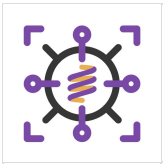


Version 6

Mar 24, 2021

SalivaDirect™: RNA extraction-free SARS-CoV-2 diagnostics V.6

Chantal Vogels[1], Orchid M Allicock[1], Doug E. Brackney[2,1], Chaney C Kalinich[1], Isabel M Ott[1], Nathan Grubaugh[1], Anne L Wyllie[1]

[1]Department of Epidemiology of Microbial Diseases, Yale School of Public Health;
[2]Department of Environmental Sciences The Connecticut Agricultural Experiment Station

1 Works for me dx.doi.org/10.17504/protocols.io.btdnni5e

Nathan Grubaugh
Department of Epidemiology of Microbial Diseases, Yale Schoo...

SUBMIT TO PLOS ONE

ABSTRACT

SalivaDirect™ is an RNA-extraction free, dual-plexed RT-qPCR method for SARS-CoV-2 detection. It can be broadly implemented as it (1) does not require saliva collection tubes containing preservatives, (2) does not require specialized equipment for RNA extraction, and (3) is validated for use with products from multiple vendors. Thus, the simplicity and flexibility of SalivaDirect™ means that it is not as affected by supply chain bottlenecks as some other assays. Our method is RNA-extraction free which enables testing of low volume and minimally processed saliva in dual-plexed RT-qPCR for SARS-CoV-2 detection. Saliva will be treated with proteinase K followed by a heat inactivation step, and is then directly used as input in the dual-plexed RT-qPCR test. Our aim was not to design new primers and probes for RT-qPCR testing, but rather to use validated primer and probe sets (N1 and RP) developed by the US CDC. The human Ribonuclease P (RP) probe was modified with a different fluorophore so that the primer/probe set could be combined in a dualplex assay, reducing the number of tests to 1 assay with 2 sets.

Version 2 includes:
- Optimized thermocycler conditions
- Locally validated alternative options for Proteinase K, RT-qPCR master mix, and thermocyclers
- Use of 8-strip tubes for sample processing step, due to contamination issues in 96-well plates.

Version 3 has been updated to remove steps for sample self-collection.

Version 4 has updated Ct thresholds for the ABI 7500 Fast Dx.

Version 5 has an updated description for use, additional RP probe with ATTO647 fluorophore, and a detailed table with catalog numbers.

Version 6 includes:
- Introducing multiple workflows for sample processing including heat Pre-treatment as an alternative to Proteinase K
- Updated list of RT-qPCR reagents, theromcyclers and protocol for 384 well format.

EXTERNAL LINK

https://covidtrackerct.com/about-salivadirect/

Citation: Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie (03/24/2021). SalivaDirectÃ¢ÂÂ¢: RNA extraction-free SARS-CoV-2 diagnostics. https://dx.doi.org/10.17504/protocols.io.btdnni5e

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Vogels CB, Watkins AE, Harden CA, Brackney DE, Shafer J, Wang J, Caraballo C, Kalinich CC, Ott IM, Fauver JR, Kudo E, Lu P, Venkataraman A, Tokuyama M, Moore AJ, Muenker MC, Casanovas-Massana A, Fournier J, Bermejo S, Campbell M, Datta R, Nelson A, , Cruz CSD, Ko AI, Iwasaki A, Krumholz HM, Matheus J, Hui P, Liu C, Farhadian SF, Sikka R, Wyllie AL, Grubaugh ND, SalivaDirect: A Simplified and Flexible Platform to Enhance SARS-CoV-2 Testing Capacity. Med (New York, N.y.) doi: 10.1016/j.medj.2020.12.010

DOI

dx.doi.org/10.17504/protocols.io.btdnni5e

EXTERNAL LINK

https://covidtrackerct.com/about-salivadirect/

PROTOCOL CITATION

Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie 2021. SalivaDirect™: RNA extraction-free SARS-CoV-2 diagnostics. **protocols.io** https://dx.doi.org/10.17504/protocols.io.btdnni5e
Version created by Nathan Grubaugh

