Erba MDx SARS-CoV-2 RT-PCR Kit

Cat. No.: PCR00001 50 reactions

Instructions for Use
Store at 15–25 °C

IVD

INTENDED USE

Erba MDx SARS-CoV-2 RT-PCR Kit is intended for the qualitative detection of nucleic acid from SARS--CoV-2 in samples collected from individuals, including those with a clinical suspicion of COVID-19, and for screening those without signs, symptoms or other reasons to suspect COVID-19 infection. Results are for the detection of SARS-CoV-2 RNA in nasopharyngeal (NP), oropharyngeal (OP) swab and saliva as an aid to clinical diagnosis.

Positive results are indicative of SARS-CoV-2 RNA detection but may not represent the presence of transmissible virus. Positive results do not rule out bacterial infections or co-infection with other viruses. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The kit is also intended for the qualitative detection of nucleic acid from SARS-CoV- 2 in pooled NP/OP swab samples. These pools may be created from up to five separate viral transport media (VTM) samples pooled into a single container.

Negative results from pooled samples should be treated as presumptive and, if inconsistent with clinical signs and symptoms, or the results are necessary for individual patient management, the unpooled samples must also be tested individually. Avoid including specimens in pools that have been previously confirmed as PCR positive, or presumptive PCR positive. Pooled samples testing positive must be re-tested individually prior to reporting an individual patient result. Specimens with low SARS-CoV-2 RNA concentrations may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Erba MDx SARS-CoV-2 RT-PCR Kit is intended for use by personnel specifically instructed and trained in the techniques of real-time PCR and/or *in vitro* diagnostic procedures, operating in an appropriate laboratory setting.

PRINCIPLES OF THE PROCEDURE

The Erba MDx SARS-CoV-2 RT-PCR Kit selectively amplifies two SARS-CoV-2 gene targets present in the purified nucleic acid sample, using primers selective for the N1 nucleocapsid (N) and the RNA dependent RNA polymerase (RdRp) gene regions of SARS-CoV-2 virus. A third primer set targets the RNase P (RP) of human genomic DNA naturally present in the sample as an endogenous Internal (extraction) Control.
During the amplification process, fluorescent probes specific for the N1, RdRp and the human RP hybridize to their specific gene target sites, resulting in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase. This results in separation of a reporter fluorophore from its quencher, generating a fluorescent signal. With each PCR cycle, the cumulative signal increases. Each target uses a characteristic fluorophore, detected at distinct wavelengths, allowing simultaneous detection and discrimination of the amplified SARS-CoV-2 targets, and the Internal Control.

MATERIALS PROVIDED

Reagent	Description	Volume (µL)	Qty
SARS-CoV-2 RT-PCR Mix	Lyophilized RT-PCR mastermix containing all reagents necessary for amplification.	N/A	1 vial (50 reactions)
SARS-CoV-2 Dilution Buffer	Used to resuspend the RT-PCR Mix, provide negative controls, and resuspend the Positive Control	800 µL	1 tube, marked blue
SARS-CoV-2 Positive Control	A foil pouch containing one vial of dried Positive Control, a synthetic RNA molecule incorporating both N1 and RdRp specific SARS-CoV-2 RNA targets	N/A (10 control reactions)	1 tube, marked red

Store the kit at 15–25 °C in a dark place.
Do not use expired reagents, or if the packed reagents are visibly damaged.
Preferably, the kit should be used immediately following rehydration, or stored refrigerated for a duration not exceeding 6 hours. Kit components can be aliquoted and stored frozen for up to 1 month at -20°C. Once thawed, do not re-freeze.

MATERIALS/EQUIPMENT REQUIRED (BUT NOT PROVIDED)

