TECHNICAL MANUAL

# Maxwell® CSC Pathogen Total Nucleic Acid Kit

Instructions for Use of Product
**AS1860**

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

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INSTRUCTIONS FOR
USE OF PRODUCT
**AS1860**

IVD

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TM706

# Maxwell® CSC Pathogen Total Nucleic Acid 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 Pathogen Total Nucleic Acid Kit is only available in certain countries.

## 1. Description

The Maxwell® CSC Pathogen Total Nucleic Acid 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 pathogen total nucleic acid. Maxwell® CSC Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification procedures, maximizing simplicity and convenience. The Maxwell® CSC Pathogen Total Nucleic Acid Kit was designed to perform automated extraction of bacterial, viral and parasite total nucleic acid from the following human biological samples: stool, sputum, bronchoalveolar lavage, urine, plasma, nasopharyngeal swabs and cervical swabs in transport medium. The Maxwell® Instrument method for the Maxwell® CSC Pathogen Total Nucleic Acid Kit can process from one to the maximum number of Maxwell® Instrument samples in approximately 40 minutes. The low 100µl elution volume results in concentrated purified nucleic acid for downstream applications such as quantitative PCR (qPCR) or quantitative RT-PCR (RT-qPCR). After a brief lysis preprocessing step, the sample is added to the Maxwell® CSC Pathogen Total Nucleic Acid 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 |

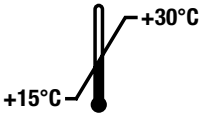
### Principle of the Method

The Maxwell® CSC Pathogen Total Nucleic Acid 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 and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **Maxwell® CSC Pathogen Total Nucleic Acid Kit** | **48 preps** | **AS1860** |

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



Includes:

- 150ml Dilution Buffer (ST1)
- 900µl 1-Thioglycerol
- 20ml Lysis Buffer
- 2 × 1ml Proteinase K (PK) Solution
- 50 CSC/RSC Plungers
- 48 Maxwell® CSC Cartridges (CSCQ)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

**Storage Conditions:** Upon receipt, remove 1-Thioglycerol and store at +2°C to +10°C. Store the remaining kit 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.

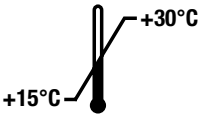
Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

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

**Additional Information:** The Maxwell® CSC Pathogen Total Nucleic Acid Kit components are qualified and quality control tested to work together. Do **not** 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**

## 2. Product Components and Storage Conditions (continued)

### 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 Pathogen Total Nucleic Acid Kit is intended for use, in combination with Maxwell® CSC instruments and the Maxwell® CSC Pathogen Total Nucleic Acid method, as an in vitro diagnostic (IVD) medical device to perform automated isolation of viral, bacterial and/or parasite total nucleic acid from the following human specimen types: stool, sputum, bronchoalveolar lavage, nasopharyngeal swabs in transport media, cervical swabs in transport media, urine and plasma. The purified total nucleic acid is suitable for use in amplification-based in vitro diagnostic assays.

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

## 4. Product Use Limitations

The performance of the Maxwell® CSC Pathogen Total Nucleic Acid Kit was evaluated for the following pathogens using the specimen types listed:

- Bacteria: stool, sputum, bronchoalveolar lavage, nasopharyngeal swabs in transport media, cervical swabs in transport media, urine.
- Parasites: stool, cervical swabs in transport media, urine.
- Viruses: stool, sputum, bronchoalveolar lavage, nasopharyngeal swabs in transport media, cervical swabs in transport media, urine, plasma.

The Maxwell® CSC Pathogen Total Nucleic Acid Kit is not intended for use with nonhuman samples.

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

## 5. Before You Begin

### Materials to Be Supplied By the User

- 1.5–2.0ml tubes for incubating samples (e.g., ClickFit Microtube, 1.5ml [Cat.# V4741]; recommended to prevent the cap from opening during heating)
- 15ml or 50ml conical tube for preparing 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
- heating block set to 95°C for sputum and BAL samples
- **optional:** 1X PBS to dilute BAL and sputum samples
- **optional:** centrifuge for urine sample preprocessing
- **optional:** 0.1–0.5mm zirconium beads for preprocessing of stool samples (e.g., ZR bashing beads, Zymo [Cat. #S6012-50])
- tubes for stool, sputum, bronchoalveolar lavage (BAL), viral transport medium (VTM), Pap medium, plasma and urine samples

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

For plasma samples, collect blood in EDTA-anticoagulant tubes. Avoid using heparin-containing blood collection tubes because heparin can inhibit downstream amplifications.

The following general recommendations are for preparing and storing samples (from references 1–4):

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. Store plasma 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.
2. For swabs in VTM, 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 inhibit PCR testing. Place swabs immediately into sterile tubes containing 2–3ml of viral transport medium. Store VTM and 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.
3. Store fresh urine samples at 4°C for up to 24 hours before processing. For longer term storage, add EDTA to a final concentration of 20mM. To avoid cell lysis, store stabilized urine at 4°C. To avoid cell lysis, urine should not be frozen. If urine is frozen and thawed, a precipitate may become visible. Mixing and/or heating of the sample should resolubilize the precipitate. Avoid centrifuging the sample to remove precipitate because this can remove intact pathogenic cells.
4. Store BAL and sputum 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 pathogen isolated.
5. Stool samples should be frozen at –20°C to –80°C before processing.

## 6. Sample Preparation

### 6.A. Preparing Stool Samples for Use with the Maxwell® CSC Pathogen Total Nucleic Acid Kit

1. Weigh 100–500mg of fecal sample into a 2ml screw-cap microcentrifuge tube.
2. Add 1ml of Dilution Buffer (ST1) to resuspend sample. Vortex vigorously for 30 seconds to 1 minute. Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
3. Centrifuge sample 1,000 × *g* for 1 minute at room temperature.
4. Transfer supernatant to appropriately sized tube and measure volume.
5. Add 2X volume of Dilution Buffer (ST1) for a 1:2 dilution of stool sample [e.g., 1ml of stool supernatant + 2ml of Dilution Buffer (ST1)]. Use this stool sample dilution for DNA purifications.

   **Note:** For extracting viral RNA, further dilute the stool sample 1:8 in nuclease-free water (e.g., 100µl of 1:2 dilution of stool sample + 700µl of nuclease-free water). Store the sample at room temperature until ready for processing.