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Vogels CB, Watkins AE, Harden CA, Brackney DE, Shafer J, Wang J, Caraballo C, Kalinich CC, Ott IM, Fauver JR, Kudo E, Lu P, Venkataraman A, Tokuyama M, Moore AJ, Muenker MC, Casanovas-Massana A, Fournier J, Bermejo S, Campbell M, Datta R, Nelson A, , Cruz CSD, Ko AI, Iwasaki A, Krumholz HM, Matheus J, Hui P, Liu C, Farhadian SF, Sikka R, Wyllie AL, Grubaugh ND, SalivaDirect: A Simplified and Flexible Platform to Enhance SARS-CoV-2 Testing Capacity. Med (New York, N.y.) doi: 10.1016/j.medj.2020.12.010

WHAT'S NEW

Version 6 includes: Introducing multiple workflows for sample processing including heat Pre-treatment as an alternative to Proteinase K Updated list of RT-qPCR reagents, theromcyclers and protocol for 384 well format.

KEYWORDS

SARS-CoV-2, COVID-19, saliva, extraction-free PCR, COVID-19 diagnostics

CREATED

Mar 16, 2021

LAST MODIFIED

Mar 24, 2021

PROTOCOL INTEGER ID

48270

MATERIALS TEXT

SalivaDirect™ ordering shortlist

A	B	C	D	E

Vendor	Item	Catalog number	Quantity	# Reactions
Order one of the following Proteinases K				
ThermoFisher Scientific	MagMAX Viral/Pathogen Proteinase K	A42363	10 mL	4,000 reactions
New England Biolabs	Proteinase K, Molecular Biology Grade	P8107S	2 mL	320 reactions
AmericanBio	Proteinase K	AB00925	100 mg	800 reactions
Order one of the following RT-qPCR kits				
New England Biolabs	Luna Universal Probe One-Step RT-qPCR Kit	E3006S	2 mL	200 reactions
		E3006L	5 mL	500 reactions
		E3006X	10 mL	1,000 reactions
		E3006E	25 mL	2,500 reactions
New England Biolabs	Luna® Probe One-Step RT-qPCR 4X Mix with UDG (for use with 384-well format PCR instruments)	M3019S	-	200 reactions
		M3019L	-	500 reactions
		M3019X	-	1,000 reactions
		M3019E	-	2,000 reactions
Bio-Rad	Reliance One-Step Multiplex RT-qPCR Supermix	12010176	1 mL	200 reactions
		12010220	5 mL	1,000 reactions
		12010221	10 mL	2,000 reactions
ThermoFisher Scientific	TaqPath 1-Step RT-qPCR Master Mix, GC	A15299	5 mL	1,000 reactions
		A15300	10 mL	2.000 reactions
Order the following primers and probes				
Integrated DNA Technologies	nCOV_N1 Forward Primer Aliquot	10006821	50 nmol	6,250 reactions
		10006830	100 nmol	12,500 reactions
	nCOV_N1 Reverse Primer Aliquot	10006822	50 nmol	6,250 reactions
		10006831	100 nmol	12,500 reactions
	nCOV_N1 Probe Aliquot	10006823	25 nmol	6,250 reactions
		10006832	50 nmol	12,500 reactions
	RNase P Forward Primer Aliquot	10006827	50 nmol	16,600 reactions
		10006836	100 nmol	33,300 reactions
	RNase P Reverse Primer Aliquot	10006828	50 nmol	16,600 reactions
		10006837	100 nmol	33,300 reactions
	RNase P Probe	Custom probe (Cy5)	25 nmol	6,250 reactions

Citation: Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie (03/24/2021). SalivaDirectÃ¢ÂÂ¢: RNA extraction-free SARS-CoV-2 diagnostics. https://dx.doi.org/10.17504/protocols.io.btdnni5e

		Custom probe (Cy5)	50 nmol	12,500 reactions
		10007061 (ATTO647)	25 nmol	6,250 reactions
		10007062 (ATTO647)	50 nmol	12,500 reactions
Order one of the following nuclease-free waters				
Integrated DNA Technologies	Nuclease-free water	11-04-02-01	20 mL	
		11-05-01-14	300 mL	
		11-05-01-04	1 L	
New England Biolabs	Nuclease-free water	B1500S	25 mL	
		B1500L	100 mL	
Order the following positive control				
Twist Bioscience	Synthetic SARS-CoV-2 RNA Control 2	102024	100 µL	

Sample collection

- Wide-mouth collection tube/container labelled with unique subject identity number and collection data.
  For example: 5 ml screw cap tube, 25 ml conical tube, 50 mL Falcon tube.

