Application note

Compatibility of QuickExtract DNA Extraction Solution with relevant human pathogen diagnostic samples and RT-qPCR systems

Introduction

A simple and time-saving protocol for extracting amplifiable nucleic acids from various sample types is crucial in pathogen detection applications. Nucleic acid extraction without further purification often negatively affects downstream pathogen detection systems.

Here, we demonstrate suitability of our QuickExtract™ DNA Extraction Solution for a range of relevant human pathogens and sample matrices and evaluate the extracted nucleic acids regarding their suitability as templates in market-leading reverse transcription qPCR (RT-qPCR) systems.

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Methods

Materials

Table 1 summarises the components used in this study.

Component	Details
Purification and extraction chemistry	a) QuickExtract DNA Extraction Solution (LGC Biosearch Technologies) b) sbeadex Pathogen Nucleic Acid Purification Kit (Biosearch Technologies)
Extraction standards and reference materials	a) Vircell Amplirun Total Respiratory Viral Panel Control (Swab), MBTC020 b) Vircell Amplirun Total Atypical Bacterial Pneumonia Control (Swab) MBTC022-R c) Vircell Amplirun Total MTB Control (Sputum), MBTC013 d) AccuPlex™ SARS-CoV-2 Verification Panel – Full Genome, Ref. 0505-0168 (LGC Clinical Diagnostics – formerly LGC SeraCare)
Transport media	a) Sigma Virocult® (Medical Wire & Equipment) b) UTM® (COPAN) c) DNA/RNA ShieldTM Collection Tube w/ Swab (Zymo Research)
Sample matrices	a) Human plasma and non-stabilised whole blood for serum preparation, Deutsches Rotes Kreuz, purchased for research use purposes b) Human Urine Innovative Research (Dunn Lab), IRHUURE50MI, pooled human urine c) Human Stool sample: MyBiosource.com, MBS170211
Oligonucleotides	Viral pathogens a) Primer and FAM-labelled probe for Influenza B. Sequences as supplied in Influenza SARS-CoV-2 Multiplex ValuPanel Reagents (Biosearch Technologies) b) 2019-nCoV ValuPanel Reagents (Biosearch Technologies) Bacterial pathogens *Please see citations[1-3] referencing the primers used for each of the bacterial pathogens tested.*
qPCR master mixes	a) RapiDxFire™ qPCR 5X Master Mix GF (Biosearch Technologies) b) Competitor A master mix c) Competitor B master mix d) Competitor C master mix
RT-qPCR master mixes	a) RapiDxFire 1-step RT-qPCR System (Biosearch Technologies) b) Competitor D master mix c) Competitor E master mix d) Competitor F master mix e) Power SYBR® Green PCR Master Mix, Ref 4367659 (Applied Biosystems)
RT-qPCR lyo-ready master mixes	a) RapiDxFire Lyo-Flex 1-step RT-qPCR 5X Master Mix (Biosearch Technologies) b) Competitor G master mix
qPCR instrument	qTOWER^3 G (Analytik Jena)
Carrier RNA	1 µg PolyA Carrier RNA used in extraction experiment Poly(A), polyadenylic acid, Roche Ref. 10108626001

Table 1. Materials used for verification of the QuickExtract DNA Extraction Solution with a range of viral and bacterial pathogens, various samples and different qPCR and RT-qPCR master mixes.

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Nucleic acid extraction from viral and bacterial extraction standards

The extraction standards (table 1) were reconstituted in molecular grade water according to the manufacturer's protocol. For each standard, 100 µL of reconstituted reagent was used as starting material for each isolation replicate. Extraction was performed as described in the QuickExtract DNA Extraction Solution manual.

Pathogen verification

For each pathogen included in the study, two isolation replicates were performed (as detailed above). For each subsequent qPCR or RT-qPCR reaction 5 µL extract was used as template in a 20 µL total reaction volume. Each sample was assayed in triplicate as neat, 1-in-2 and 1-in-4 dilutions, respectively.

