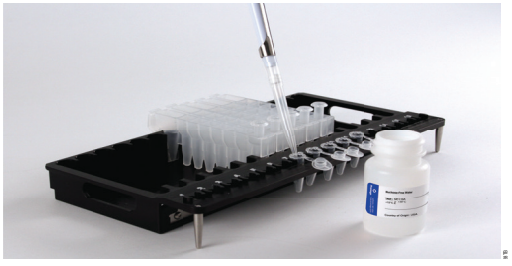


Figure 2. Setup and configuration of the deck tray(s). Nuclease-Free Water is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

7. Maxwell® Instrument Setup and Run

For detailed information, refer to the Technical Manual specific to your Maxwell® Instrument (see Table 1).

1. Turn on the Maxwell® Instrument and Tablet PC. Sign in to the Tablet PC, and start the Maxwell® IVD mode software by double-touching the icon on the desktop. The instrument will power up, proceed through a self test and home all moving parts.
2. Touch **Start** to begin the process of running a method.
3. Scan or enter the method bar code in the upper right corner of the Maxwell® CSC Viral Total Nucleic Acid Purification Kit label to automatically select the method to be run (Figure 3).

   Note: The Maxwell® CSC Viral Total Nucleic Acid Purification Kit bar code is required for purification on the Maxwell® CSC Instruments. The kit label contains two bar codes. The method bar code is indicated in Figure 3 below. If the bar code cannot be scanned, contact Promega Technical Services.

Figure 3. Kit label indicating the method bar code to scan. Scan the bar code shown in the red box, upper right of the kit label, to start a purification run.

4. On the 'Cartridge Setup' screen, touch the cartridge positions to select or deselect any positions to be used for this extraction run. Enter any required sample tracking information and touch the **Proceed** button to continue.

   Note: When using 48-position Maxwell® Instruments, touch the **Front** and **Back** buttons to select or deselect cartridge positions on each deck tray.

7. Maxwell® Instrument Setup and Run (continued)

5. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with Nuclease-Free Water and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® Instrument platform.

   Inserting the Maxwell® Deck Tray: Hold the deck tray by the sides to avoid dislodging cartridges from the deck tray. Ensure that the deck tray is placed in the Maxwell® Instrument with the elution tubes closest to the door. Angle the back of the deck tray downward and place into the instrument so that the back of the deck tray is against the back of the instrument platform. Press down on the front of the deck tray to firmly seat the deck tray on the instrument platform. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation. Ensure the deck tray is level on the instrument platform and fully seated.

   Note: Check the identifier on the 24-position Maxwell® deck tray(s) to determine whether they should be placed in the front or back of the instrument.

6. Confirm all indicated preprocessing has been performed, and touch **Start** to close the instrument door and start processing.

   Note: When using a 48-position Maxwell® Instrument, if the Vision System has been enabled, the deck tray(s) will be scanned as the door retracts. Any errors in deck tray setup (e.g., plungers not in well #8, elution tubes not present and open) will cause the software to return to the 'Cartridge Setup' screen, and problem positions will be marked with an exclamation point in a red circle. Touch the exclamation point for a description of the error and resolve all error states. Touch the **Start** button again to repeat deck tray scanning and begin the extraction run.

   Warning: Pinch point hazard.

   The Maxwell® Instrument will immediately begin the purification run. The screen will display information including the user who started the run, the current method step being performed and the approximate time remaining in the run.

   Notes:

   1. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.
   2. If the run is abandoned before completion, you may be prompted to check whether plungers are still loaded on the plunger bar. If plungers are present on the plunger bar, you should perform **Clean Up** when requested. If plungers are not present on the plunger bar, you can choose to skip **Clean Up**. The samples will be lost. Do not attempt to repurify samples if an instrument run has been aborted.

7. Follow on-screen instructions at the end of the method to open the door. Verify that plungers are located in well #8 of the cartridge at the end of the run. If plungers are not removed from the plunger bar, follow the instructions in the Technical Manual appropriate to your Maxwell® Instrument (Table 1) to perform a **Clean Up** process to attempt to unload the plungers.

8. Remove the deck tray(s) from the instrument. Remove elution tubes containing viral total nucleic acid, and cap the tubes. If paramagnetic particles are present in the elution tubes, centrifuge at 10,000–20,000 × *g* for 30 seconds to 1 minute. After the run is complete, the extraction run report will be displayed. From the 'Report View' screen, you can print or export this report or both.

9. Remove the cartridges and plungers from the deck tray(s), and discard as hazardous waste following your institution's recommended guidelines. Do not reuse reagent cartridges, plungers or elution tubes.

Note: Ensure samples are removed before performing any required ultraviolet (UV) light treatment to avoid damage to the nucleic acid.

8. Storing Eluted Nucleic Acid

If samples are not processed immediately, store eluted viral DNA on ice or at 4°C for up to 24 hours. For longer term storage, freeze at –20°C or –70°C. Viral RNA is less stable and preferably tested in downstream assays immediately after isolation. Alternatively, store eluted viral RNA at –70°C. Consult the instructions for downstream applications for specific sample storage and handling recommendations.

9. Analytical Performance Evaluation

Analytical performance evaluation of the the Maxwell® CSC Viral Total Nucleic Acid Purification Kit was performed on Maxwell® CSC and Maxwell® CSC 48 instruments using universal transport media (UTM), saliva and plasma samples.