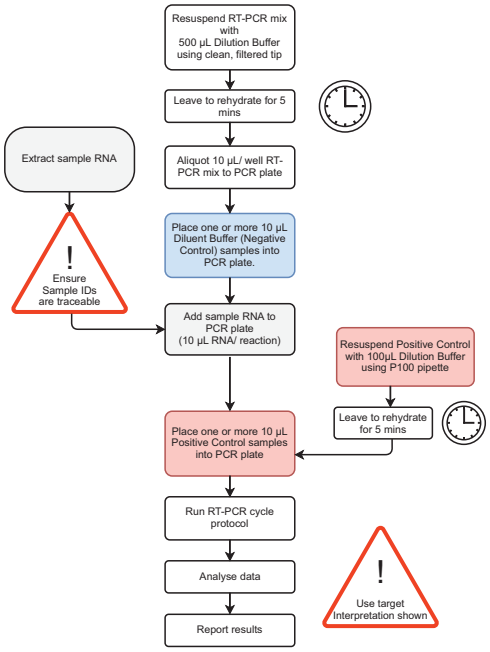
Nucleic acid extraction

Product	Cat. No.	Manufacturer
QIAamp® Viral RNA Mini Kit	52904 or 52906	Qiagen
OR		
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	A42352 or A48310	ThermoFisher Scientific
Zybio Nucleic Acid Extraction Kit	A-200 series T-200 series	Zybio Inc
Macherey-Nagel NucleoMag® Dx Pathogen kit	744215.4	Macherey-Nagel
KingFisher™ Duo Prime system KingFisher™ Flex system	5400110 5400630	ThermoFisher Scientific
OR		
MagCore® triXact RNA Kit	MRX-01 or MRX-03	RBC Bioscience
MagCore® Plus II Automated Nucleic Acid Extractor	MCA1605	RBC Bioscience

General equipment

Product	Cat. No.	Manufacturer
Calibrated micropipettes (P20, P200, P1000)	-	-
Aerosol resistant barrier pipette tips	-	-
Compatible 0.2 mL PCR reaction plates (or tubes)	-	-
Compatible PCR plate sealing films (or tube caps)	-	-
Centrifuge (saliva specimens)	-	-
One of the following PCR instruments:		
Applied Biosystems 7500, 7500 Fast or 7500 Fast Dx Real Time PCR instrument. PC running SDS software version 1.4 or later.	4351104 4406984 4406985	ThermoFisher Scientific
CFX96 / CFX96 Touch / CFX Opus	-	BioRad
Stratagene MX3000/MX3005P	-	Agilent
BMS Mic Real Time PCR cycler	-	Bio Molecular Systems
Qiagen Rotor-Gene Q 5plex / 5plex HRM	9001570 9001580	Qiagen
QuantStudio™ Real-Time PCR systems: 1, 3, 5, 6 pro, 7 pro	A40426 A28567 A28139 A44288 A43162	ThermoFisher Scientific
LightCycler® 480 Instrument 96-well LightCycler® 480 Instrument II 96-well	04 640 268 001 05 015 278 001	Roche

PROCEDURE



WARNINGS AND PRECAUTIONS

Only professional laboratory personnel proficient in handling infectious materials and trained to perform RT-PCR should perform this procedure.
Take care to confirm PCR instrument cycling conditions, detection channels and data analysis settings are correctly applied. Where necessary, PCR instruments must be calibrated (colour compensation) to distinguish between HEX (RdRp) and ROX (internal control) signals. Refer to PCR instrument user manuals for guidance.
Performance characteristics have been determined with RNA extractions from artificialy spiked saliva, human nasopharyngeal (NP) or oropharyngeal (OP) swab samples stored in appropriate transport medium, from patient samples with signs and symptoms of respiratory infection. Positive results are only indicative of the presence of SARS-CoV-2 RNA. Some positive NP/OP swab VTM samples may not be detected when diluted and tested in pools.

As with any diagnostic test procedure, good laboratory practice is essential to ensure its correct performance. Due to the high sensitivity of this test, care should be taken to store and handle reagents and materials correctly, and free of sources of contamination. The following guidance should be followed:

1. Wear powder-free laboratory gloves, protective laboratory coats, and eye protection during all processing steps.
2. All patient samples should be handled as potentially infectious, using good laboratory procedures as described in CLSI Document M29-A4 or local biosafety regulations.
3. If sample spillage occurs, immediately disinfect with 0.5% sodium hypochlorite (dilute household bleach 1:10) or follow appropriate local procedures.
4. Wash hands thoroughly after handling samples and reagents.
5. Always consult user manuals and IFUs, use only manufacturer specified PCR consumables as deviations may affect test performance and result validity.

Hazards identification in accordance with Regulation (EC) No 1272/2008

Dilution Buffer:
SARS-CoV-2 Dilution Buffer
UFI: 6CGW-HW0N-KJ51-TGAT

Warning

Contains: 2-Methyl-2H-isothiazol-3-one
H317 May cause an allergic skin reaction.
P261 Avoid breathing vapours.
P280 Wear protective gloves/protective clothing.
P302 + P352 IF ON SKIN: Wash with plenty of water and soap.
P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

Safety Data Sheets (SDS) are available from your local customer support representative.