Viral panel sample extracts underwent RT-qPCR using the RapiDxFire 1-step RT-qPCR system (Biosearch Technologies) in conjunction with Influenza SARS-CoV-2 Multiplex ValuPanel reagents for the Influenza B target. Each reaction contained 5 pmole of each primer and 1.25 pmole of a FAM-labelled Influenza B probe following the manufacturer's thermal cycling protocol guidelines. Adenovirus target was assayed by qPCR using RapiDxFire qPCR 5X Master Mix GF in conjunction with the appropriate primers and probe (8 pmole per each Ad4-primer and 4 pmole of a FAM-labelled Ad4-probe per reaction)[1]. Extracted bacterial samples were assayed by qPCR using Power SYBR® Green PCR Master Mix (Applied Biosystems) in conjunction with the appropriate primers (2.5 pmole for each primer per reaction) for each bacterial pathogen[2,3] following manufacturer's guidelines and thermal cycling protocols.

C_q values were determined for each reaction and used to calculate the average C_q value for each pathogen.

Sample matrix experiments using the SARS-CoV-2 model system

Preparation of matrix materials

Human plasma was purchased from Deutsches Rotes Kreuz (DRK) for research use. Each plasma sample was aliquoted and stored at -20 °C. A pre-frozen aliquot was used for the experiments.

For serum preparation, non-stabilised whole blood was purchased from DRK. The blood bottle was stored upright for one hour at room temperature. After sedimentation, the supernatant was removed, aliquoted into 50 mL tubes and stored for 30 minutes at room temperature with air exposure. All aliquots were subsequently centrifuged at 300 × g for 10 minutes at room temperature and the clear supernatant was used in the sample matrix experiments.

From stool, a 5 mm diameter sample was collected using a spatula and diluted in 1 mL 1X PBS. The sample was vigorously vortexed and spun down. Supernatant was taken for use in the matrix experiments, avoiding solid particles.

Urine and saliva were applied to each reaction undiluted. Saliva samples were collected and subsequently pooled from a single donor.

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Matrix material spiking

To assess the compatibility of the QuickExtract DNA Extraction Solution with common matrices for human diagnostics, spiking experiments were performed. The matrices used for this study were urine, stool, plasma, serum and saliva. A volume of 100 µL of each matrix was spiked with 10 µL AccuPlex SARS-CoV-2 Verification Panel (100k copies/mL member, approximately 1000 copies) as a model pathogen and extracted in duplicate as described in the QuickExtract DNA Extraction Solution manual. UTM was used as a comparison matrix. Subsequent RT-qPCRs were performed as described in section SARS-CoV-2 RT-qPCR.

SARS-CoV-2 RT-qPCR

For the detection of SARS-CoV-2 reference material, the 2019-nCoV ValuPanel Reagents (Biosearch Technologies, N1 target) were used in conjunction with RapiDxFire 1-step RT-qPCR system following manufacturer's cycling conditions. A 5 µL volume of each extract was added to each reaction. For each isolation replicate, PCR was performed in triplicate. The RT-qPCR experiments were run on the qTOWER3 G instrument (Analytik Jena).

Transport media comparison

To assess the compatibility of the QuickExtract DNA Extraction Solution with common transport media, spiking experiments were performed. For this test, the Sigma Virocult® (mwe), UTM® (COPAN) and DNA/RNA ShieldTM Collection Tube w/Swab (Zymo Research) were used. To evaluate a successful extraction using QuickExtract DNA Extraction Solution, parallel nucleic acid purifications with the sbeadex Pathogen Nucleic Acid Purification Kit were performed. For each test, 100 µL of the corresponding transport medium was spiked with 10 µL AccuPlex SARS-CoV-2 Verification Panel (100k copies/mL member, approximately 1000 copies per reaction) as a model pathogen. For each test, 100 µL of spiked transport medium was used in duplicate isolation experiments according to manufacturer's protocols. Subsequent RT-qPCRs were performed as described in section SARS-CoV-2 RT-qPCR.