   **Optional Bead Beating Method for Extracting DNA from Difficult-to-Lyse Pathogens in Stool Samples**

   **Note:** For extracting nucleic acids from difficult-to-lyse pathogens such as Gram-positive bacteria or protozoa in stool specimens, an optional bead-beating step can be performed. This additional homogenization can increase nucleic acid purification yield if the standard lysis method is insufficient.

   a. Transfer 600µl to 1ml of diluted stool sample to a 2ml tube containing beads and close tube.
   b. Place tubes on plate vortexer or equivalent and vortex or bead beat at maximum speed for 10 minutes.
   c. Centrifuge tubes in a microcentrifuge for 1 minute at full speed to separate beads from sample.
6. Add 300µl of stool lysate to new 1.5ml tube.
7. Add 300µl of Lysis Buffer and mix by vortex for 10 seconds.
8. Add 30µl of Proteinase K Solution and vortex briefly to mix.
9. Incubate at 56°C for 20 minutes.
10. Proceed to Maxwell® CSC Pathogen Total Nucleic Acid cartridge preparation (Section 6.E).

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### 6.B. Preparing BAL or Sputum Samples for Use with the Maxwell® CSC Pathogen Total Nucleic Acid Kit

1. If frozen, thaw BAL or sputum sample at room temperature.

   **Note:** To reduce viscosity of BAL and sputum samples, dilute in an equal volume of 1X PBS, mechanically homogenize, or dilute and homogenize prior to processing.

2. For each sample, add 100–300µl to a 1.5ml microcentrifuge tube. (We recommend using a wide-bore pipette). Use fresh pipette tips for each sample to prevent sample-to-sample contamination.

3. Add 12µl of 1-Thioglycerol and pipet to mix.

   **Note:** Pipet slowly because the solution is viscous.

4. Add 300µl of Lysis Buffer and mix by vortexing for 10 seconds.

5. Incubate at 95°C for 5 minutes. Cool on the benchtop for 2 minutes.

6. Add 30µl of Proteinase K Solution and vortex briefly to mix.

7. Incubate at 56°C for 20 minutes.

8. Proceed to Maxwell® CSC Pathogen Total Nucleic Acid cartridge preparation (Section 6.E).

## 6.C. Preparing Urine Samples for Use with the Maxwell® CSC Pathogen Total Nucleic Acid Kit

**Preparing small sample volumes (300µl of urine):**

1. Vortex urine sample briefly to resuspend any settled cells.
2. For each sample, add 300µl of urine to a 1.5ml microcentrifuge tube. Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
3. Add 300µl of Lysis Buffer and mix by vortexing for 10 seconds.
4. Add 30µl of Proteinase K Solution and vortex briefly to mix.
5. Incubate at 56°C for 20 minutes.
6. Proceed to Maxwell® CSC Pathogen Total Nucleic Acid cartridge preparation (Section 6.E).

**Preparing large sample volumes (30ml of urine):**

1. Vortex urine sample briefly to resuspend any settled cells.
2. For each sample, add 30ml of urine to a 50ml tube. Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
3. Centrifuge urine sample at 2,000 × *g* for 10 minutes.
4. Remove supernatant with a pipette, being careful not to disturb the pellet.
   **Note:** Some liquid can be left behind to preserve pellet integrity (up to 300µl).
5. Add 300µl of Lysis Buffer to tube and resuspend pellet by pipetting.
6. Transfer the lysate to a 1.5ml tube.
7. Add 30µl of Proteinase K Solution and vortex briefly to mix.
8. Incubate at 56°C for 20 minutes.
9. Proceed to Maxwell® CSC Pathogen Total Nucleic Acid cartridge preparation (Section 6.E).

### 6.D. Preparing Plasma, Viral Transport Media or Pap Media Samples for Use with the Maxwell® CSC Pathogen Total Nucleic Acid Kit

1. If frozen, thaw plasma or media at room temperature and vortex briefly to mix.
2. For each sample, add 300µl of plasma or medium containing biological sample to a 1.5ml microcentrifuge tube. Use fresh pipette tips for each sample to prevent sample-to-sample contamination.
3. Add 300µl of Lysis Buffer and mix by vortexing for 10 seconds.
4. Add 30µl of Proteinase K Solution and vortex briefly to mix.
5. Incubate at 56°C for 20 minutes.
6. Proceed to Maxwell® CSC Pathogen Total Nucleic Acid cartridge preparation (Section 6.E).

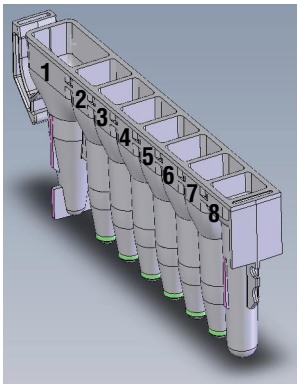
### 6.E. Preparing the Maxwell® CSC Pathogen Total Nucleic Acid Cartridge

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 is removed from 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 (0.5ml) into the elution tube position for each cartridge in the deck tray(s).
4. Add 100µl of Elution Buffer to the bottom of each Elution Tube.
5. Briefly spin sample lysates in a microcentrifuge to collect liquid at the bottom of the tube. Transfer sample lysate to well #1 (the largest well) of the cartridge. Do not add anything other than the sample lysate to the cartridge.
6. Proceed to Section 7, Maxwell® Instrument Setup and Run.

   **Notes:**

   a. 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.

   b. 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 lysate

8. CSC/RSC Plunger

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

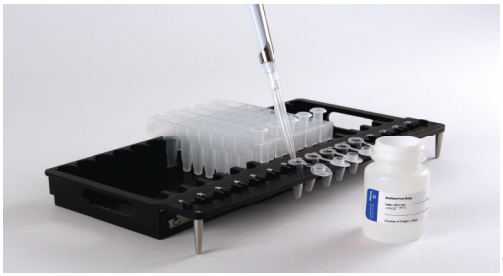


**Figure 2. Setup and configuration of the deck tray(s).** Elution Buffer is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

## 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 Pathogen Total Nucleic Acid Kit label to automatically select the method to be run (Figure 3).

   **Note:** The Maxwell® CSC Pathogen Total Nucleic Acid 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.

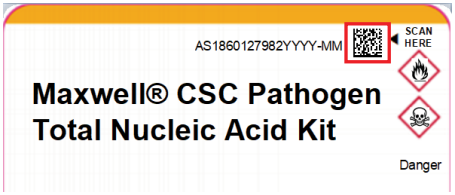



**Figure 3. Kit label indicating the method bar code to scan.** The bar code to scan for starting a purification run is shown in the red box, on the upper right of the kit label.

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 press the **Proceed** button to continue.

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

   a. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.

   b. 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 not to perform **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 pathogen 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, then transfer the supernatant to storage tubes (not provided). 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 UV light treatment of the instrument to avoid damage to the nucleic acid.