9.A. RNA Quantity, Quality and Amplifiability

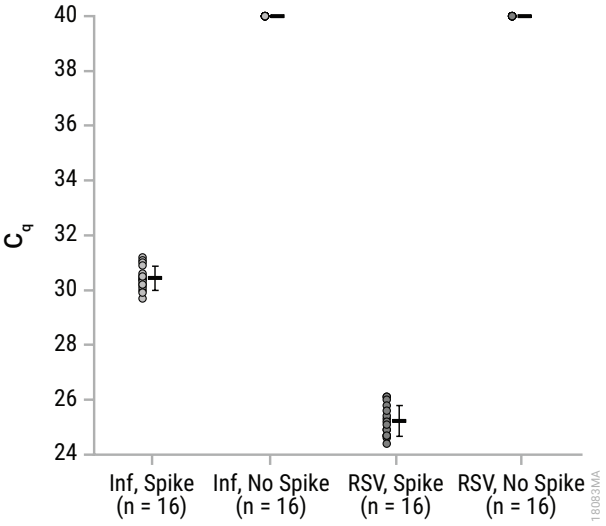


Figure 4. RT-qPCR C_q values for eluates prepared from universal transport medium (UTM). "Spike" samples were UTM spiked with inactivated influenza (Inf) or respiratory syncytial virus (RSV). "No spike" samples were UTM controls with no inactivated virus added. For each data set, dots on the left represent individual sample C_q values, while the mean with standard deviation is shown on the right. Samples with no C_q were assigned a C_q of 40 for averaging purposes. The influenza spiked sample eluates had an average C_q of 30.4 and the RSV spiked sample eluates had an average C_q of 25.2. Controls with no spike had a C_q of 40.

9.A. RNA Quantity, Quality and Amplifiability (continued)

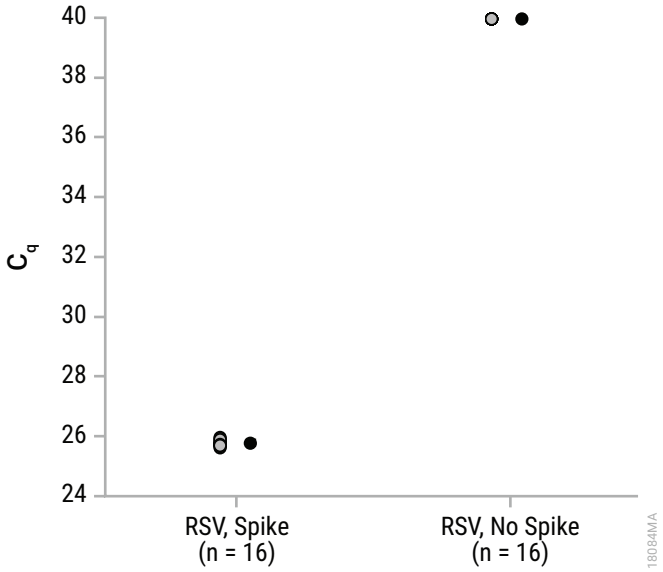


Figure 5. RT-qPCR C_q values for eluates prepared from saliva. "Spike" samples were saliva spiked with respiratory syncytial virus (RSV). "No spike" samples were saliva with no inactivated virus added. For each data set, dots on the left represent individual sample C_q values, while the mean with standard deviation is shown on the right. Samples with no C_q were assigned a C_q of 40 for averaging purposes. The RSV spiked sample eluates had an average C_q of 25.8 and controls with no spike had a C_q of 40.

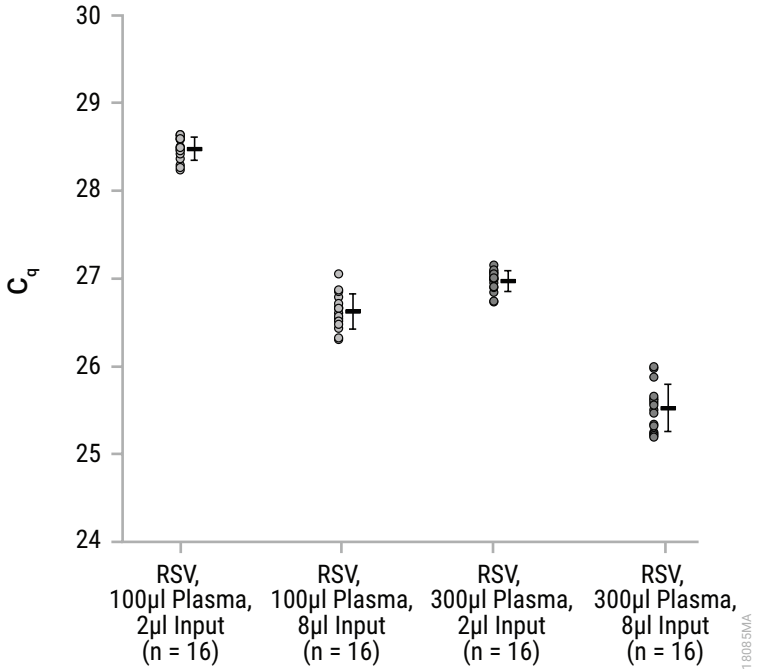


Figure 6. RT-qPCR C_q values for eluates prepared from plasma. Plasma samples (100µl or 300µl) were spiked with respiratory syncytial virus (RSV) then used for purification. Inhibition was assessed using 2µl and 8µl as a fourfold input difference that should result in a C_q difference of approximately 2 cycles. Eluates (2µl or 8µl input) from each plasma purification were amplified by RT-qPCR. For each data set, dots on the left represent individual sample C_q values, while the mean with standard deviation is shown on the right. The 100µl plasma sample with 2µl input in RT-qPCR had an average C_q of 28.5 and 8µl input in the RT-qPCR had an average of 26.6. The 300µl plasma sample with 2µl input in RT-qPCR had an average C_q of 27.0 and 8µl input in RT-qPCR had an average of 25.5.