SPECIMEN COLLECTION, HANDLING, AND STORAGE

Samples should only be collected by an appropriately trained clinician. Inappropriate specimen collection, pooling sizes greater than 5, storage, and transport are likely to yield false test results. Follow specimen collection device manufacturer instructions for proper collection methods.
For nasopharyngeal (NP) or oropharyngeal (OP) samples, use only nylon or polyester (Dacron®) tipped NP/OP swabs. Calcium alginate swabs are not acceptable. Cotton swabs are not recommended.
Place swabs immediately into sterile tubes containing 1–3 mL of VTM.
Saliva samples of around 1ml collected in a sterile container are suitable.
Samples may be stored at 2–8 °C and tested within 72 hours post-collection.
Fresh pipette tips and pooling containers must be used whenever handling individual samples/pools, in order to avoid cross-contamination between samples.

Swab specimen pooling guidelines

When performing swab VTM specimen pooling, consider a pooling strategy based on the last 7-10 day COVID-19 percent positivity rate ($P_{individual}$) in the testing population. The Erba MDx SARS-CoV-2 RT-PCR Kit has been validated for pool sizes of 5 samples per pool, although pools of 4, 3 and 2 specimens are also suitable. Based on a Dorfman pooling strategy, guideline maximum pooling efficiency values are:

$P_{individual}$*	Recommend sample pool size	Efficiency of sample pooling
5–6%	5	2.35–2.15
7–12%	4	1.99–1.54
13–25%**	3	1.48–1.10

*Percent positive individuals within the testing population over previous 7–10 days.

**Where $P_{individual}$ is >25%, efficiency is reduced to where individual testing should be considered.

When pooling VTM swab samples, identify a uniquely labelled tube and maintain traceability to

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the individual sample identifiers (where available). When pooling VTMs, ensure that the volume ratio of each specimen is kept consistent, and the minimum total pooled volume available is >200 µL (a minimum of 200 µL is required per nucleic acid extraction). For guidance:

Pool size	Recommended volume of individual specimen (µL)	Total pooled volume (µL)	Minimum total pooled volume required for nucleic acid extraction
5	50	250	200
4	60	240	200
3	80	240	200
2	120	240	200

Until pooling test results are known, ensure that the original unpooled samples are retained and stored at 2–8°C for subsequent individual retesting as required.

NUCLEIC ACID EXTRACTION

Performance of the Erba MDx SARS-CoV-2 RT-PCR Kit has been validated for use with the kits/ equipment listed above. Follow the manufacturers documented procedures with the specific recommendations for use below.

Nucleic acid extraction kit	NP/OP Swab		Saliva supernatant	
	Sample Volume (µL)	Elution volume (µL)	Sample Volume (µL)	Elution volume (µL)
QIAamp® Viral RNA Mini Kit	200	60	200	60
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	200	60	200	60
MagCore® triXact RNA Kit	200	60	200	60
Zybio Nucleic Acid Extraction Kit	200	50	200	50
Macherey-Nagel NucleoMag® Dx Pathogen kit	200	100	200	100

REAGENT PREPARATION

The Erba MDx kit should only be opened immediately prior to use. Do NOT open the positive control pouch until all the PCR plate set-up steps have been completed.
1. Tap-down or centrifuge the blue Dilution Buffer vial briefly, to collect liquid at the bottom.
2. Resuspend the glass vial of dried RT-PCR mastermix using 500 µL of Dilution Buffer.
3. Allow to rehydrate for 5 min at room temperature.
4. Mix the reagent thoroughly by pipetting. Avoid foaming.

Following rehydration, the kit should preferably be used immediately, or aliquoted and frozen at -20°C for up to 1 month.
Rehydrated kit components may be stored at 2–8°C for a maximum of 6 hours prior to use but must then be frozen or discarded. Sensitivity of the test will decline if stored for longer periods.
Suggested frozen aliquot sizes are: ≥100µl SARS-CoV-2 RT-PCR mastermix, ≥20µl Positive Control. Once thawed, aliquots should be used immediately and not refrozen. Sensitivity of the test will decline if subjected to multiple freeze-thaw cycles.