## 8. Storing Eluted Nucleic Acid

If samples are not processed immediately, store eluted pathogen DNA on ice or at 4°C for up to 24 hours. For longer term storage, freeze at –20°C or –70°C. RNA is less stable and preferably tested in downstream assays immediately after isolation. If testing soon after isolation, store pathogen RNA on ice or at 4°C. Alternatively, store eluted pathogen 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 Maxwell® CSC Pathogen Total Nucleic Acid Kit was performed using various sample types spiked with inactivated pathogens processed on the Maxwell® CSC and Maxwell® CSC 48 Instruments.

### 9.A. DNA Quantity, Quality and Amplifiability

Total nucleic acid was extracted from 300µl replicates of a stool suspension, each spiked with approximately $2.5 \times 10^4$ copies of inactivated *Giardia lamblia* using either the standard or the bead-beating preprocessing methods. Total nucleic acid was eluted using 100µl of Elution Buffer. Five microliters of each undiluted eluate were used in a qPCR assay to amplify a DNA target specific for *G. lamblia*. Average $C_q$ generated using the standard method was 34.39 cycles. Average $C_q$ generated using the bead-beating method was 29.35 cycles.

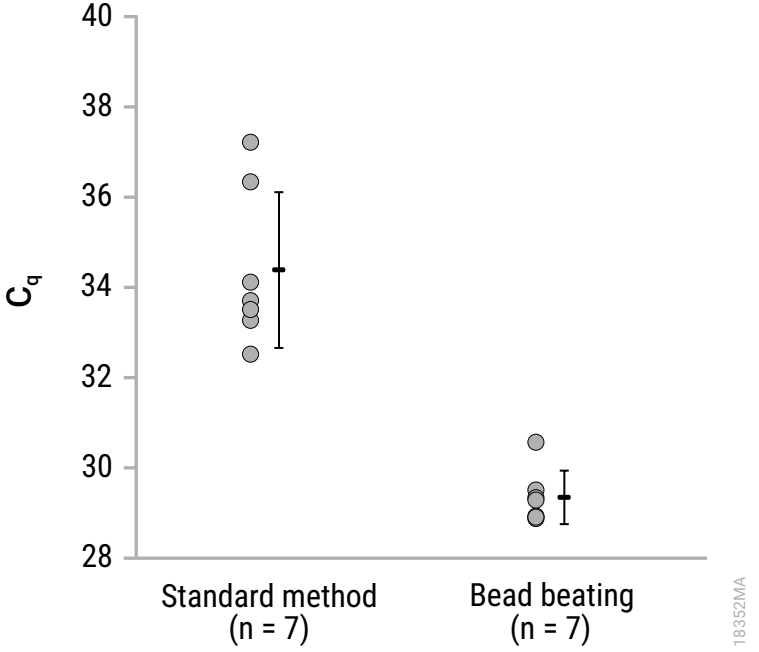



**Figure 4. Amplification of *G. lamblia* target DNA sequence using total nucleic acid extracted from stool samples of using the standard versus bead-beating methods.**

### 9.B. RNA Quantity, Quality and Amplifiability

To demonstrate that the performance of the Maxwell® CSC Pathogen Total Nucleic Acid Kit is similar when used with either the Maxwell® CSC or the Maxwell® CSC 48 Instrument, total nucleic acid was extracted using the kit with both instruments from eight 300µl replicates of sputum samples spiked with $1 \times 10^4$ copies of inactivated SARS CoV-2 virus. Total nucleic acid was eluted in 100µl of Elution Buffer, and 5µl of each eluate was used in an RT-qPCR assay to amplify a target RNA sequence specific to SARS CoV-2. Comparison of the RT-qPCR results obtained from total nucleic acid extracted using the Maxwell® CSC Instrument and Maxwell® CSC 48 Instrument showed that all $|\Delta C_q|$ values were ≤0.59 cycles, with an average $|\Delta C_q|$ of 0.16 cycles.

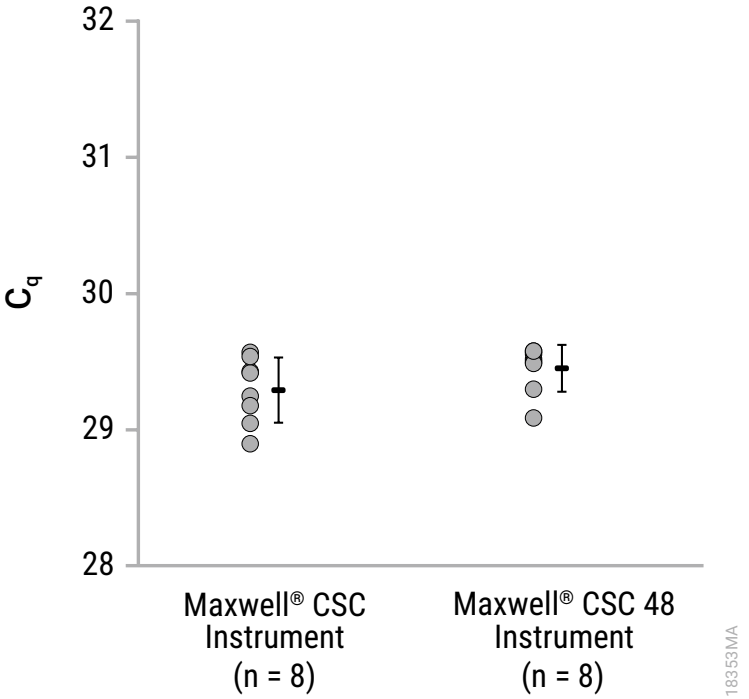



**Figure 5. Amplifiability of total nucleic acid extracted from sputum samples containing SARS CoV-2 using either the Maxwell® CSC or Maxwell® CSC 48 Instrument.**

**9.B. RNA Quantity, Quality and Amplifiability (continued)**

Total nucleic acid was purified in parallel from 300µl as well as 100µl of bronchoalveolar lavage (BAL) samples, each spiked with $1 \times 10^4$ copies of inactivated SARS CoV-2 virus to evaluate the effect of different amounts of sample matrix on amplifiability. Total nucleic acid was eluted in 100µl of Elution Buffer, and 5µl of each undiluted eluate was used in an RT-qPCR assay for amplifying a target RNA sequence specific for SARS CoV-2. All $C_q$ values for total nucleic acid purified from 300µl of sputum were ≤30.86 cycles, with an average $C_q$ of 30.56 cycles. All $C_q$ values for total nucleic acid purified from 100µl of BAL were ≤29.93 cycles, with an average $C_q$ of 29.74 cycles.