9.A. RNA Quantity, Quality and Amplifiability (continued)

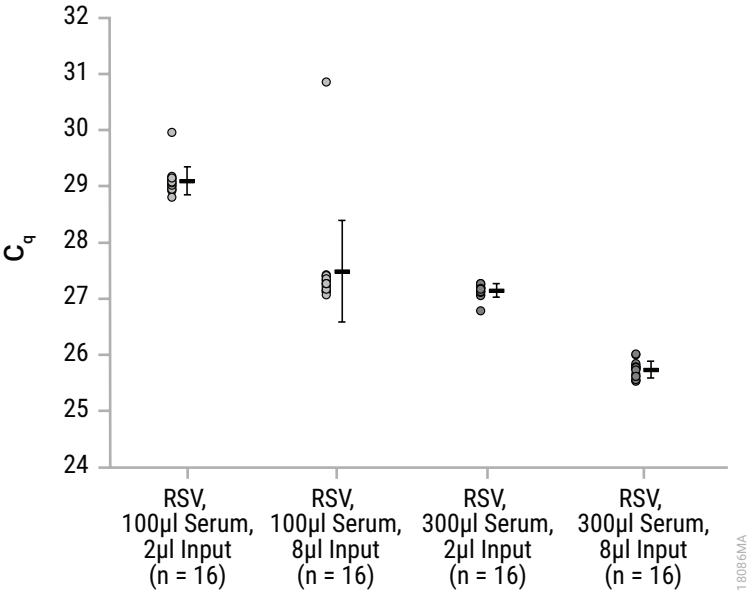


Figure 7. RT-qPCR C_q values for eluates prepared from serum. Serum samples (100µl or 300µl) were spiked with respiratory syncytial virus (RSV) then used for purification. Inhibition was assessed using 2µl and 8µl as a fourfold input difference that should result in a C_q difference of approximately 2 cycles. Eluates (2µl or 8µl input) from each serum purification were amplified by RT-qPCR. For each data set, dots on the left represent individual sample C_q values, while the mean with standard deviation is shown on the right. The 100µl serum sample with 2µl input in RT-qPCR had an average C_q of 29.1 and 8µl input in RT-qPCR had an average C_q of 27.5. The 300µl serum sample with 2µl input in RT-qPCR had an average C_q of 27.1 and 8µl input in RT-qPCR had an average C_q of 25.7.

9.B. DNA Quantity, Quality and Amplifiability

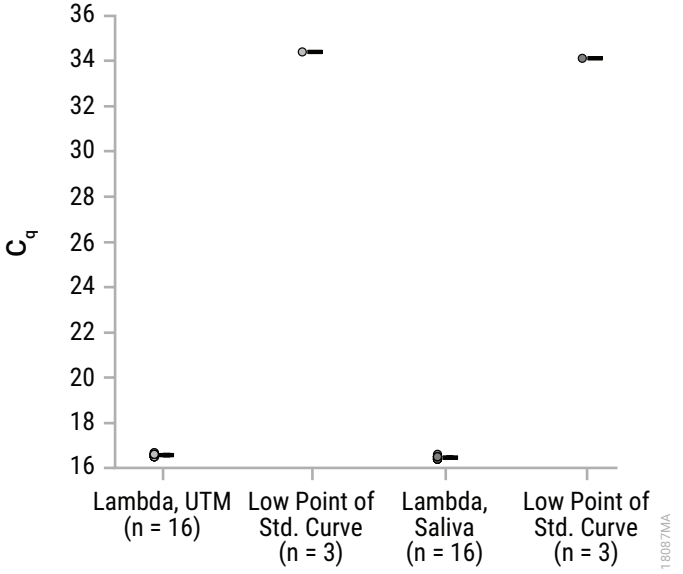


Figure 8. qPCR C_q values for eluates prepared from UTM or saliva. UTM or saliva samples were spiked with lambda phage for "lambda" samples. The low point of the standard ("Std.") curve is shown as a relative quantitation control. For each data set, dots on the left represent individual sample C_q values, while the mean with standard deviation is shown on the right. The lambda spiked UTM sample eluates had an average C_q of 16.6 and the lambda spiked saliva sample eluates had an average C_q of 16.5. The lowest point on the lambda standard curve in the UTM experiment had a C_q of 34.4, and in the saliva experiment the C_q of the lowest point was 34.1.