Master mix comparison

To assess compatibility of our QuickExtract DNA Extraction Solution with different downstream RT-qPCR systems, we evaluated both, RNA and DNA model pathogen spiked extracts, using the different master mixes detailed in table 1. For assessing (lyo-ready) RT-qPCR master mixes, molecular grade water was spiked with 10 µL AccuPlex SARS-CoV-2 Verification Panel (100k copies/mL member, approximately 1000 copies per reaction), extracted using QuickExtract DNA Extraction Solution and the N1 target was subsequently amplified as described in section SARS-CoV-2-RT-qPCR according to manufacturer`s recommendations. For evaluation of qPCR master mixes, Adenovirus was used as model pathogen. A volume of 100 µL of Vircell Amplirun Total Respiratory Viral Panel control was used for extraction with QuickExtract DNA Extraction Solution and the Adenovirus target was subsequently amplified using appropriate primers and probe for the detection of Adenovirus (see section Pathogen detection for detailed PCR protocol description) in conjunction with different qPCR master mixes (table 1). Cycling conditions were applied according to manufacturers' protocols.

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Results and discussion

Nucleic acid extraction from different pathogens

RT-qPCR and qPCR results for the viral and bacterial targets demonstrate successful nucleic acid extraction using QuickExtract DNA Extraction Solution for both viral pathogen (figure 1) and bacterial pathogen (figure 2) extraction standards.

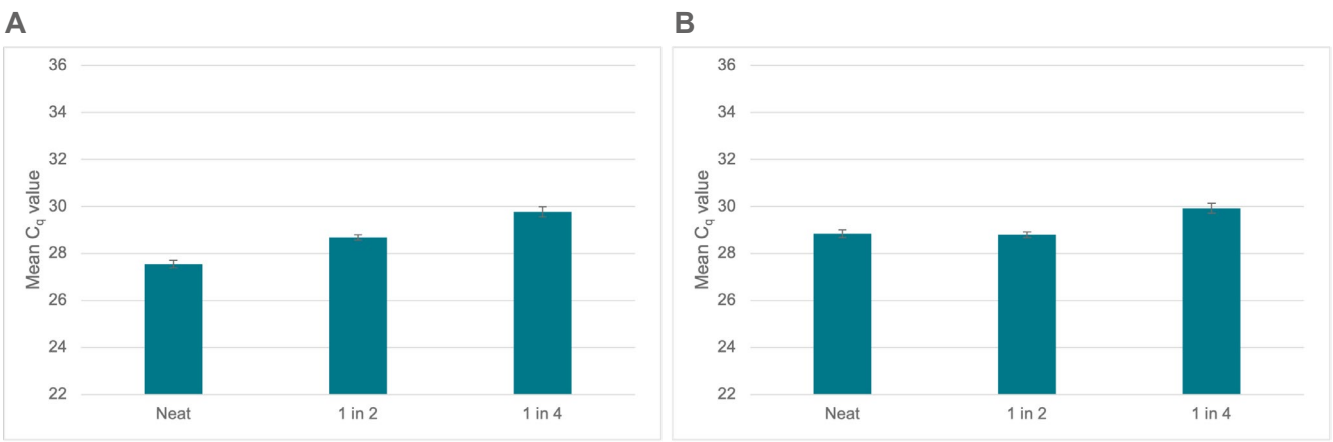

Figure 1. RT-qPCR results for viral standards extracted using the QuickExtract DNA Extraction Solution. A) Influenza B and B) Adenovirus demonstrating compatibility of extracts applied directly to RT-qPCR reactions. One out of the six neat Adenovirus extracts failed to amplify during qPCR. The data shown represent averages from two replicate extractions. Error bars represent standard deviation.