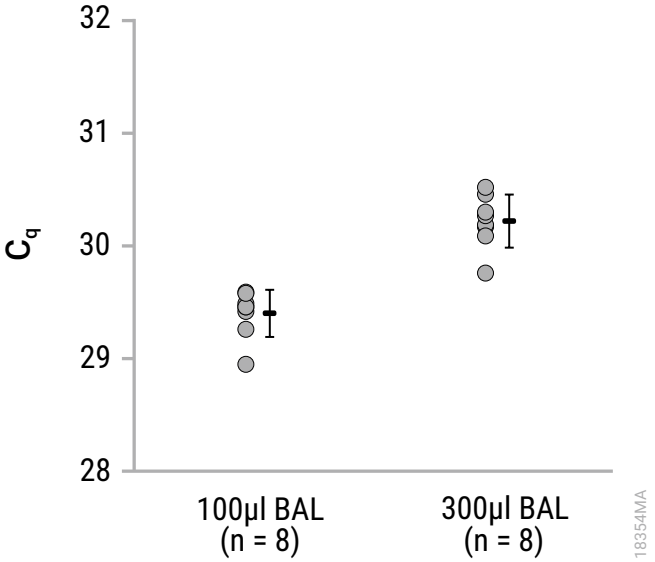



**Figure 6. Amplification of SARS CoV-2 target RNA sequence using total nucleic acid extracted from different sample input volumes.**

### 9.C. Reproducibility

To assess the reproducibility of total nucleic acid extraction using the Maxwell® CSC Pathogen Total Nucleic Acid Kit between users, three users each preprocessed five 300µl replicates of universal transport medium (UTM) spiked with *Bordetella pertussis*, and extracted total nucleic acid in the same Maxwell® CSC Instrument run. The nucleic acids were eluted in 100µl of Elution Buffer. Five microliters of the undiluted eluate were used in a qPCR assay to amplify a target DNA sequence specific to *B. pertussis*, and the average $C_q$ and standard deviation were calculated. Total nucleic acid purification was reproducible, with standard deviations of 0.31 cycles (User 1), 0.23 cycles (User 2) and 0.40 cycles (User 3). Across all three users, the standard deviation was 0.32 cycles.

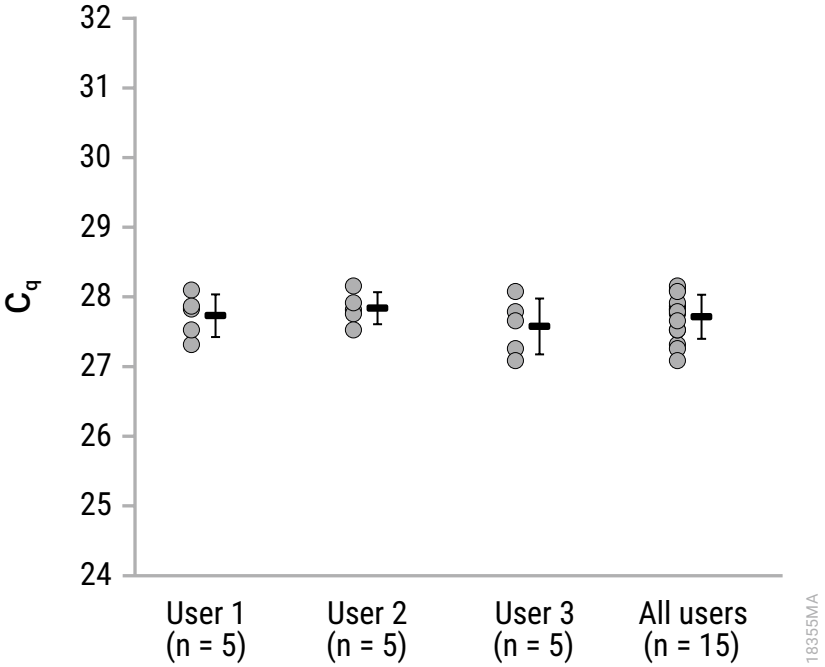



**Figure 7. Reproducibility of total nucleic extraction among three users.**

### 9.C. Reproducibility (continued)

To assess the reproducibility of total nucleic acid extraction between different extraction runs, a single user performed three different total nucleic acid extraction runs each with eight 300µl replicates of UTM spiked with *B. pertussis* using the Maxwell® CSC Pathogen Total Nucleic Acid Kit with the Maxwell® CSC Instruments. Total nucleic acid was eluted in 100µl of Elution Buffer during each of the three separate runs. Five microliters of the undiluted eluate was used in a qPCR assay to amplify a *B. pertussis*-specific target DNA sequence, and the average $C_q$ and standard deviation were calculated. Total nucleic acid purification was reproducible, with standard deviations of 0.25 cycles (Run 1), 0.40 cycles (Run 2) and 0.22 cycles (Run 3). Across all three runs, the standard deviation was 0.43 cycles.

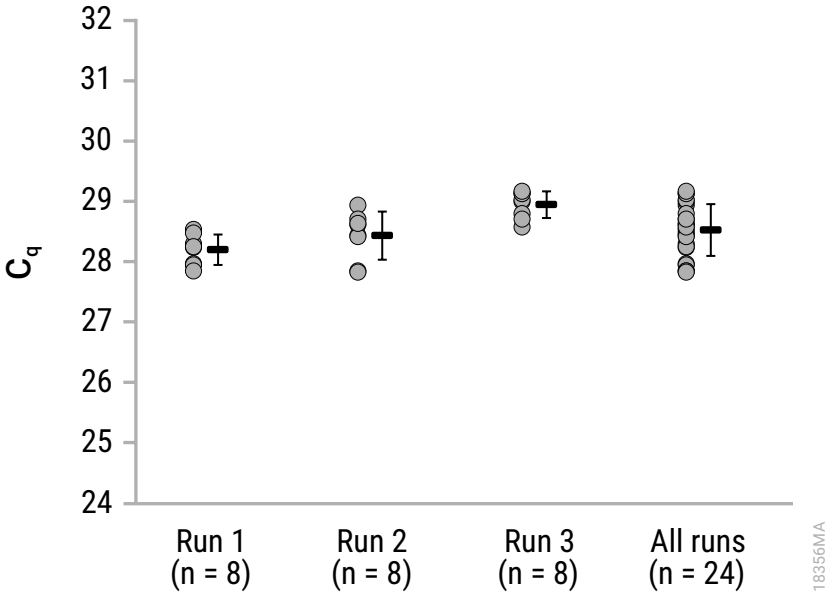



**Figure 8. Reproducibility of total nucleic extraction among three extraction runs performed by a single user.**

To assess the reproducibility of total nucleic acid extraction between different lots of the Maxwell® CSC Pathogen Total Nucleic Acid Kit, total nucleic acid was purified from 300µl replicates of UTM spiked with *B. pertussis* using three lots of kits, each containing unique lots of critical reagents. Each undiluted eluate was used in a qPCR assay to amplify a target DNA sequence specific for *B. pertussis*, and the average $C_q$ and standard deviation were calculated. The standard deviation for $C_q$ values generated using Lot 1 was 0.38 cycles; Lot 2 was 0.30 cycles; and Lot 3 was 0.35 cycles. The standard deviation for $C_q$ across all the three lots was 0.34 cycles.