15 mL conical tubes are not recommended due to their long, narrow opening. This design increases the risk of contaminating pipettes when aliquoting the collected sample.
Urine cups can also be used for sample collection; however, they are difficult to vortex during the processing step, and thus we do not recommend them.

- Personal protective equipment (PPE) for **sample collector**. (at minimum, gloves and face mask)

Extraction-free sample processing

- Thermocycler or real-time PCR (qPCR) instrument
- Vortex mixer
- Plate centrifuge or spinner
- Pipette (P20 and P200)
- Pipette tips (20 µL and 200 µL)
- 8-strip PCR tubes (200 µL capacity)
- Proteinase K (volumes for different concentrations given in text)
  ThermoFisher
  NEB
  AmericanBio
- Saliva samples (see “sample collection”)

SARS-CoV-2 RNA Detection by Dual-Plex RT-qPCR

- Real-time PCR (qPCR) instrument. Validation data are currently available for:
  Bio-Rad CFX96
  ABI 7500 Fast
  ABI 7500 Fast Dx
  ABI QuantStudio 5
  ABI QuantStudio 6
  ABI QuantStudio 7 Pro
  ABI QuantStudio 7 Flex
  ABI QuantStudio 12K Flex
  Agilent AriaMX

Citation: Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie (03/24/2021). SalivaDirectÃ¢ÂÂ¢: RNA extraction-free SARS-CoV-2 diagnostics. https://dx.doi.org/10.17504/protocols.io.btdnni5e

- RT-qPCR kit. Validation data are currently available for:
  NEB Luna Universal Probe One-Step RT-qPCR kit
  NEB Luna Probe One-Step RT-qPCR 4X Mix with UDG
  Reliance One-Step Multiplex RT-qPCR Supermix
  TaqPath™ 1-Step RT-qPCR Master Mix
- 96 well optical PCR plate and adhesive film
  For Bio-Rad CFX96: plates and seals
  For ABI 7500 Fast (Dx): plates and seals
- Plate centrifuge or spinner
- 1.5 mL tube centrifuge
- Vortex
- Pipettes (P10, P20, P200, and P1000 - optional P10 multichannel)
- Pipette tips (10 µL, 20 µL, 200 µL, and 1000 µL)
- PCR cooler
- Adhesive film applicator
- 1.5 mL tubes (clear and LightSafe)
- Nuclease-free water
- 70% ethanol, for cleaning
- Twist synthetic SARS-CoV-2 RNA controls at 100 copies/uL
- Extraction-free saliva samples (see “extraction-free sample processing”)
- Primers and probes, as follows.

A	B	C
Target	**Primer/probe**	**Sequence**
CDC-N1	2019-nCoV_N1-F	GACCCCAAAATCAGCGAAAT
	2019-nCoV_N1-R	TCTGGTTACTGCCAGTTGAATCTG
	2019-nCoV_N1-P	FAM-ACCCCGCATTACGTTTGGTGGACC-IBFQ
Human RNase P	RP-F	AGATTTGGACCTGCGAGCG
	RP-R	GAGCGGCTGTCTCCACAAGT
	RP-P*	Cy5-TTCTGACCTGAAGGCTCTGCGCG-IBRQ
		or
		ATTO647-TTCTGACCTGAAGGCTCTGCGCG-IBRQ

Stocks can be kept at 100 uM, and will be diluted to working concentrations of 20 uM at the beginning of the RT-qPCR protocol section.

* Use either the Cy5 or ATTO647 fluorophore for the RP probe.

SAFETY WARNINGS

Processing of any sample type which could potentially be positive for SARS-CoV-2 should be conducted in BSL2+ settings. Before starting work with these samples, please contact your local EHS (environment, health and safety) or biosafety office for proper guidance on how to work with these samples in your laboratory.

DISCLAIMER:

BEFORE STARTING

Citation: Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie (03/24/2021). SalivaDirectÃ¢ÂÂ¢: RNA extraction-free SARS-CoV-2 diagnostics. https://dx.doi.org/10.17504/protocols.io.btdnni5e

The FDA issued Emergency Use Authorization (EUA) for SalivaDirect™ as a Laboratory Developed Test (LDT) on 15 August 2020. This version of the SalivaDirect™ protocol is for **RESEARCH ONLY**. Prior authorization and the official Instructions For Use are needed to use SalivaDirect™ as an FDA-authorized LDT. See our website for authorization instructions.