RT-PCR REACTION SET UP

PCR plate/tube set-up will vary depending on the number of specimens being analysed. Each kit has sufficient mastermix for 50x reactions (20 µL final reaction volume). At least 1 negative control (NTC) and Positive Control (PC) reaction should be included in each PCR run.
1. Dispense the RT-PCR reaction mix to a PCR plate (or PCR tubes), 10 µL per well, for the total number of reactions required.
2. For negative controls, add 10 µL of Dilution Buffer to any chosen **NTC well.**

RNA SAMPLE ADDITION

To avoid carry over contamination, pipette tips must be changed between samples, avoid foaming when mixing.
1. Pipette mix a purified RNA sample, then add 10 µL to a PCR well.
2. Pipette mix the completed reaction 3 times.
3. Change pipette tip.
4. Repeat for all RNA samples.

Positive Control Preparation
1. Open Positive Control Pouch.
2. Open the red Positive Control vial and add 100 µL of Dilution Buffer.
3. Allow to rehydrate for 5 min.
4. Mix the Positive Control briefly by pipetting. Avoid foaming.
5. Add 10 µL of Positive Control to any chosen **PC well.**

Seal the PCR plate/tubes with sealing film or caps, and transfer to the PCR instrument.

PROGRAMMING THE PCR INSTRUMEN

Refer to instrument user manual for information on the real time PCR instrument set-up.
Note: PCR cycling conditions between instruments may vary, check the instrument model and cycling parameters carefully before use.

Block-based cyclers (ABI7500, Bio-Rad CFX, Stratagene, QuantStudio, LightCycler480)
For ABI7500 only, set passive reference to none. A ROX passive reference is not required.

1. Enter the following amplification program.

Run Method		
Steps	**Temp (°C)***	**Time**
Holding_ RT 1 cycle	45	10 min
	95	3 min
Cycling 45 cycles	95	15 sec
	60	30 sec**

Detection parameters		
Target Name	**Reporter**	**Quencher (ABI only)**
SARS-CoV-2 **N1** gene	**FAM**	NFQ-MGB
SARS-CoV-2 **RdRp** gene	**HEX (JOE / VIC / Yellow 555)**	NFQ-MGB
Human **RNaseP** Internal Control	**ROX**	NFQ-MGB

** Use default (100%) temperature ramping rate for all cycling steps.*
***Fluorescence acquisition must be performed at the end of this phase.*
ǂ HEX is the preferred detection channel for RdRp detection, however calibrated channels for VIC, JOE or Yellow 555 (Lightcycler) should produce outcomes within 0.1Ct and should therefore be suitable. LightCycler 480 Instrument II must have a universal colour compensation file applied containing calibration data for the VIC/HEX/Yellow555 channel, or a custom calibration must be performed specific for HEX, see Roche LightCycler 480 user manual for instructions. Failure to apply this calibration will prevent correct interpretation of the RNaseP control in the presence of an RdRp positive signal.

2. Insert the PCR plate into the instrument and select **Start Run**.

Rotary cyclers (BMS Mic, Qiagen Rotor-Gene Q)

1. Enter the following amplification program.

Run Method		
Steps	**Temp (°C)***	**Time**
Holding_RT 1 cycle	45	10 min
	95	3 min
Cycling 45 cycles	95	15 sec
	64	30 sec**

Detection parameters		
Target Name	**Reporter**	**Quencher (ABI only)**
SARS-CoV-2 **N1** gene	**FAM**	Green (470/510nm)
SARS-CoV-2 **RdRp** gene	**HEX**	Yellow (530/555nm)
Human **RNaseP** Internal Control	**ROX**	Orange (585/610nm)

** Use default (100%) temperature ramping rate for all cycling steps.*
***Fluorescence acquisition must be performed at the end of this phase.*

2. Insert the PCR plate into the instrument and select **Start Run**.

INTERPRETATION OF RESULTS AND REPORTING

Follow instrument specific guidance on correctly assigning Ct values. All amplification data should be manually inspected for all samples to verify the presence or absence of SARS-CoV-2 positive amplification characteristics.

The following information provides guidance for correct interpretation of Erba MDx SARS-CoV-2 RT--PCR Kit data on the ABI7500 series instruments.

Setting threshold values and baseline to find amplifications (ABI7500)
For ease of manual data inspection on ABI7500, always compare a suspected SARS-CoV-2 positive amplification curve, against a known negative control (NTC) well.

1. After the PCR run has completed, select the Analysis window.
2. Select the Amplification Plot tab to view raw data.
3. Highlight all the wells from the run in the View Plate layout tab. Amplification data from all samples and detection channels should appear on the Amplification Plot graph.
4. Select Target (N1) from the Options panel. Ensure Show Threshold and Auto Baseline are both ticked.

Note: each detection channel must be analysed individually to reflect the amplification profile of each gene target: Target 1 = N1 (FAM-channel), Target = RdRp (JOE/VIC-channel), Target 3 = RNaseP (ROX-channel).