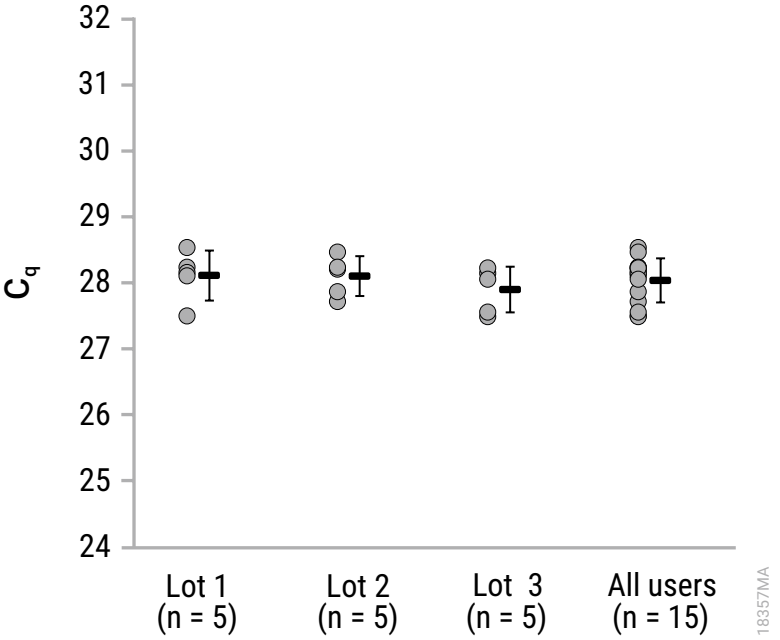



**Figure 9. Total nucleic acid extraction reproducibility across three different lots of the Maxwell® CSC Pathogen Total Nucleic Acid Kit.**

### 9.D. Amplification Inhibition Due to Interfering Substances

To assess for any inhibition in amplification due to interfering substances copurifying with the nucleic acid, total nucleic acid was purified from eight 300µl replicates of a stool suspension spiked with $3 \times 10^4$ cells of inactivated *Clostridium difficile*. Five microliters of each undiluted total nucleic acid eluate and 5µl of a tenfold dilution of each eluate were used in a qPCR assay to amplify a DNA target specific for *C. difficile* to evaluate the effect of interfering substances. For the Maxwell® CSC Instrument, all $C_q$ values for the undiluted eluates were ≤27.57 cycles, with an average $C_q$ of 26.93 cycles. For the Maxwell® CSC 48 Instrument, all $C_q$ values for the undiluted eluates were ≤27.24 cycles, with an average $C_q$ of 27.03 cycles. $C_q$ values from undiluted eluates and tenfold diluted eluates were compared to assess inhibition within individual eluates with an expected $\Delta C_q$ of >2.5 cycles, indicating no inhibition. The $\Delta C_q$ of undiluted and tenfold diluted eluates for individual samples ranged from 3.24 to 3.59, indicating that any interfering substances present in the eluate cause minimal inhibition.

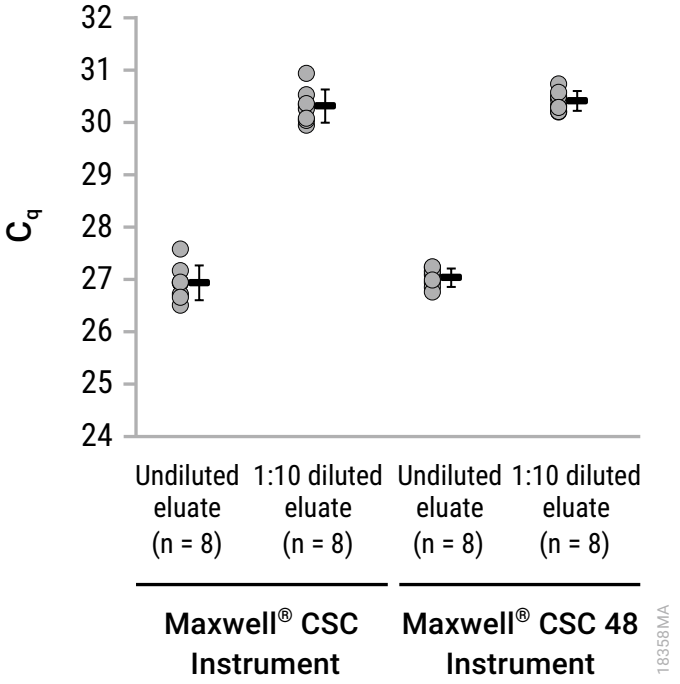



**Figure 10. Amplification inhibition assessment of total nucleic acid extracted using the Maxwell® CSC or the Maxwell® CSC 48 Instrument.**

Total nucleic acid was extracted from eight 300µl replicates of either sputum or BAL samples spiked with $9 \times 10^4$ copies of inactivated *Chlamydia pneumoniae* and eluted in 100µl of Elution Buffer. Five microliters of each undiluted total nucleic acid eluate and 5µl of a tenfold dilution of each eluate were used in a qPCR assay to amplify a DNA target specific for *C. pneumoniae* to evaluate the effect of interfering substances. All $C_q$ values for the undiluted total nucleic acid eluates from sputum were ≤26.59 cycles, with an average $C_q$ of 26.48 cycles. The average $C_q$ value for 1:10 dilution of total nucleic acid eluates from sputum was 29.83 cycles, with a difference between the average $C_q$ values for diluted and undiluted samples of 3.35 cycles. All $C_q$ values for undiluted eluates from BAL were ≤26.64 cycles, with an average $C_q$ of 26.41 cycles. The average $C_q$ value for 1:10 dilution of total nucleic acid eluates from BAL was 29.83 cycles, with a difference of 3.42 cycles between the average $C_q$ values for diluted and undiluted samples, indicating that any interfering substances present in the eluate cause minimal inhibition.