9.B. DNA Quantity, Quality and Amplifiability (continued)

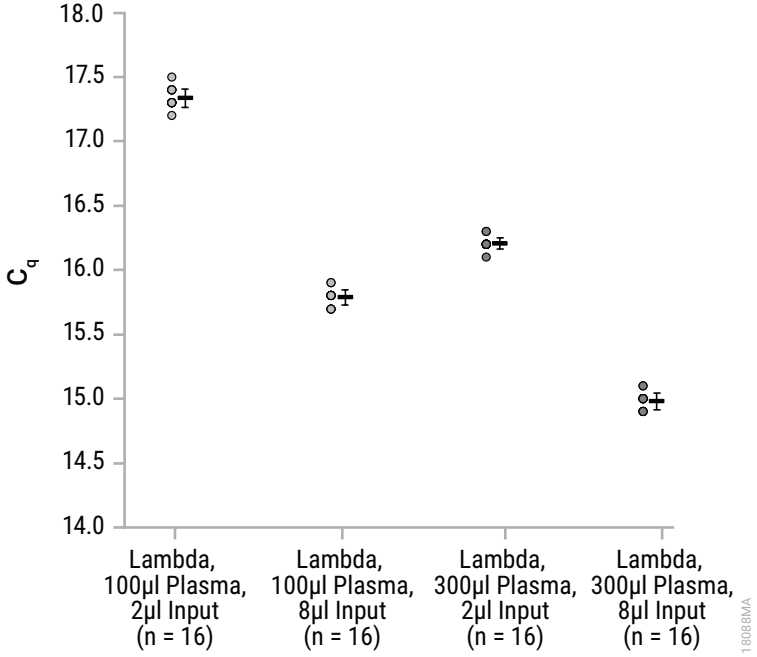


Figure 9. qPCR C_q values for eluates prepared from plasma. Plasma samples (100µl or 300µl) were spiked with lambda phage then used for purification. Inhibition was assessed using 2µl and 8µl as a fourfold input difference that should result in a C_q difference of approximately 2 cycles. Eluates (2µl or 8µl input) from each plasma purification were amplified by qPCR. For each data set, dots on the left represent individual sample C_q values, while the mean with standard deviation is shown on the right. The 100µl plasma sample with 2µl input in qPCR had an average C_q of 17.3 and 8µl input in qPCR had an average of 15.8. The 300µl plasma sample with 2µl input in qPCR had an average C_q of 16.2 and 8µl input in qPCR had an average of 15.0.

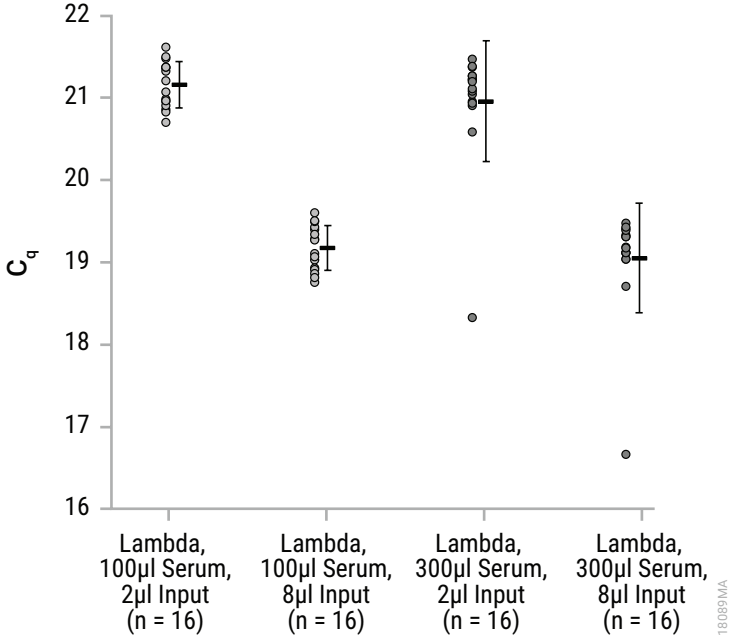


Figure 10. qPCR C_q values for eluates prepared from serum. Serum samples (100µl or 300µl) were spiked with lambda phage then used for purification. Inhibition was assessed using 2µl and 8µl as a fourfold input difference that should result in a C_q difference of approximately 2 cycles. Eluates (2µl or 8µl input) from each serum purification were amplified by qPCR. For each data set, dots on the left represent individual sample C_q values, while the mean with standard deviation is shown on the right. The 100µl serum sample with 2µl input in qPCR had an average C_q of 21.2 and 8µl input in qPCR had an average of 19.2. The 300µl serum sample with 2µl input in qPCR had an average C_q of 21.0 and 8µl input in qPCR had an average of 19.1.

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9.C. Reproducibility

Table 3. RT-qPCR C_q Values. Eluates were prepared by extracting viral total nucleic acid from PBS samples spiked with an in vitro transcript. Three kit lots, three users and three instrument runs were tested to examine intra-run and inter-run reproducibility. Each sample set contained 8 replicates. Results are shown in the table.