Latest information on SalivaDirect™ is available here.

While collecting saliva is significantly easier than swabs, saliva samples can be difficult to work with. It is important to follow the sample collection guidelines to ensure that saliva, not sputum, is being collected.

Sample collection

1 Saliva should be collected with the assistance of a healthcare worker or technician.

2 Before collection, clean hands using alcohol-based sanitizer or soap and water (no fragrances) and don appropriate PPE (at minimum, gloves and a mask).

3 Ensure all collection materials are labelled with the correct identifying information.

3.1 While preparing collection materials, direct the sample provider to begin pooling saliva in their mouth. Saliva production can be stimulated by thinking about food (favorite foods, upcoming meals, etc.) or about the saliva collection itself.

This protocol is intended for the collection of the **normal** saliva that naturally pools into the mouth. **No coughing or sniffing prior to sample collection is required.**
Ideally, water should be avoided 10 minutes prior to collection. Other drinks, food, and nasal sprays should be avoided for half an hour before sample collection.

4 Remove the lid of the collection container, and direct the sample provider to gently expel saliva into the container until at least 0.5 mL has been collected.

The total volume measured is to exclude any bubbles.

4.1 Once at least 0.5 mL has been collected, securely replace the lid of the collection container.

4.2 Sterilize the container surface with 70% ethanol or a disinfecting wipe, and place the sample in a secondary container or an appropriately labeled biohazard bag.

4.3 Dispose of gloves, and register the sample collection (including date and time).

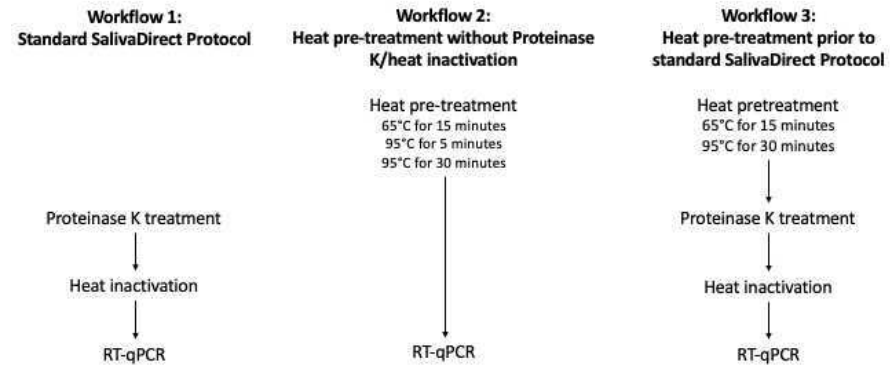
Citation: Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie (03/24/2021). SalivaDirectÃ¢ÂÂ¢: RNA extraction-free SARS-CoV-2 diagnostics. https://dx.doi.org/10.17504/protocols.io.btdnni5e

5 Transfer the sample to the laboratory for sample processing. If the time between sample collection and the initial processing steps (aliquoting) is likely to exceed 6 hours, samples can be stored at 2-8°C for up to 7 days, or -80°C for long-term storage, then later thawed on ice for testing.

Sample pretreatment

6 Samples can either be treated with either:
- Workflow 1: Standard SalivaDirect protocol
- Workflow 2: Pre-Treatment Heat step prior to SalivaDirect protocol without the addition of Proteinase K and heat inactivation step
- Workflow 3: Pre-Treatment Heat step prior to standard SalivaDirect protocol with Proteinase K and heat inactivation step

Select the appropriate option below.