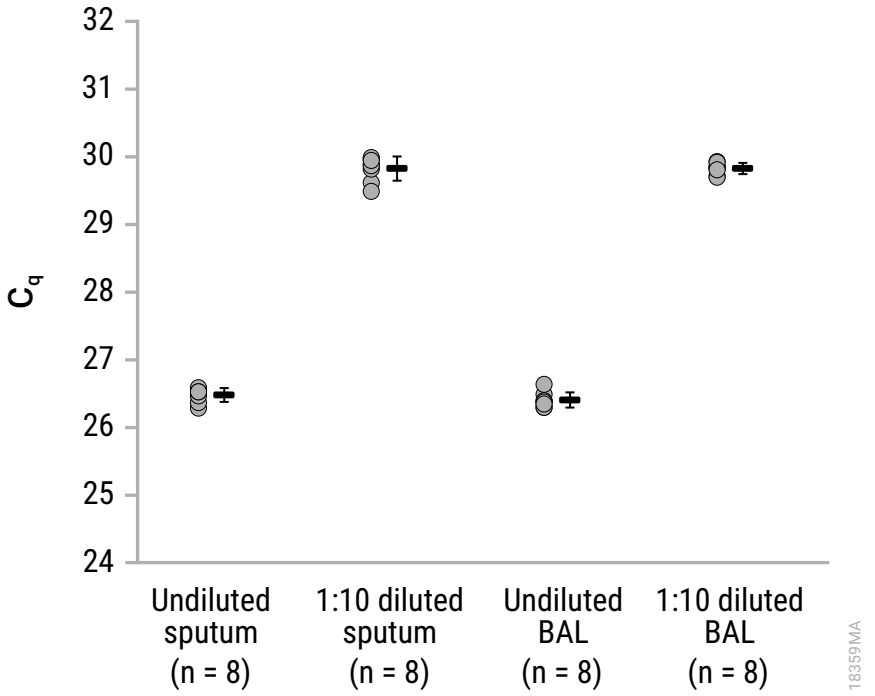



**Figure 11. Amplification inhibition assessment of total nucleic acid extracted from sputum and BAL samples.**

### 9.D. Amplification Inhibition Due to Interfering Substances (continued)

Amplification inhibition was assessed by extracting total nucleic acid from UTM samples spiked with inactivated *B. pertussis* and amplifying the resulting total nucleic acid by qPCR. Briefly, total nucleic acid was extracted from eight 300µl replicates of UTM spiked with approximately $1 \times 10^4$ copies of inactivated *B. pertussis* and eluted in 100µl of Elution Buffer. Five microliters of each undiluted total nucleic acid eluate and 5µl of a tenfold dilution of each eluate were used in a qPCR assay to amplify a DNA target specific for *B. pertussis* to evaluate the effect of interfering substances. The average $C_q$ for undiluted eluates obtained from UTM samples was 25.97 cycles. The average $C_q$ for a 1:10 dilution of the eluates was 29.49 cycles, with a difference of 3.52 cycles between the average $C_q$ values for diluted and undiluted samples, indicating that any interfering substances present in the eluate cause minimal inhibition.

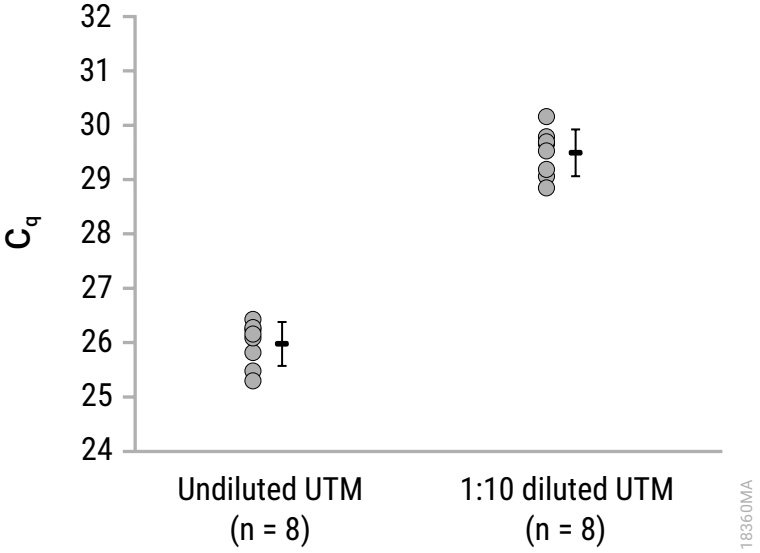



**Figure 12. Amplification inhibition assessment of total nucleic acid extracted from UTM samples spiked with *B. pertussis*.**

Amplification inhibition was assessed by extracting total nucleic acid from UTM samples spiked with inactivated Herpes simplex virus 1 (HSV-1). Total nucleic acid was extracted from eight 300µl replicates of UTM spiked with approximately $1 \times 10^5$ copies of inactivated HSV-1 and eluted in 100µl of Elution Buffer. Five microliters of each undiluted total nucleic acid eluate and 5µl of a tenfold dilution of each eluate were used in a qPCR assay to amplify a DNA target specific to HSV-1 to evaluate the effect of interfering substances. All $C_q$ values for undiluted eluates were ≤27.25 cycles, with an average $C_q$ of 26.88 cycles. The $\Delta C_q$ of undiluted and tenfold diluted eluates for individual samples ranged from 3.44 to 3.91, indicating that any interfering substances present in the eluate cause minimal inhibition.

