Quick SARS-CoV-2 Multiplex Kit

Catalog no. **R3013, R3013-1K, R3013-10K**

For simultaneous detection of the SARS-CoV-2 virus and a host internal control

Instructions for Use

For *in vitro* diagnostic (IVD) use

IVD

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Introduction

This package insert must be read carefully prior to use. Instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Intended Use

Quick SARS-CoV-2 Multiplex Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in upper respiratory specimens (such as nasal, nasopharyngeal, mid-turbinate or oropharyngeal swabs), and lower respiratory specimens (such as sputum, tracheal aspirates, and bronchoalveolar lavage) from patients suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper and lower respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

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 *Quick* SARS-CoV-2 Multiplex Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR assays.

Summary and Explanation of Test

The *Quick* SARS-CoV-2 Multiplex Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test on the Bio-Rad CFX96 or CFX384 Touch™ Real-Time PCR Detection System using the Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 software (or higher). The SARS-CoV-2 primer and probe set is designed to detect RNA from the SARS-CoV-2 N gene in upper and lower respiratory samples from patients who are suspected of COVID-19 by their healthcare provider.

Principles of the Procedure

Nucleic acid from patient samples is extracted and purified as described in the procedural steps. Selective amplification of target nucleic acid from the sample is achieved by reverse transcription of the SARS-CoV-2 RNA as well as the host specific RNase P RNA and subsequent PCR amplification using the target-specific forward and reverse primers.

The *Quick* SARS-CoV-2 Multiplex Kit detects one SARS-CoV-2 specific target sequence from the Nucleocapsid gene (N) of the virus and one host specific target sequence from the RNase P gene. The primers and probes used in the rRT-PCR assay are based on the CDC published primer and probe sequences with some modification. In addition to the RNase P gene, the test uses both a no template control which is taken through all procedural steps, including the extraction, and a positive control that monitor integrity of reagents and correct performance of the testing procedure.

Reagents and Materials

Materials Provided

Component Name	Description	Concentration	Component Cat #	Volume	Quantity	Kit Cat #
CV Positive Control	SARS-CoV-2 positive control	2,5 copies/µl of *in vitro* transcribed SARS-CoV-2 N gene fragment spiked into 1 ng/µl human cell RNA	R3011-3-100	100 µl	1	R3013
					3	R3013-1K
			R3011-3-3	3 ml	1	R3013-10K
2X CV Mix	Cocktail for one-step rRT-PCR detection of SARS-CoV-2 and RNase P. Includes: enzymes, dNTPs, $MgCl_2$, salts, additives, and the SARS-CoV-2 and RNase P primers and probes	2X	R3013-1-1	1 ml	1	R3013
					10	R3013-1K
			R3013-1-100	100 ml	1	R3013-10K
No Template Control (NTC)	Nuclease-free water	N/A	R3011-4-1	1 ml	3	R3013
					9	R3013-1K
			R3011-4-100	100 ml	1	R3013-10K

Note - Integrity of kit components are guaranteed for up to the claimed expiration date on the kit under proper storage conditions. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

Reagent Storage and Handling

The *Quick* SARS-CoV-2 Multiplex Kit is to be shipped on dry ice.

If received in a condition other than the label indicates, or that are damaged, contact Zymo Research Corp. directly.

Upon receipt, all components of the kit should be stored at ≤ -20°C

Store at -20°C for 30 days or for longer storage, store at ≤ -70°C.

Reagents are stable for up to five (5) freeze-thaw cycles.

Reagents and Materials Required (NOT provided):

Product Name	Catalog No.	Manufacturer
CFX96 Touch™ Real-Time PCR Detection System (with optics capable of detecting HEX and Quasar 670) with CFX Maestro software	1855195	Bio-Rad
CFX384 Touch™ Real-Time PCR Detection System (with optics capable of detecting HEX and Quasar 670) with CFX Maestro software	1855485	Bio-Rad
Microcentrifuge	Non-specific	Non-specific
Mini Plate Spinner	Non-specific	Non-specific
Hard Shell PCR Plate, 96-well, thin wall	HSP9601	Bio-Rad
Hard Shell PCR Plate, 384-well, thin wall	HSP3805	Bio-Rad
Microseal 'B' seal	MSB1001	Bio-Rad
Aerosol barrier pipette tips (Nuclease-Free)	Non-specific	Non-specific
Micropipettes (2, 10, 200, 1000 µl)	Non-specific	Non-specific
Vortex mixer	Non-specific	Non-specific
Freezer (≤ -70°C)	Non-specific	Non-specific
Disposable gloves, powder-free	Non-specific	Non-specific

General Laboratory Warnings and Precautions

This assay is for *in vitro* diagnostic use.

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of soap and water (i.e., 20% aqueous solution of Sodium Dodecyl Sulfate disinfectant (SDS))
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.

Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet. Safety Data Sheets are available from Zymo Research Corp. Inquire directly.

Special Precautions
As with any test procedure, good laboratory practice is essential to the proper performance of this test. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

Only personnel proficient in handling infectious materials and the use of the *Quick* SARS-CoV-2 Multiplex Kit and the Bio-Rad CFX96 or CFX384 Touch™ Real-Time PCR Detection System together with the Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 (or higher) software should perform this procedure.

Handling Precautions for Specimens
All patient samples should be handled as if infectious, using good laboratory procedures.

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality.

Compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of RNases into samples.

Amplification technologies such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the reagents used become contaminated by accidental introduction of even a few molecules of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the activities involved in performing PCR in compliance with good laboratory practices.