Step 6 includes a Step case.
Workflow 1: Standard SalivaDirect protocol
Workflow 2: Heat pre-treatment only
Workflow 3: Heat pre-treatment then proteinase K

step case

Workflow 1: Standard SalivaDirect protocol

Standard SalivaDirect protocol with Proteinase K and heat inactivation step

7 Add Proteinase K (see table for volume per sample) to designated 8-strip PCR tubes (200 µL capacity).

A	B	C
Vendor	**Concentration**	**Volume**
ThermoFisher Scientific MagMAX™ Viral/Pathogen Proteinase K	50 mg/mL	2.5 µL
New England Biolabs Proteinase K, Molecular Biology Grade	20 mg/mL	6.25 µL
AmericanBio Proteinase K	Lyophilized (add 50 mg per 1 mL of nuclease-free water)	2.5 µL

Results of validation experiments for different Proteinase K options are available here.
Reagent order info: ThermoFisher, NEB, AmericanBio

This work should be completed under BSL-2 conditions, and samples potentially containing

Citation: Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie (03/24/2021). SalivaDirectÃ¢ÂÂ¢: RNA extraction-free SARS-CoV-2 diagnostics. https://dx.doi.org/10.17504/protocols.io.btdnni5e

SARS-CoV-2 should only be handled in a biosafety cabinet. Please seek guidance from your local biosafety office on specific recommendations for working with samples which could contain SARS-CoV-2.

8 Vortex each saliva sample until homogeneous, and immediately transfer 50 µL saliva to each 8-strip PCR tube containing proteinase K. Add 50 µL of nuclease-free water to one 8-strip PCR tube (negative extraction control).

8.1 Close the 8-strip tube lids tightly.

8.2 Place the 8-strip tubes in a rack and vortex for 1 minute at 3000-5000 RPM.

8.3 Briefly spin down the rack/tubes using a plate spinner or 8-strip tube microcentrifuge.

If no plate centrifuge or spinner is available, the plate can be gently tapped to get the samples at the bottom of each well.

9 Inactivate the proteinase K by heating samples for 5 minutes at 95°C on a PCR instrument or equivalent thermocycler.

9.1 Briefly spin down the tubes using a plate spinner or 8-strip tube microcentrifuge.

Tubes should only be centrifuged for **a few seconds**, to spin down condensation in the tubes.

10 Store samples at -80°C or proceed immediately to RT-qPCR testing.

RT-qPCR

11 Prepare 20 uM working stocks of the primers and probes (sequences provided in Materials) by adding 20 µL of 100 uM stock to 80 µL nuclease-free water.

Briefly vortex and centrifuge reagents before use.
Probes are photosensitive and should be stored in the dark.

11.1 Use the 20 uM working stocks to prepare dualplex primer-probe-water mix containing the following:

Citation: Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie (03/24/2021). SalivaDirectÃçÂÂç: RNA extraction-free SARS-CoV-2 diagnostics. https://dx.doi.org/10.17504/protocols.io.btdnni5e

A	B	C
Component	**Volume (1 reaction)**	**Volume (100 reactions)**
2019-nCoV_N1-F (400 nM/reaction)	0.4 µL	40 µL
2019-nCoV_N1-R (400 nM/reaction)	0.4 µL	40 µL
2019-nCoV_N1-P (200 nM/reaction)	0.2 µL	20 µL
RP-F (150 nM/reaction)	0.15 µL	15 µL
RP-R (150 nM/reaction)	0.15 µL	15 µL
RP-P (200 nM/reaction)	0.2 µL	20 µL
Nuclease-free water	2.5 µL	250 µL

A larger volume of primer-probe-water mix can be prepared in advance, aliquoted in LightSafe microcentrifuge tubes, and stored at -20°C.

12 Bring the processed samples and the PCR mastermix plate to a biosafety cabinet.

Most institutions will require samples potentially containing full-length SARS-CoV-2 RNA to be handled in a biosafety cabinet. Please seek guidance from your local biosafety office on specific recommendations for working with samples which could contain full-length SARS-CoV-2 RNA.

13 Select the appropriate option below if you are using 96 well plates or 384 well plates.
Step 13 includes a Step case.
96 Well format
384 Well format

step case

96 Well format

14 Prepare a master mix from one of the approved vendors containing the following (account for 10% extra lost during pipetting), excluding processed saliva samples or control

A	B	C	D
Component	**NEB Luna (2x)**	**Bio-Rad Reliance**	**Thermo TaqPath**
Master mix	10 µL	5 µL	5 µL
RT	1 µL	-	-
Primer-probe- water mix (see **Step 11.1**)	4 µL	4 µL	4 µL
Nuclease-free water	-	6 µL	6 µL
Treated sample or control	5 µL (do not add to master mix)	5 µL (do not add to master mix)	5 µL (do not add to master mix)

15 Place the 96-well PCR plate on the PCR cooler and add 15 µL of mastermix to each designated well.

Citation: Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie (03/24/2021). SalivaDirectÃ¢ÂÂ¢: RNA extraction-free SARS-CoV-2 diagnostics. https://dx.doi.org/10.17504/protocols.io.btdnni5e

16 Add 5 µL of processed saliva sample (including the processed negative extraction control) to each designated well. Mix by pipetting (avoid bubbles).