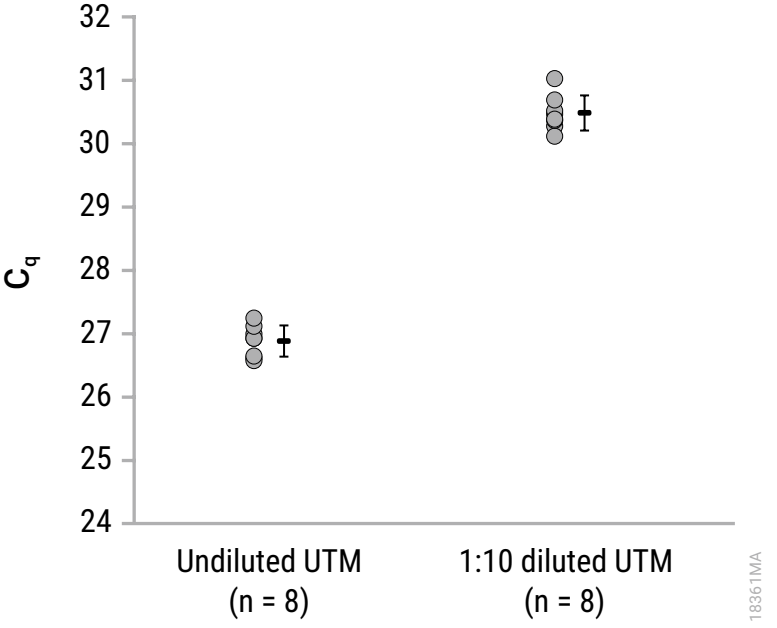



**Figure 13. Amplification inhibition assessment of total nucleic acid extracted from UTM samples spiked with HSV-1.**

### 9.E. Cross Contamination

Cross contamination was assessed using the Maxwell® CSC Pathogen Total Nucleic Acid Kit with the Maxwell® CSC Instrument by alternating the Maxwell® CSC cartridges containing UTM samples spiked with inactivated *B. pertussis* and negative UTM samples without added *B. pertussis* on the Maxwell® deck tray in a single total nucleic acid extraction run. Eluates from both *B. pertussis* positive and negative UTM samples were used in a qPCR assay to amplify a DNA target specific to *B. pertussis*, and the resulting $C_q$ values were compared to a standard curve ($10^1$–$10^6$ copies) generated using serial dilutions of a synthetic DNA with the *B. pertussis* target sequence. All of the UTM eluates with added *B. pertussis* had $C_q$ values within the standard curve, with an average $C_q$ of 25.42 cycles. All of the negative UTM eluates without added *B. pertussis* had $C_q$ values higher than the $C_q$ obtained for the lowest DNA concentration of the standard curve (10 copies).

## 10. Clinical Performance Evaluation

Clinical performance evaluation of the Maxwell® CSC Pathogen Total Nucleic Acid Kit was performed by an external clinical laboratory using the Maxwell® CSC 48 instrument to extract pathogen nucleic acid from the clinical sample types specified below, and amplifying the nucleic acid in a clinically relevant assay.

**Table 2. *Clostridium difficile* Total Nucleic Acid Extraction from Stool Specimens.** Total nucleic acid was extracted by one tester from 200 mg each of ten *Clostridium difficile* (*C. difficile*)-positive and ten *C. difficile*-negative human stool specimens using the Maxwell® CSC Pathogen Total Nucleic Acid Kit. Nucleic acid was also purified from these specimens using the laboratory's standard purification method for reference. The resulting eluates were analyzed by amplifying the *C. difficile* Toxin A and Toxin B DNA targets in a qPCR assay. Nucleic acid extracted from all presumed *C. difficile*-negative and *C. difficile*-positive specimens purified with the Maxwell® CSC Pathogen Total Nucleic Acid Kit gave the expected result and demonstrated concordance with results obtained from nucleic acid extracted using the laboratory reference extraction method.

| | Maxwell® CSC Pathogen Total Nucleic Acid System | Laboratory Reference Method |
|---|---|---|
| **Specimens matching *C. difficile*-negative status** | 10/10 | 10/10 |
| **Specimens matching *C. difficile*-positive status** | 10/10 | 10/10 |

**Table 3. Cytomegalovirus Total Nucleic Acid Extraction from Bronchoalveolar Lavage (BAL) Specimens.** Total nucleic acid was extracted by one tester from ten cytomegalovirus (CMV)-positive (100µl and 300µl) and ten CMV-negative (300µl) human BAL specimens using the Maxwell® CSC Pathogen Total Nucleic Acid Kit. In addition, total nucleic acid was extracted from 300µl of the same ten CMV-positive and ten CMV-negative BAL specimens using the laboratory's standard purification method for reference. The resulting eluates were analyzed by amplifying the CMV major immediate early gene DNA target in a qPCR assay. Nucleic acid extracted from all presumed CMV-negative and CMV-positive specimens (both 100µl and 300µl) purified with the Maxwell® CSC Pathogen Total Nucleic Acid Kit gave the expected result and demonstrated concordance with results obtained from nucleic acid extracted using the laboratory reference extraction method.

| | Maxwell® CSC Pathogen Total Nucleic Acid System | Laboratory Reference Method |
|---|---|---|
| **Specimens matching CMV-negative status** | 10/10 for 300µl input volume | 10/10 for 300µl input volume |
| **Specimens matching CMV-positive status** | 10/10 for 100µl input volume<br>10/10 for 300µl input volume | 10/10 for 300µl input volume |

**Table 4. SARS-CoV-2 Total Nucleic Acid Extraction from Viral Transport Medium (VTM) Specimens.** Two testers extracted total nucleic acid from 300ul of VTM that contained a nasopharyngeal swab specimen. Total nucleic acid was extracted from ten SARS CoV-2-positive and ten SARS CoV-2-negative human VTM specimens by two testers using the Maxwell® CSC Pathogen Total Nucleic Acid Kit. One tester also extracted nucleic acid from the same specimens using the laboratory's standard purification method for reference. The resulting eluates were analyzed by amplifying the SARS CoV-2 RdRP and N genes in an RT-qPCR assay. Nucleic acid extracted from all SARS CoV-2-negative and SARS CoV-2-positive specimens purified by both the testers with the Maxwell® CSC Pathogen Total Nucleic Acid Kit gave the expected result and demonstrated concordance with results obtained from nucleic acid extracted using the laboratory reference extraction method.

| | Maxwell® CSC Pathogen Total Nucleic Acid System | Laboratory Reference Method |
|---|---|---|
| **Specimens matching SARS-CoV-2-negative status** | 10/10 for Tester A<br>10/10 for Tester B | 10/10 |
| **Specimens matching SARS-CoV-2-positive status** | 10/10 for Tester A<br>10/10 for Tester B | 10/10 |

**Table 5. Human Papillomavirus Total Nucleic Acid Extraction from Pap Medium Specimens.** One tester extracted total nucleic acid from 300µl of ThinPrep® PAP medium specimens that contained cervical brushing collected from human patients. Total nucleic acid was extracted from nine human papillomavirus (HPV)-positive and nine HPV-negative Pap medium specimens with the Maxwell® CSC Pathogen Total Nucleic Acid Kit. Nucleic acid was also extracted from the same specimens using the laboratory's standard purification method for reference. The resulting eluates were analyzed by amplifying the HPV-16, HPV-18 and high-risk HPV DNA targets in a qPCR assay. Nucleic acid extracted from all HPV-positive Pap medium specimens extracted with the Maxwell® CSC Pathogen Total Nucleic Acid Kit gave the expected result and demonstrated concordance with results obtained from nucleic acid extracted using the laboratory reference extraction method.

| | Maxwell® CSC Pathogen Total Nucleic Acid System | Laboratory Reference Method |
|---|---|---|
| **Specimens matching HPV-negative status** | 9/9 | 9/9 |
| **Specimens matching HPV-positive status** | 9/9 | 9/9 |