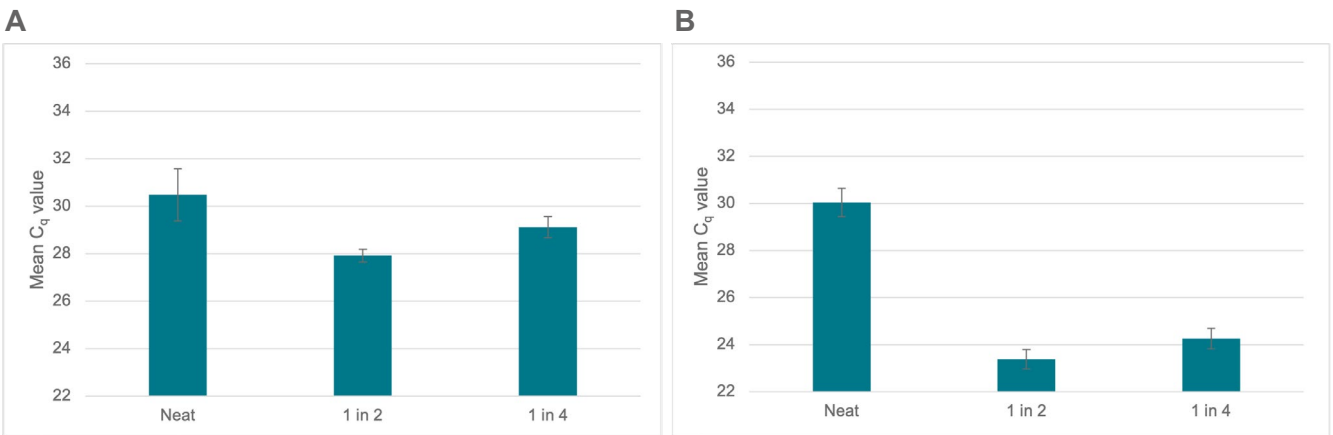

Figure 2. qPCR results for A) *Bordetella pertussis*[1] and B) *Coxiella burnetii*[2] targets from bacterial standards extracted using the QuickExtract DNA Extraction Solution demonstrate the requirement for dilution of some matrices for efficient pathogen detection when extracts are applied directly to qPCR. The data shown represent averages from two replicate extractions. Error bars represent standard deviation.

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Nucleic acid isolation from different matrices

RT-qPCR results for the SARS-CoV-2 model demonstrate the suitability of QuickExtract DNA Extraction Solution for RNA extraction from a range of spiked human sample matrices. SARS-CoV-2 reference material was successfully extracted from human urine, plasma, stool and saliva (figure 3).