Extraction-free saliva samples can be added using a multichannel pipette for high-throughput testing.

17 Add 5 µL of positive control (Twist synthetic SARS-CoV-2 RNA controls at 100 copies/uL) and no-template control (NTC - water) to designated PCR wells for the controls (1 NTC, and 2 positive controls per plate). Mix by pipetting, taking care to avoid introducing bubbles.

18 Seal with a transparent plastic qPCR seal. Centrifuge briefly to remove bubbles, if present.

19 **For the Agilent AriaMX**: After loading the plate in the thermocycler, place a compression pad securely on the top of the plate and assure the position is aligned with the plate. On the AriaMX screen select “graphical displays” and then adjust or confirm that the Cycle Range for Background Based Threshold is: “8 thru 12”.

20 Load the plate into the qPCR machine, and run the following thermocycler conditions on standard mode:

Step	Temperature	Time
1	52°C	10 min
2	95°C	2 min
3	95°C	10 sec
4	55°C	30 sec
5	Read plate (FAM & Cy5 channels)	
Repeat steps 3-5 for 44 cycles.		

Real-time PCR (qPCR) instruments currently locally validated: Bio-Rad CFX96, ABI 7500 Fast, and ABI 7500 Fast Dx. Validation data, including demonstrated compatibility between kits and thermocyclers, are available here.

For the ABI 7500 Fast Dx the threshold should be manually set to 100,000 for N1 and to 10,000 for RP. For other PCR instruments this will be automatically determined.

21 Report results per the following criteria:

For Bio-Rad CFX96 Touch, ABI 7500 Fast, ABI 7500 Fast Dx and ABI QuantStudio 5

A	B	C
Result	**Ct value N1**	**Ct value RP**
Positive	<40.0	Any value
Negative	≥40.0	<35.0
*Invalid	≥40.0	≥35.0

*Invalid test results will be repeated by retesting the primary specimen from the beginning of the protocol. Results from retested samples will follow the same interpretation as listed in the table above.

For ABI QuantStudio 6 and ABI Quant Studio 7 Pro

Citation: Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie (03/24/2021). SalivaDirectÃ¢ÂÂ¢: RNA extraction-free SARS-CoV-2 diagnostics. https://dx.doi.org/10.17504/protocols.io.btdnni5e

A	B	C
Result	**Ct value N1**	**Ct value RP**
Positive	<37.0	Any value
Negative	≥37.0	<35.0
*Invalid	≥37.0	≥35.0

*Invalid test results will be repeated by retesting the primary specimen from the beginning of the protocol. Results from retested samples will follow the same interpretation as listed in the table above.

For Agilent AriaMX

A	B	C
Result	**Cq*** value N1**	**Ct value RP**
Positive	<34.0	Any Value
Negative	≥36.0	<30.0
**Inconclusive	≥34.0 - <36.0	<30.0
*Invalid	≥34.0	≥30.0

*Invalid test results will be repeated by retesting the primary specimen from the beginning of the protocol. Results from retested samples will follow the same interpretation as listed in the table above.

**When the Cq value for RP is <30 and the Cq is in the range of ≥34.0 - <36.0 for N1, the sample will be retested from the beginning of the protocol to potentially convert an inconclusive result to a confirmed negative or positive, if desired by the requesting healthcare provider. Results from retested samples will follow the same interpretation as listed in the table above.

***Cq values are qualified cycle thresholds in the Agilent AriaMX system and can be interpreted synonymously to Ct values.

Citation: Chantal Vogels, Orchid M Allicock, Doug E. Brackney, Chaney C Kalinich, Isabel M Ott, Nathan Grubaugh, Anne L Wyllie (03/24/2021). SalivaDirectÃ¢ÂÂ¢: RNA extraction-free SARS-CoV-2 diagnostics. https://dx.doi.org/10.17504/protocols.io.btdnni5e