**Table 6. Cytomegalovirus Total Nucleic Acid Extraction from Urine Specimens.** Total nucleic acid was extracted by one tester from 300µl each of ten CMV-positive and ten CMV-negative human urine specimens using the Maxwell® CSC Pathogen Total Nucleic Acid Kit. Nucleic acid was also extracted from the same specimens using the laboratory's standard purification method for reference. The resulting eluates were analyzed by amplifying the CMV major immediate early gene DNA target in a qPCR assay. All of the CMV-negative human urine specimens and nine of the ten CMV-positive human urine specimens demonstrated concordance with the results obtained using nucleic acid extracted by the laboratory reference extraction method. One presumed CMV-positive urine specimen was discordant between the two purification methods with a negative result for the Maxwell® CSC purification system and a weak positive result (lowest $C_q$ of 36.26 for four replicates with a positive result out of a total of five replicates tested) using the laboratory reference method.

| | Maxwell® CSC Pathogen Total Nucleic Acid System | Laboratory Reference Method |
|---|---|---|
| **Specimens matching CMV-negative status** | 10/10 | 10/10 |
| **Specimens matching CMV-positive status** | 9/10 | 10/10 |

**Table 7. Cytomegalovirus Total Nucleic Acid Extraction from Plasma Specimens.** Total nucleic acid was extracted by one tester from 300µl each of ten CMV positive and ten CMV-negative human plasma specimens using the Maxwell® CSC Pathogen Total Nucleic Acid Kit. Nucleic acid was also extracted from the same specimens using the laboratory's standard purification method for reference. The resulting eluates were analyzed by amplification of the CMV major immediate early gene DNA target in a qPCR assay. Nucleic acid extracted from all CMV-negative and CMV-positive human plasma specimens purified with the Maxwell® CSC Pathogen Total Nucleic Acid Kit gave the expected result and demonstrated concordance with results obtained from nucleic acid extracted using the laboratory reference extraction method.

| | Maxwell® CSC Pathogen Total Nucleic Acid System | Laboratory Reference Method |
|---|---|---|
| **Specimens matching CMV-negative status** | 10/10 | 10/10 |
| **Specimens matching CMV-positive status** | 10/10 | 10/10 |

Cross contamination occurring in the intended user environment using the Maxwell® CSC Pathogen Total Nucleic Acid Kit was assessed by alternating the Maxwell® CSC cartridges containing CMV-positive and CMV-negative human plasma specimens on the Maxwell® CSC 48 Instrument deck tray in a single total nucleic acid extraction run. No cross contamination was observed as the negative samples gave a negative result in a qPCR assay for a CMV DNA target.

## 11. Creating a Ribonuclease-Free Environment

Ribonucleases are extremely difficult to inactivate. Take care to avoid introducing RNase activity into the samples of total nucleic acid that contain RNA during and after isolation. This is especially important if the starting material was difficult to obtain or is irreplaceable. The following notes may help prevent accidental RNase contamination of your samples.

1. Two of the most common sources of RNase contamination are the user's hands and bacteria or molds that may be present on airborne dust particles. To prevent contamination from these sources, use sterile technique when handling the reagents supplied with this system. Wear gloves at all times. Change gloves whenever ribonucleases may have been contacted.
2. Whenever possible, sterile, disposable plasticware should be used for handling total nucleic acid that contains RNA. These materials are generally RNase-free and do not require pretreatment to inactivate RNase.
3. Treat non-sterile glassware, plasticware and electrophoresis chambers before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plasticware with 0.1N NaOH, 1mM EDTA, followed by RNase-free water. Commercially available RNase removal products also may be used, following the manufacturer's instructions.

   **Note:** Electrophoresis chambers may be contaminated with ribonucleases, particularly RNase A, from analysis of DNA samples. Whenever possible, set aside a new or decontaminated apparatus for RNA analysis only.
4. Treat solutions not supplied with the system by adding diethyl pyrocarbonate (DEPC) to 0.1%v/v in a fume hood. Incubate overnight with stirring at room temperature in the hood. Autoclave for 30 minutes to remove any trace of DEPC.

**Caution:** DEPC is a suspected carcinogen and should only be used in a chemical fume hood. DEPC reacts rapidly with amines and cannot be used to treat Tris buffers.

**Note:** For all downstream applications, it is essential that you continue to protect your RNA samples from RNases. Wear clean gloves and use RNase-free solutions and centrifuge tubes.

## 12. References


1. Clinical Laboratory Standards Institute (2020) CLSI MM13–Collection, transport, preparation and storage of specimens for molecular methods. Second Edition**.**
2. Baron, E.J. (2015) Specimen collection, transport, and processing: Bacteriology. In: *Manual of Clinical Microbiology, 11th Edition*, edited by Jorgensen, J.H. *et al*. 270–315. Washington, D.C., ASM Press.
3. Centers for Disease Control and Prevention. *Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing*. Accessed November 2, 2022: **www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html**
4. Centers for Disease Control and Prevention. *Stool Specimens - Molecular Diagnosis*. Accessed November 2, 2022: **www.cdc.gov/dpdx/diagnosticprocedures/stool/moleculardx.html**

## 13. 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 pathogen 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.<br>• Use only the Lysis Buffer provided with this kit.<br>• Incomplete mixing may reduce lysis. Vortex sample with Lysis Solution as recommended.<br>• Incomplete protease treatment to remove viral capsids and/or proteins from bacteria and parasites. Check the heat block or water bath temperature, and incubate for the full time recommended.<br>• When working with difficult to lyse pathogens (Gram positive bacteria or parasites) in stool, the optional bead beating step can be used.<br>• Make sure that 1-Thioglycerol has been added for BAL or sputum sample preprocessing. Omitting 1-Thioglycerol may negatively affect yields.<br>• Adding more sample than recommended may reduce nucleic acid recovery. |
| | Check that a plunger was added to the cartridge. |
| | Post-purification storage issues.<br>• Remove eluates, and store at the recommended temperature immediately after the Maxwell® Instrument run.<br>• 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. |

## 13. Troubleshooting (continued)

| Symptoms | Causes and Comments |
|---|---|
| Poor amplification | Paramagnetic particle carryover may cause interference in amplification reactions. Remove particles in elution tube by centrifugation or magnetic separation. |
| | Wrong elution buffer was added. Use only the Elution Buffer supplied with the Maxwell® CSC Pathogen Total Nucleic Acid 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 Maxwell® 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.

## 14. Related Products

### Instrument and Accessories

| 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**

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



Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information. All prices and specifications are subject to change without prior notice.

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