Variable		Average C_q (Cycles) n = 8	Relative Standard Deviation (Cycles)
Lot-to-Lot Variability	Lot A	30.9	0.4
	Lot B	30.9	0.2
	Lot C	31.1	0.5
Average of three different lots		**31.0**	**0.4**
Between User Variability	User A	32.4	0.1
	User B	31.7	0.3
	User C	31.7	0.5
Average of three different users		**31.9**	**0.4**
Inter-Run Variability (1 user, 3 instrument runs)	Run 1	30.9	0.4
	Run 2	31.7	0.3
	Run 3	31.0	0.5
Average of three different extraction runs		**31.2**	**0.5**

9.D. Interfering Substances (Inhibition)

Table 4. RT-qPCR C_q Values for Viral RNA. The effect of interfering substances was tested by looking for amplification inhibition when comparing the C_q obtained from a fourfold increase of input nucleic acid to the C_q of the original input. Viral RNA was purified from UTM spiked with inactivated respiratory syncytial virus and preprocessed without heating or Proteinase K treatment. Results are shown for RT-qPCR containing 2µl or 8µl of viral RNA. Inhibition was assessed using 2µl and 8µl as a fourfold input difference that should result in a C_q difference of approximately 2 cycles. The ΔC_q between 2µl and 8µl inputs averaged 1.2 on the Maxwell® CSC Instrument (Cat.# AS6000) and averaged 1.5 on the Maxwell® CSC 48 Instrument (Cat.# AS8000).

Instrument	Sample ID	2µl C_q (Cycles)	8µl C_q (Cycles)	ΔC_q for 2µl and 8µl (Cycles)	NTC C_q* (Cycles)	ΔC_q for 2µl and NTC (Cycles)
Maxwell® CSC Instrument	U17	28.1	26.8	1.3	40	11.9
	U18	28.1	26.8	1.3	40	11.9
	U19	28.1	27.0	1.1	40	11.9
	U20	28.0	26.7	1.3	40	12.0
	U21	27.8	26.5	1.3	40	12.2
	U22	28.1	27.0	1.1	40	11.9
	U23	28.0	26.7	1.3	40	12.0
	U24	28.1	27.0	1.1	40	11.9
	Average	**28.0**	**26.8**	**1.2**	**40**	**12.0**
Maxwell® CSC 48 Instrument	U25	27.9	26.3	1.6	40	12.1
	U26	28.2	26.8	1.4	40	11.8
	U27	28.4	26.9	1.5	40	11.6
	U28	28.1	26.7	1.4	40	11.9
	U29	27.7	26.2	1.5	40	12.3
	U30	27.9	26.3	1.6	40	12.1
	U31	28.3	27.2	1.1	40	11.7
	U32	28.2	26.7	1.5	40	11.8
	Average	**28.1**	**26.6**	**1.4**	**40**	**11.9**

*All no-template controls (NTC) had no C_q value and were assigned a C_q value of 40 cycles.

9.E. Cross Contamination

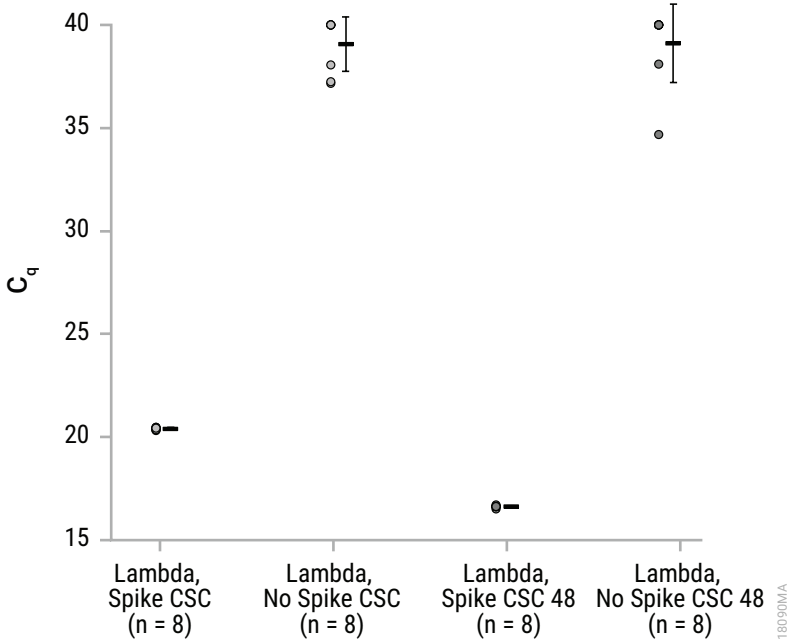

Figure 11. C_q values for DNA purified from universal transport medium. DNA was purified from UTM samples with and without added lambda DNA using the Maxwell® CSC Total Viral Nucleic Acid Kit and Maxwell® CSC and Maxwell® CSC 48 Instruments. The samples with and without added lambda DNA were alternated on the deck of the instruments. Results are shown for qPCRs containing 2µl of lambda DNA. For each data set, the dots on the left represent individual replicates while the standard deviation is shown on the right. The average C_q for the spiked samples purified on the Maxwell® CSC and Maxwell® CSC 48 were 20.4 and 16.6, respectively. Samples with no C_q were assigned a C_q of 40 for averaging purposes. The average C_q for the negative samples was 39.1 in each of the experiments.

10. Clinical Performance Evaluation

Clinical performance was evaluated by extracting viral RNA or DNA from the specified clinical sample types using the Maxwell® CSC Viral Total Nucleic Acid Purification Kit and the Maxwell® CSC 48 Instrument, and amplifying the nucleic acid in a clincally relevant assay.