5. Under Plot Settings select Plot Type = ΔRn vs Cycle, graph type = Log, colour = Target.
6. To set the threshold, drag the threshold bar to within the exponential phase and above any background signals.
7. Click the Reanalyze button in the upper right corner of the window. Amplification curves and Ct values (presented on the right-hand pane) must be manually inspected for every sample.
8. Repeat for all targets, setting the threshold for RdRp and RNaseP detection channels as above and inspecting the data for all samples.
9. For all gene targets, a PCR amplification is positive if Ct ≤40.
10. Save the analysis file using Save As from the main menu.

For other PCR systems (CFX96, MX300 etc), use the default automatic threshold assignment. Manually inspect the data for each sample, to confirm validity of the amplification plot and any Ct value calculation.

Assigning a test result to patient samples
Once a sample has been assigned a positive or negative amplification status in each of the 3 detection channels, the following interpretation of channel results should be used for assigning the SARS-CoV-2 status:

FAM N1 gene	HEX (or JOE / VIC / Yellow555) RdRp gene	ROX IC	Test Result
Positive (Ct ≤40)	Positive (Ct ≤40)	Any Positive or Negative	**SARS-CoV-2 POSITIVE**
Positive (Ct ≤40)	Negative		
Negative	Positive (Ct ≤40)		
Negative	Negative	Positive (Ct ≤40)	**SARS-CoV-2 NEGATIVE**
Negative	Negative	Negative	**INVALID – RETEST**

QUALITY CONTROL

Adhere to Good Laboratory Practice and any local/national clinical laboratory Quality Control requirements/ guidelines. Always include Positive and Negative control reactions during each PCR run.

Internal Control (IC)
Human genomic DNA present in the clinical sample is used as an Internal Control to confirm successful extraction of nucleic acid, and PCR amplification – protecting against false negative results. Failure to detect any gene targets in any clinical specimen may be due to:
- Poor nucleic acid recovery or nucleic acid degradation.
- Insufficient recovery of cells during patient sample collection.
- Degradation of the clinical sample.
- Incompatible sample types.
- Reagent or equipment malfunction.

Negative Control (NTC)
NTC reactions (using 10 µL of the Dilution Buffer in place of a RNA sample) should not generate positive amplification curve motifs in either the FAM (N1) or JOE/VIC (RdRp) channel. If NTC reactions show positive amplification curve characteristics with a Ct ≤40, carryover contamination may have occurred. Invalidate the run and re-test all samples.

Positive Control (PC)
The Positive Control contains synthetic SARS-CoV-2 RNA expected to yield a positive detection in both N1 (FAM) and RdRp (HEX/JOE/VICP/Yellow555) detection channels, but none in the IC (ROX) channel.

Expected Performance of External Controls Included in the kit

Control Type	Used to Monitor	SARS-CoV-2 N1 gene	SARS-CoV-2 RdRp gene	Human RNaseP gene (IC)
Positive Control	Reagent or process failure	Positive (Ct ≤40)	Positive (Ct ≤40)	Negative (Ct >40)
Negative Control	Carryover contamination	Negative (Ct >40)	Negative (Ct >40)	Negative (Ct >40)

A positive result from the IC in an NTC does **not** invalidate the run.
If positive and negative controls do not exhibit the expected performance, retest all samples.

LIMITATIONS

- Use only supported clinical sample types and manufacturer's recommended consumables.
- Negative results do not preclude SARS-CoV-2 infection.
- Test results should not be used as the sole basis for patient management or treatment decisions.
- Failure to collect, store or test clinical samples correctly may invalidate the test result.

PERFORMANCE CHARACTERISTICS

Analytical LoD (RT-PCR only)
An estimate of the analytical LoD of the N1 and RdRp targets in the RT-PCR assay was conducted on ABI7500 using molecular grade water spiked with dilutions of Droplet Digital PCR (ddPCR™) quantified SARS-CoV-2 genomic RNA[1]. A dilution series to indicate the LoD was performed on a single kit lot

2027501:

Copy level/rxn	N1 LoD confirmation		RdRp LoD confirmation	
	Reps detected	(%) detection	Reps detected	(%) detection
40	24/24	100	24/24	100
20	44/44	100	41/44	93.2
10	44/44	100	38/44	86.4
5	43/44	97.8	19/44	43.2
2.5	19/20	95	9/20	45

This LoD was confirmed for three separate kit lots:

Lot Number		2027501			2025501			2024701		
Target	cps/rxn	Reps positive		Mean Ct	Reps positive		Mean Ct	Reps positive		Mean Ct
N1	**5**	32/32	100%	34.4	31/32	96.9%	35.9	32/32	100%	35.3
RdRp	**30**	32/32	100%	36.3	32/32	100%	36.3	32/32	100%	36.4

Analytical LoD was also confirmed on the MX3000, CFX96, BMS Mic and Rotor-Gene Q instruments.