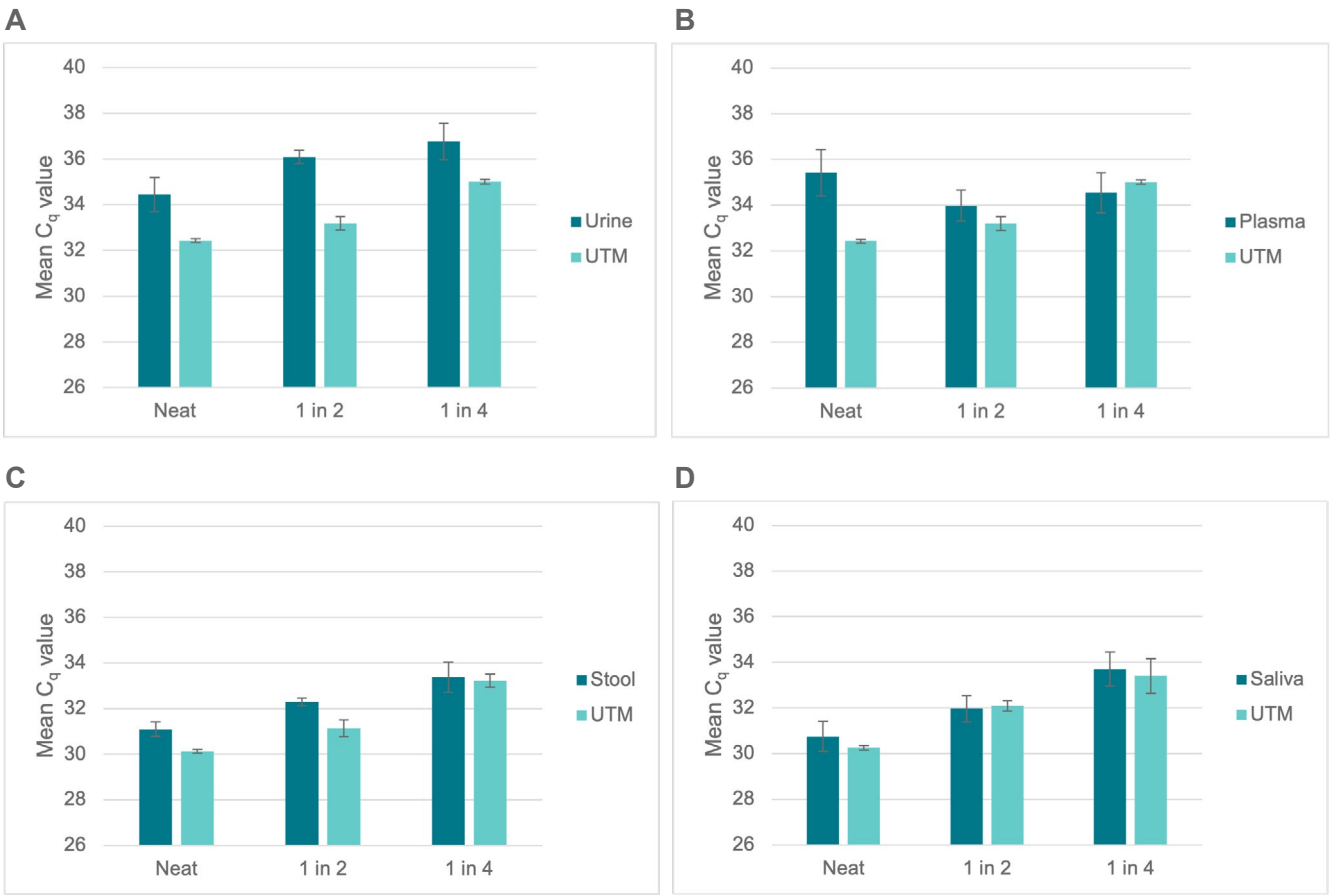

Figure 3. RT-qPCR results for the SARS-CoV-2 standard spiked in and extracted from urine (A), plasma (B), stool (C), and saliva (D) using QuickExtract DNA Extraction Solution with UTM as a comparison sample matrix. Data demonstrate overall equivalent performance of applied sample matrices to our QuickExtract system when diluted 1 in 4. The data shown represents averages from two replicate extractions. Error bars represent standard deviation.

Results show C_q values for extractions from plasma, stool and saliva comparable to the UTM extractions for the 1-in-4 dilutions. The neat and 1-in-2 dilutions show slight deviations to the UTM extraction which is likely due to the presence of higher levels of PCR inhibitors affecting the PCR. The extraction from urine shows deviation to the UTM extraction across all dilutions which might be due to insufficient dilution of inhibitors. Therefore, for urine samples further dilution is recommended.

The addition of QuickExtract DNA Extraction Solution to the serum sample led to gelatinisation of the sample, which made subsequent analysis impossible. This demonstrates that QuickExtract DNA Extraction Solution is incompatible with serum as a sample matrix.

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Transport media comparison

RT-qPCR results for the SARS-CoV-2 model demonstrate the suitability of QuickExtract DNA Extraction Solution 1.0 for RNA purification from two different non-inactivating transport media (figure 4).