10.A. Extraction of Viral RNA from UTM Samples

Table 5. SARS-CoV-2 Viral RNA in UTM Samples. Ten positive and 10 negative SARS-CoV-2 UTM samples were purified using the Maxwell® CSC Viral Total Nucleic Acid Purification Kit and a Maxwell® CSC 48 Instrument. RNA was also purified from these samples using the laboratory's standard purification method for reference. Nine of 10 positive samples and 10 of 10 negative samples matched results between the Maxwell® System and laboratory reference method. All Maxwell® samples matched the presumed sample status, which was based on a previous SARS-CoV-2 test run on the sample.

Specimen ID	Presumed Status	Maxwell® System	Laboratory Reference Method	Maxwell® Matches Reference Method	Maxwell® Matches Presumed Status
21432233	Positive	Positive	Positive	Yes	Yes
21880339	Positive	Positive	Positive	Yes	Yes
21202162	Positive	Positive	Positive	Yes	Yes
21213630	Positive	Positive	Positive	Yes	Yes
21590664	Positive	Positive	Positive	Yes	Yes
21315054	Positive	Positive	Positive	Yes	Yes
21823123	Positive	Positive	Positive	Yes	Yes
21180346	Positive	Positive	Positive	Yes	Yes
21102471	Positive	Positive	Positive	Yes	Yes
21147196	Positive	Positive	Negative	No	Yes
21182913	Negative	Negative	Negative	Yes	Yes
21296504	Negative	Negative	Negative	Yes	Yes
21189671	Negative	Negative	Negative	Yes	Yes
21676213	Negative	Negative	Negative	Yes	Yes
21396949	Negative	Negative	Negative	Yes	Yes
21856471	Negative	Negative	Negative	Yes	Yes
21152493	Negative	Negative	Negative	Yes	Yes
21960831	Negative	Negative	Negative	Yes	Yes
21618705	Negative	Negative	Negative	Yes	Yes
21530939	Negative	Negative	Negative	Yes	Yes

10.B. Extraction of Viral RNA from Saliva Samples

Table 6. Viral RNA Purified from SARS-CoV-2 Saliva Samples. Ten positive and 10 negative SARS-CoV-2 saliva samples were purified using the Maxwell® CSC Viral Total Nucleic Acid Purification Kit on a Maxwell® CSC 48 Instrument. RNA was also purified from these samples using the laboratory's standard purification method for reference. All Maxwell® samples matched results between the Maxwell® System and laboratory reference method. All Maxwell® System samples matched the presumed sample status, which was based on a previous SARS-CoV-2 test run on the sample.

Specimen ID	Presumed Status	Maxwell® System	Laboratory Reference Method	Maxwell® Matches Reference Method	Maxwell® Matches Presumed Status
12204502	Positive	Positive	Positive	Yes	Yes
12207992	Positive	Positive	Positive	Yes	Yes
12200960	Positive	Positive	Positive	Yes	Yes
12203868	Positive	Positive	Positive	Yes	Yes
12206897	Positive	Positive	Positive	Yes	Yes
12200453	Positive	Positive	Positive	Yes	Yes
12208750	Positive	Positive	Positive	Yes	Yes
12209126	Positive	Positive	Positive	Yes	Yes
12201677	Positive	Positive	Positive	Yes	Yes
21744360	Positive	Positive	Positive	Yes	Yes
12204630	Negative	Negative	Negative	Yes	Yes
12203230	Negative	Negative	Negative	Yes	Yes
12202781	Negative	Negative	Negative	Yes	Yes
12202953	Negative	Negative	Negative	Yes	Yes
12204617	Negative	Negative	Negative	Yes	Yes
12206702	Negative	Negative	Negative	Yes	Yes
12209395	Negative	Negative	Negative	Yes	Yes
12201994	Negative	Negative	Negative	Yes	Yes
12205532	Negative	Negative	Negative	Yes	Yes
12206575	Negative	Negative	Negative	Yes	Yes

10.C. Extraction of Viral RNA from Plasma Samples

Table 7. Dengue Fever Virus RNA in Plasma Samples. Ten positive and 10 negative Dengue fever virus plasma samples were purified using the Maxwell® CSC Viral Total Nucleic Acid Purification Kit on a Maxwell® CSC 48 Instrument. RNA was also purified from these samples using the laboratory's standard purification method for reference. Ten of 10 positive samples and 8 of 10 negative samples matched results between the Maxwell® System and laboratory reference method. All Maxwell® samples matched the presumed sample status, which was based on a previous Dengue fever virus test run on the sample.

		Maxwell® System		Laboratory Reference Method		
Specimen ID	Presumed Status	100µl Input	300µl Input	300µl Input	Maxwell® Matches Reference Method	Maxwell® Matches Presumed Status
21364611	Positive	Positive	Positive	Positive	Yes	Yes
21964895	Positive	Positive	Positive	Positive	Yes	Yes
21836674	Positive	Positive	Positive	Positive	Yes	Yes
21485868	Positive	Positive	Positive	Positive	Yes	Yes
21949507	Positive	Positive	Positive	Positive	Yes	Yes
21232505	Positive	Positive	Positive	Positive	Yes	Yes
21092389	Positive	Positive	Positive	Positive	Yes	Yes
21443444	Positive	Positive	Positive	Positive	Yes	Yes
21839389	Positive	Positive	Positive	Positive	Yes	Yes
21960608	Positive	Positive	Positive	Positive	Yes	Yes
21017143	Negative	NT*	Negative	Negative	Yes	Yes
21478268	Negative	NT*	Negative	Negative	Yes	Yes
21598671	Negative	NT*	Negative	Positive	No	Yes
21363671	Negative	NT*	Negative	Positive	No	Yes
21323109	Negative	NT*	Negative	Negative	Yes	Yes
21004789	Negative	NT*	Negative	Negative	Yes	Yes
21893607	Negative	NT*	Negative	Negative	Yes	Yes
21993638	Negative	NT*	Negative	Negative	Yes	Yes
21121581	Negative	NT*	Negative	Negative	Yes	Yes
21514345	Negative	NT*	Negative	Negative	Yes	Yes

*NT: Not tested.