LIMIT OF DETECTION (NUCLEIC EXTRACTION AND RT-PCR)

Nasopharyngeal (NP) swab

Inactivated SARS-CoV-2 virus diluted in VTM at the LoD, was spiked into retrospective (frozen) nasopharyngeal specimens previously tested PCR negative for COVID-19. LoD values were confirmed with these samples by spiking at 200 and 450 copies/ml. The samples were extracted using the QIAamp® Viral RNA Mini Kit. These specimens were then detected using the ABI 7500 according to the method described above.

	N1			RdRp		
Virus cps/ml	**Detection**	**(%) positivity**	**Mean Ct**	**Detection**	**(%) positivity**	**Mean Ct**
200	20 of 20	100	33.70	N/A	N/A	N/A
450	N/A	N/A	N/A	20 of 20	100	36.30

NP swab LoD was confirmed at 200 virus copies/ml (N1 target), 450 copies/ml (RdRp) in nasopharyngeal specimens when extracted using the QIAamp® Viral RNA Mini Kit. Performance of the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, Zybio kit, RBC Bioscience MagCore® triXact RNA Kit, and Macherey-Nagel NucleoMag® Dx Pathogen kit were then subsequently validated and gave an equivalent analytical LoD performance.

Oropharyngeal (OP) swab

Inactivated SARS-CoV-2 virus diluted in VTM at the LoD, was spiked into retrospective (frozen) oropharyngeal specimens previously testing PCR negative for COVID-19. The samples were extracted using the QIAamp® Viral RNA Mini Kit. These specimens were detected using the ABI 7500 as above.

	N1			RdRp		
Virus cps/ml	**Detection**	**(%) positivity**	**Mean Ct**	**Detection**	**(%) positivity**	**Mean Ct**
200	20 of 20	100	35.21	N/A	N/A	N/A
400	N/A	N/A	N/A	17 of 20	85	37.39
450	N/A	N/A	N/A	19 of 20	95	36.66

OP swab LoD was confirmed at 200 virus copies/ml (N1 target), 450 copies/ml (RdRp) in oropharyngeal specimens when extracted using the QIAamp® Viral RNA Mini Kit. Performance of the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, Zybio kit, RBC Bioscience MagCore® triXact RNA Kit, and Macherey-Nagel NucleoMag® Dx Pathogen kit were then subsequently validated and gave an equivalent analytical LoD performance.

Saliva specimens

Inactivated SARS-CoV-2 virus in VTM at the LoD was spiked into saliva specimens from healthy individuals testing negative for SARS-CoV-2.
The sample was centrifuged, the supernatant spiked with inactivated virus and extracted using the Qiagen QIAamp® Viral RNA Mini Kit. These specimens were then detected using ABI 7500 as above.

	N1			RdRp		
Virus cps/ml	**Detection**	**(%) positivity**	**Mean Ct**	**Detection**	**(%) positivity**	**Mean Ct**
200	20 of 20	100	36.18	N/A	N/A	N/A
400	N/A	N/A	N/A	18 of 20	90	37.46
450	N/A	N/A	N/A	18 of 20	90	37.18
500	N/A	N/A	N/A	19 of 20	95	37.78

The LoD was confirmed at 200 virus copies/ml (N1 target), 500 copies/ml (RdRp) in saliva supernatant specimens when extracted using the QIAamp® Viral RNA Mini Kit. Performance of the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, Zybio kit, RBC Bioscience MagCore® triXact RNA Kit, and Macherey-Nagel NucleoMag® Dx Pathogen kit were then subsequently validated and gave an equivalent analytical LoD performance.

INCLUSIVITY

The primers used were compared to published SARS-CoV-2 sequences available in the public domain in GenBank® as of 24th Sep 2020.
A search was conducted on the NCBI virus database[2] for complete SARS-CoV-2 genomes, between 28,000 and 35,000 bases long. 16811 sequences were identified. 16808 sequences had 100 (%) identity and 100 (%) base coverage in comparison to the reference sequence for at least one target. Three sequences did not 100% match in either target. Their mismatches were analysed (method from Stadhouders, Ralph et al. 2010[3]) and found to have sufficient affinity to be likely to be detected.