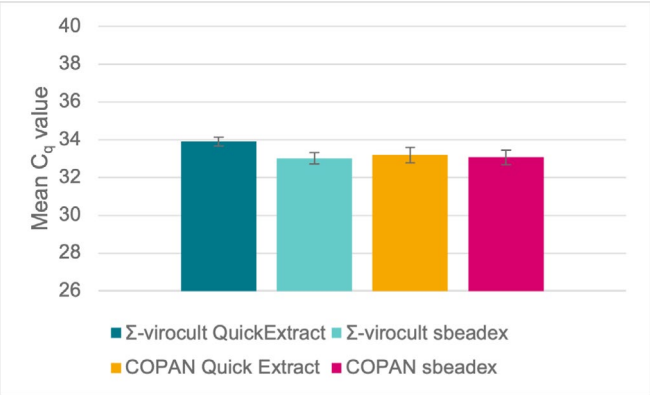

Figure 4. RT-qPCR results for N1 target from the SARS-CoV-2 standard isolated from Virocult transport media using the QuickExtract DNA Extraction Solution and the sbeadex Pathogen Nucleic Acid Purification Kit with Copan media as a comparison sample matrix. Results demonstrate suitability of QuickExtract for this transport media. The data shown represents averages from two replicate isolations. Error bars represent standard deviation.

Using QuickExtract, the RNA extraction from inactivating transport medium DNA/RNA shieldTM Collection Tube w/ Swab (Zymo Research) led to complete failure of subsequent RT-qPCR reactions. Inactivating transport media likely contain enzyme activity inhibiting reagents and may not be suitable to be applied to a rather crude DNA/RNA extraction method with subsequent direct application to RT-qPCR. Since the DNA/RNA released with the QuickExtract DNA Extraction Solution is used for direct PCR/RT-PCR analysis without further purification, inactivating transport media should be tested for suitability when working with QuickExtract.

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Master mix comparison

RT-qPCR and qPCR results for the viral panel and the SARS-CoV-2 reference material extracted with QuickExtract DNA Extraction Solution demonstrate the compatibility of this solution with different market-leading qPCR (figure 5), RT-qPCR (figure 6) and lyo-ready RT-qPCR (figure 7) master mixes.

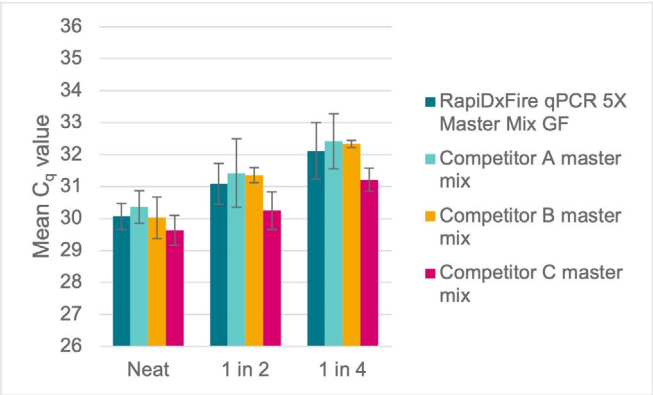

Figure 5. qPCR results for the Vircell Amplirun Total Respiratory Viral Panel control extracted with QuickExtract DNA Extraction Solution. Subsequent amplification was performed using Adenovirus primers and probe[1] in conjunction with four different qPCR master mixes, showing compatibility of the QuickExtract Solution with different qPCR-master mixes. The data shown represents averages from two replicate extractions. Error bars represent standard deviation.

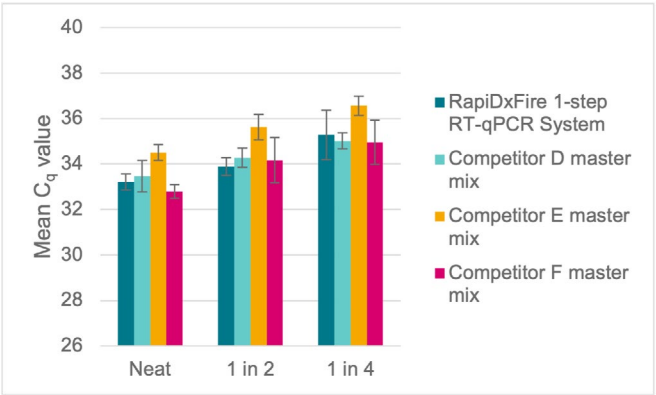

Figure 6. RT-qPCR results for N1 target from extracts from the AccuPlex SARS-CoV-2 Reference Material extracted with QuickExtract DNA Extraction Solution in serial dilutions. Results represent compatibility of QuickExtract with the different RT-qPCR master mixes. The data shown represents averages from two replicate extractions. Error bars represent standard deviation.