10.D. Extraction of Viral DNA from Plasma Samples

Table 8. Cytomegalovirus (CMV) DNA in Plasma Samples. Ten positive and 10 negative CMV plasma samples were purified using the Maxwell® CSC Viral Total Nucleic Acid Purification Kit on a Maxwell® CSC 48 Instrument. DNA was also purified from these samples using the laboratory's standard purification method for reference. All Maxwell® samples matched results between the Maxwell® System and laboratory reference method. All Maxwell® samples matched the presumed sample status, which was based on a previous CMV test run on the sample.

		Maxwell® System		Laboratory Reference Method	Maxwell® Matches Reference Method	Maxwell® Matches Presumed Status
Specimen ID	Presumed Status	100µl Input	300µl Input	300µl Input		
38375075	Positive	Positive	Positive	Positive	Yes	Yes
38535155	Positive	Positive	Positive	Positive	Yes	Yes
37293873	Positive	Positive	Positive	Positive	Yes	Yes
37271420	Positive	Positive	Positive	Positive	Yes	Yes
38133737	Positive	Positive	Positive	Positive	Yes	Yes
38212566	Positive	Positive	Positive	Positive	Yes	Yes
38228092	Positive	Positive	Positive	Positive	Yes	Yes
37975220	Positive	Positive	Positive	Positive	Yes	Yes
37924077	Positive	Positive	Positive	Positive	Yes	Yes
38757118	Positive	Positive	Positive	Positive	Yes	Yes
30615407	Negative	NT*	Negative	Negative	Yes	Yes
23916496	Negative	NT*	Negative	Negative	Yes	Yes
22380697	Negative	NT*	Negative	Negative	Yes	Yes
33545486	Negative	NT*	Negative	Negative	Yes	Yes
40639511	Negative	NT*	Negative	Negative	Yes	Yes
40346295	Negative	NT*	Negative	Negative	Yes	Yes
21423543	Negative	NT*	Negative	Negative	Yes	Yes
20341215	Negative	NT*	Negative	Negative	Yes	Yes
215139202	Negative	NT*	Negative	Negative	Yes	Yes
40503484	Negative	NT*	Negative	Negative	Yes	Yes

*NT: Not tested.

10.E. Extraction of Viral RNA from Serum Samples

Table 9. Dengue Fever Virus RNA in Serum Samples. Ten positive and 10 negative Dengue fever virus serum samples were purified using the Maxwell® CSC Viral Total Nucleic Acid Purification Kit on a Maxwell® CSC 48 Instrument. RNA was also purified from these samples using the laboratory's standard purification method for reference. All samples matched results between the Maxwell® System and laboratory reference method. All Maxwell® System samples matched the presumed sample status, which was based on a previous Dengue fever virus test run on the sample.

Specimen ID	Presumed Status	Maxwell® System		Laboratory Reference Method	Maxwell® Matches Reference Method	Maxwell® Matches Presumed Status
		100µl Input	300µl Input	300µl Input		
21837552	Positive	Positive	Positive	Positive	Yes	Yes
21923921	Positive	Positive	Positive	Positive	Yes	Yes
21489704	Positive	Positive	Positive	Positive	Yes	Yes
21125739	Positive	Positive	Positive	Positive	Yes	Yes
21095976	Positive	Positive	Positive	Positive	Yes	Yes
21783122	Positive	Positive	Positive	Positive	Yes	Yes
21936932	Positive	Positive	Positive	Positive	Yes	Yes
21738559	Positive	Positive	Positive	Positive	Yes	Yes
21176258	Positive	Positive	Positive	Positive	Yes	Yes
21542794	Positive	Positive	Positive	Positive	Yes	Yes
21441970	Negative	NT*	Negative	Negative	Yes	Yes
21090946	Negative	NT*	Negative	Negative	Yes	Yes
21247913	Negative	NT*	Negative	Negative	Yes	Yes
21109632	Negative	NT*	Negative	Negative	Yes	Yes
21792527	Negative	NT*	Negative	Negative	Yes	Yes
21905523	Negative	NT*	Negative	Negative	Yes	Yes
21165524	Negative	NT*	Negative	Negative	Yes	Yes
21510977	Negative	NT*	Negative	Negative	Yes	Yes
21826187	Negative	NT*	Negative	Negative	Yes	Yes
21117238	Negative	NT*	Negative	Negative	Yes	Yes

*NT: Not tested.

10.F. Reproducibility

Table 10. Reproducibility of RNA Purification. Ten positive and 10 negative Dengue fever virus plasma samples were purified using the Maxwell® CSC Viral Total Nucleic Acid Purification Kit on a Maxwell® CSC 48 Instrument by two testers. All samples result matched between the Tester A and Tester B.