A further search for sequence alignment of oligonucleotide primer and probes contained within the Erba MDx SARS-CoV-2 RT-PCR Kit was performed on using search tools based on GISEAD data from 25 Apr 2021. No strains of Covid at this time represented a challenge to the N1 or RdRp targets used in the Erba MDx SARS COV-2 RT-PCR Kit.

EXCLUSIVITY

The selected primers were compared in a BLAST search against sequences held on the on the NCBI database[4] of a selection of organisms and other coronaviruses that could be present in relevant samples. Human genomic DNA is demonstrably not detected as negative swabs with human DNA present return SARS-CoV-2 negative results.

Taxonomic ID for BLAST	Detection?
Adenoviridae (taxid:10508)	No
Bacillus anthracis (taxid:1392)	No
Bordetella pertussis (taxid:520)	No
Candida albicans (taxid:5476)	No
Chlamydia pneumoniae (taxid:83558)	No
Chlamydia psittaci (taxid:83554)	No
Corynebacterium diphtheriae (taxid:1717)	No
Coxiella burnetii (taxid:777)	No
Human enterovirus (taxid:1193974)	No
Haemophilus influenzae (taxid:727)	No
Human metapneumovirus (taxid:162145)	No
Influenza A virus (taxid:11320)	No
Influenza B virus (taxid:11520)	No
Legionella (taxid:445) Exclude L. pneumophila (taxid:446)	No
Legionella pneumophila (taxid:446)	No
Leptospira (taxid:171)	No
Moraxella catarrhalis (taxid:480)	No
Mycobacterium tuberculosis (taxid:1773)	No
Mycoplasma pneumoniae (taxid:2104)	No
Neisseria elongata (taxid:495)	No
Neisseria meningitidis (taxid:487)	No
Parainfluenza 1-3 (taxid:186938)	No
Human rubulavirus 4 (taxid:1979161)	No
Parechovirus (taxid:138954)	No
Pneumocystis jirovecii (taxid:42068)	No
Pseudomonas aeruginosa (taxid:287)	No
Human orthopneumovirus (taxid:11250)	No
Rhinoviruses (taxid:12059)	No
Staphylococcus aureus (taxid:1280)	No
Staphylococcus epidermidis (taxid:1282)	No
Streptococcus pyogenes (taxid:1314)	No
Streptococcus pneumoniae (taxid:1313)	No
Streptococcus salivarius (taxid:1304)	No
Human coronavirus 229E	No
Human coronavirus OC43	No
Human coronavirus HKU1	No
Human coronavirus NL63	No
SARS-coronavirus	No[5]
MERS-coronavirus	No

None of these organisms are expected to cross-react with the assay.

SYMBOLS USED

Symbol	Meaning	Symbol	Meaning
IVD	*In vitro* diagnostic medical device		Storage Temperature
LOT	Lot Number		See Instructions for Use
	Protect from Sunlight		Do not reuse
	Manufacturer	Σ	Content is sufficient for <n> tests
REF	Catalogue Number	CE	CE marking of conformity – IVD complies with the Directive 98/79/EC
	Expiry Date		Do not use if packaging damaged

CLINICAL PERFORMANCE EVALUATION

180 retrospective clinical specimens (-80 °C frozen samples, a combination of SARS-CoV-2 PCR positive and negative UTM or VTMs), were tested using a third-party CE-IVD/FDA EUA cleared RT-PCR kit at Erba Molecular (Ely, Cambridge, UK) as a predicate. All specimens were re-extracted using the QIAamp® Viral RNA Mini Kit according to the manufacturer instructions and re-tested using the third-party RT-PCR kit on ABI7500 according to manufacturer instructions. 31 specimens gave discordant results by the second third-party test compared with the original diagnostic result and were rejected from the study. 39 were confirmed as positive and 110 were confirmed negative specimens. These samples were then tested using the Erba MDx SARS-CoV-2 RT-PCR Kit. Clinical Performance was as follows:

		Specimen status	
		Positive	**Negative**
Erba MDx SARS-CoV-2 RT-PCR Kit result	**Positive**	39	0
	Negative	0	110
Positive Percent Agreement	100% (39/39) 90.9–100% C.I.[6]		
Negative Percent Agreement	100% (110/110) 96.7–100% C.I.		