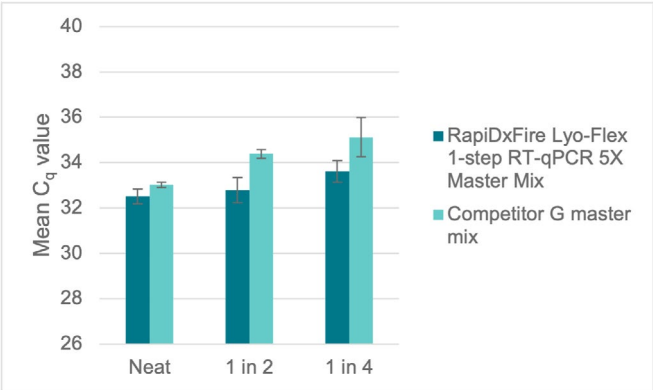

Figure 7. RT-qPCR results for N1 target from extracts of the AccuPlex SARS-CoV-2 Reference Material extracted with QuickExtract DNA Extraction Solution. Subsequent amplification was performed using 2019-nCoV ValuPanel reagents in conjunction with two different lyo-ready RT-qPCR master mixes. Results represent compatibility of QuickExtract with the two lyo-ready RT-qPCR master mixes. Error bars represent standard deviation.

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Summary

The QuickExtract DNA Extraction Solution has been successfully verified for fast and simple extraction of DNA and RNA from common viral and bacterial pathogens from swab-like sample matrices. However, each pathogen-of-interest will require evaluation to ensure efficient lysis and release of nucleic acids.

In addition, compatibility of QuickExtract DNA Extraction Solution was shown with a broad range of matrices relevant to human disease diagnostics including saliva, stool and plasma. Serum proved to be incompatible with QuickExtract chemistry; for this sample matrix we recommend our sbeadex Pathogen Nucleic Acid Purification Kit which performed well with serum.

Pathogens	Sample matrices	Detection systems
✓ Bacteria* ✓ Virus*	✓ Plasma ✓ Urine ✓ Stool ✓ Saliva ✓ UTM ✓ Other non-inactivating transport media	✓ QuickExtract is compatible with different, commonly used market-leading qPCR and RT-qPCR kits (including lyo-ready master mixes)
*Each pathogen-of-interest must be evaluated for sufficient lysis upon usage of QuickExtract DNA Extraction Solution.	Some extracts might require further dilution when applied to RT-qPCR reactions.	

Table 2. Summary of the scope of applications for the QuickExtract DNA Extraction Solution.

The QuickExtract DNA Extraction Solution offers a quick and simple extraction method for direct PCR of a broad range of viral and bacterial pathogens and is compatible with many relevant sample matrices for human diagnostics. Subsequent PCR reactions benefit from dilution of the extracts to reduce inhibitory influence, especially when working with challenging sample matrices like urine. However, samples with high concentrations of potential enzyme inhibitors may limit diagnostic sensitivity of downstream PCR/RT-PCR applications as QuickExtract does not effectively remove such PCR inhibitors. In such cases, a DNA/RNA purification step is recommended.

QuickExtract also shows good compatibility with market-leading transport media, except inactivating transport media where an additional purification step might be required.

QuickExtract is also perfectly compatible with many well-established RT-qPCR systems, including our RapiDxFire master mixes.

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References

The references below relate specifically to the published primer sets used for the detection of bacterial respiratory pathogens. The relevant pathogen is detailed in parenthesis at the end of the reference.

1. Wong, S., *et al.* (2008) Journal of medical virology 80.5: 856-865. (Human adenovirus)
2. Grogan, J.A., *et al.* (2011). J. Med Microbiol, 60:722-729 *(Bordetella pertussis)*
3. De Bruin, A., *et al.* (2011). Appl Environ Microbiol, 77(18):6516-6523 *(Coxiella burnetii)*

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