		Maxwell® System		Tester A Result Matches Tester B Result
Specimen ID	Presumed Status	Tester A	Tester B	
21364611	Positive	Positive	Positive	Yes
21964895	Positive	Positive	Positive	Yes
21836674	Positive	Positive	Positive	Yes
21485868	Positive	Positive	Positive	Yes
21949507	Positive	Positive	Positive	Yes
21232505	Positive	Positive	Positive	Yes
21092389	Positive	Positive	Positive	Yes
21443444	Positive	Positive	Positive	Yes
21839389	Positive	Positive	Positive	Yes
21960608	Positive	Positive	Positive	Yes
21017143	Negative	Negative	Negative	Yes
21478268	Negative	Negative	Negative	Yes
21598671	Negative	Negative	Negative	Yes
21363671	Negative	Negative	Negative	Yes
21323109	Negative	Negative	Negative	Yes
21004789	Negative	Negative	Negative	Yes
21893607	Negative	Negative	Negative	Yes
21993638	Negative	Negative	Negative	Yes
21121581	Negative	Negative	Negative	Yes
21514345	Negative	Negative	Negative	Yes

10.G. Cross Contamination

Table 11. Viral DNA from CMV Plasma Samples. Nine presumed negative CMV plasma samples were alternated with 10 positive CMV plasma samples on the deck of the Maxwell® CSC 48 instrument. Nine of 9 negative samples alternated with positive samples were found to be negative, showing that there was no detectable cross contamination.

		Maxwell® System	
Specimen ID	**Presumed Status**	**C_q 300µl input**	**CMV Result**
30615407	Negative	no C_q	Negative
23916496	Negative	no C_q	Negative
22380697	Negative	no C_q	Negative
33545486	Negative	no C_q	Negative
40639511	Negative	no C_q	Negative
40346295	Negative	no C_q	Negative
21423543	Negative	no C_q	Negative
20341215	Negative	no C_q	Negative
40503484	Negative	no C_q	Negative

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11. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Lower viral nucleic acid recovery than expected (e.g., for customer-provided internal controls)	The starting samples were compromised. Ensure that samples were collected, shipped and stored according to recommended guidelines.
	For RNA viral samples, ensure RNase-free conditions are used for sample preparation and assay setup, including RNase-free tubes and pipette tips.
	Processing step was not optimal. • Prepare Lysis Buffer and Proteinase K immediately before use, and discard unused solutions following your institution's recommended guidelines. • Use only the Lysis Buffer provided with this kit. • Incomplete mixing may reduce lysis. Vortex sample with Lysis Solution as recommended. • Incomplete protease treatment to remove viral capsids. Check the heat block or water bath temperature, and incubate for the full time recommended. • Incubation for 10 minutes at room temperature before the 56°C incubation may improve recovery for some plasma samples. • Some viruses may need higher incubation temperatures. • Adding more sample than recommended may reduce nucleic acid recovery.
Lower viral nucleic acid recovery than expected (e.g., for customer-provided internal controls)	Check that a plunger was added to the cartridge.
	Ensure that all cartridges are snapped into the deck tray properly before processing.
	Post-purification storage issues. • Remove eluates, and store at the recommended temperature immediately after the Maxwell® Instrument run. • Do not subject eluates to multiple freeze-thaw cycles before downstream assays.
	Nucleic acid internal controls smaller than 100bp may not be efficiently purified using the system. The user is responsible for establishing performance of any internal control.

Symptoms	Causes and Comments
Poor amplification	Paramagnetic particle carryover may cause interference in amplification reactions. Remove particles in elution tube by centrifugation.
	Wrong elution buffer was added. Use only the Nuclease-Free Water supplied with the Maxwell® CSC Viral Total Nucleic Acid Purification Kit.
Cross contamination	Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
	Avoid splashing when adding lysates to cartridges. Cartridges may be removed from the deck tray for sample addition to minimize contamination of adjacent cartridges.
Instrument unable to pick up plungers	Make sure you are using a CSC-specific chemistry kit; the plungers for the Maxwell® CSC reagent kits are specific to the supported Maxwell® Instruments for this kit.

Any serious incident that occurred in relation to the device that led to, or might lead to, death or serious injury of a user or patient should be immediately reported to the manufacturer. Users based in the European Union should also report any serious incidents to the Competent Authority of the Member State in which the user and/or the patient is established.

12. References

1. Clinical Laboratory Standards Institute (2007). Handling, transport, and storage of specimens for molecular methods. This can be viewed online at: **www.clsi.org**
2. Murray, P.R. *et al*. (2007) *Manual of Clinical Microbiology*, 9th Edition, ASM Press.

13. Related Products

Product	Size	Cat.#
Maxwell® CSC Instrument	1 each	AS6000
Maxwell® CSC 48 Instrument	1 each	AS8000
Maxwell® RSC/CSC Plungers, 50pk	1 each	AS1331
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
ClickFit Microtube, 1.5ml	1,000/pack	V4741

Maxwell® CSC Reagent Kits

For a list of available Maxwell® CSC purification kits, visit: **www.promega.com**

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14. Summary of Changes

The following changes were made to the 10/22 revision of this document:

1. Section 3 was renamed Product Intended Purpose/Intended Use.
2. Sections 9 and 10 were added and subsequent sections renumbered.
3. Document updated for compliance with Regulation (EU) 2017/746 on in vitro diagnostic medical devices.

[a]U.S. Pat. No. 7,329,488 and S. Korean Pat. No. 100483684.