CLINICAL PERFORMANCE USING N=5 POOLED VTM FROM NP SWABS

45 retrospective SARS-CoV-2 PCR positive and 35 negative NP clinical specimens (-80 °C frozen VTM samples) were tested unpooled, to verify the original patient diagnostic result using the Erba MDx SARS-CoV-2 RT-PCR Kit.
Of these, 30 negatives and 30 positives were selected for inclusion in the pooling study. In accordance with FDA pooling guidelines, 8 positive samples were selected on the basis of giving PCR Ct values within 2–4 cycles (≈1log$_{10}$) of the expected Erba MDx kit LoD, and 22 positive samples giving PCR Ct >4 cycles above LoD. The 30 positive samples were then blinded, and randomised with 30 negative NP samples and re-tested individually using a third-party CE-IVD/FDA EUA cleared RT-PCR kit against the Erba MDx SARS-CoV-2 RT-PCR Kit.

The blinded, and randomised samples were then n=5 pooled using additional negative NP clinical specimens as diluent (50 µL of blinded NP swab VTM added to 4x 50 µL of negative NP swab VTM). 200 µL of each sample extracted using the ThermoFisher MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit on the KingFisher Duo Prime instrument according to manufacturer instructions.

The resulting nucleic acid eluates were then tested using the Erba MDx SARS-CoV-2 RT-PCR Kit on the Bio-Rad CFX Opus 96 Real-Time PCR System, according to manufacturer instructions. Clinical performance of NP swab VTM pooling against unpooled specimens was as follows:

		Specimen status	
		Positive	**Negative**
Erba MDx SARS-CoV-2 RT-PCR Kit result	**Positive**	30	0
	Negative	0	30
Positive Percent Agreement	100% (30/30)		
Negative Percent Agreement	100% (30/30)		

INTERFERING SUBSTANCES

Saliva specimens collected from healthy individuals testing negative for SARS-CoV-2 were centrifuged, and the supernatant spiked with inactivated SARS-CoV-2 virus at the 3x LoD level, together with representative quantities of the interfering substances expected in salivary samples.

For simulated nasal matrix, inactivated SARS-CoV-2 virus spiked VTM was used (rather than saliva). SARS-CoV-2 negative specimens with the interfering substances were also ested to assess robustness of the internal process control (Human RNaseP target). All samples were extracted using the QIAamp® Viral RNA Mini Kit and detected using ABI 7500 according to the method described above.

Substance	Final conc.	Negative correct	3x LoD detected
Human whole blood	2% v/v	1/1	3/3
Albuterol sulfate	830 µg/ml	1/1	3/3
Ultra Chloraseptic spray	1% v/v	1/1	3/3
Mupirocin	10 mg/ml	1/1	3/3
NaCl (as in 0.65% nasal spray)	0.1% w/v	1/1	3/3
Vicks Sinex Soother	15% v/v	1/1	3/3
Phenylephrine	0.0075%	1/1	3/3
Zanamivir (GSK)	7.5 mg/ml	1/1	3/3
Tobramycin (Dextramethasone)	0.6 mg/ml	1/1	3/3
Fluticasone Propionate (Flovent etc)	5 µg/ml	1/1	3/3
Heel Luffeel nasal spray Bio Pathica	15% v/v	1/1	3/3
NeilMed Nasogel NeilMed	1% w/v	1/1	3/3
Nasacort spray	10% v/v	1/1	3/3
Mucin (bovine submaxillary glands)*	2.5% w/v	1/1	3/3
Simulated Nasal Matrix*	100%	1/1	3/3

*Simulated Nasal Matrix and 2.5% bovine mucin may have an inhibitory effect on RNA recovery when using this QIAamp® Viral RNA Mini Kit, this may result in a higher Ct values depending on sample type used.

DISPOSAL

Dispose of unused kit reagents, human specimens, and sealed post-amplification plates according to local clinical laboratory waste disposal routes.
Avoid autoclaving spent PCR plates.

1 Genomic RNA from SARS-Related Coronavirus 2, Isolate Italy-INMI1 BEI Resources NR-52498 Lot # 70035261

2 https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/

3 The effect of primer-template mismatches on the detection and quantification of nucleic acidsusing the 5' nuclease assay.
The Journal of Molecular Diagnostics: JMD vol. 12,1 (2010): 109-117.

4 https://blast.ncbi.nlm.nih.gov/

5 Corman, Victor M et al. "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR." Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin vol. 25,3 (2020): 2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045

6 Clopper-Pearson 5% level.