Work Areas
The use of 2 dedicated areas (Sample Preparation Area and Amplification Area) within the laboratory is recommended when performing the *Quick* SARS-CoV-2 Multiplex Kit.

All reagents used in the Sample Preparation Area should remain in the dedicated area at all times. Do not bring amplification product into the Sample Preparation Area.

The Amplification Area is dedicated to the amplification and detection of amplified product. Laboratory coats and equipment used in the Amplification Area must remain in this area and not be moved to the Sample Preparation Area.

Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, no template control, positive control, or specimens.

Decontaminate and dispose of all potentially biohazardous materials in accordance with local, national, and European regulations.

Prevention of Nucleic Acid Contamination
Nucleic acid contamination is minimized through:

- Reverse transcription, PCR amplification, and oligonucleotide hybridization occur in a sealed 96- or 384-Well PCR Plate.
- Detection is carried out automatically without the need to open the 96-Well Reaction Plate.
- Pipettes with aerosol barrier tips are used for all pipetting, which are discarded after use.
- Separate, dedicated areas are used to perform the *Quick* SARS-CoV-2 Multiplex Kit. Refer to the **Special Precautions** section of this package insert.

Procedure

Quick SARS-CoV-2 Multiplex Kit Procedure

This insert contains instructions for the *Quick* SARS-CoV-2 Multiplex Kit. The *Quick* SARS-CoV-2 Multiplex Kit has been validated using upper and lower respiratory tract specimens collected in DNA/RNA Shield™ (Zymo Research, cat. R1107-E, R1109-E, R1210-E). All samples were extracted using the *Quick*-DNA/RNA Viral Magbead (Zymo Research, cat. R2140-E, R2141-E) extraction kit by both manual and automated procedures. Automated extraction was performed on the KingFisher Flex Purification System (Thermo Fisher Scientific). Real-time RT-PCR was performed using the CFX96 and CFX384 Touch Real-Time PCR Detection Systems (Bio-Rad) with CFX Maestro™ 1.1 Version 4.1.2433.1219 software.

Deviation from the validated workflow described above may results in changes to the performance of the *Quick* SARS-CoV-2 Multiplex Kit.

Kit Protocol

Laboratory personnel should be trained to operate the Bio-Rad CFX96 or CFX384 Touch™ Real-Time PCR Detection Systems. Operators should have a thorough knowledge of the software run on the instrument/s and must follow good laboratory practices.

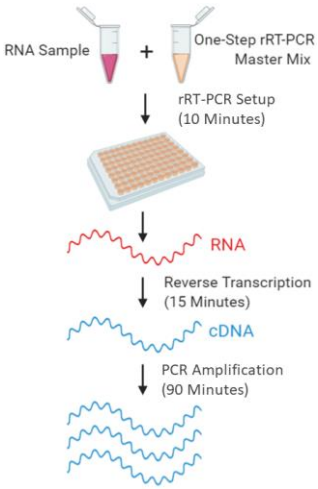
The Bio-Rad CFX96 or CFX384 Touch™ Real-Time PCR Detection Systems must be linked together with the Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 software and installed prior to performing the assay. For a detailed description of how to use the application refer to the software guide: https://www.bio-rad.com/en-us/product/cfx-maestro-software-for-cfx-real-time-pcr-instruments?ID=OKZP7E15

For a detailed description of how to use the Bio-Rad CFX96 or CFX384 Touch™ Real-Time PCR Detection System, refer to the instrument guide. (https://www.biorad.com/webroot/web/pdf/lsr/literature/10000068706.pdf).

Amplification Reaction Set-up Procedure

Overview:
The assay can be used with RNA extracted by both manual and automated protocols.

As illustrated in the workflow figure below, extracted RNA samples are mixed directly with the 2X CV Mix and analyzed using a real-time PCR system. Results are ready to be interpreted after the reverse transcription and real-time PCR steps are complete.



I. rRT-PCR Reaction Setup:

96-Well rRT-PCR Setup:

- ✓ Before starting, thaw frozen reagents on ice, mix 10 times by inversion, centrifuge briefly, and place back on ice.
- ✓ Avoid exposing the 2X CV Mix and reactions to direct light and keep the PCR plate on ice during preparation.
- ✓ To prevent contamination, aliquot the 2X CV Mix in the 96-well PCR plate before handling any test samples or controls.

1. Add 10 µl of **2X CV Mix** to each well.
2. Add 10 µl of every RNA sample to be tested except CV Positive Control and NTC.
3. Add 10 µl of **CV Positive Control** to one dedicated well in each plate.
4. Add 10 µl of extracted **No Template Control** (NTC) to one dedicated well in each plate.

Example plate layout for a Quick SARS-CoV-2 Multiplex Kit assay in 96-well format is illustrated below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	NTC
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	PC

S# = Test Sample
NTC = No Template Control
PC = CV Positive Control

5. Firmly seal the PCR plate with an optical sealing film.
6. Briefly vortex the PCR plate and centrifuge to eliminate bubbles and bring any droplets to the bottom of the well.
7. Place the PCR plate on ice.

384-Well rRT-PCR Setup:

- ✓ Before starting, thaw frozen reagents on ice, mix 10 times by inversion, centrifuge briefly, and place back on ice.
- ✓ Avoid exposing the 2X CV Mix and reactions to direct light and keep the PCR plate on ice during preparation.
- ✓ To prevent contamination, aliquot the 2X CV Mix in the 384-well PCR plate before handling any test samples or controls.

1. Add 5 µl of **2X CV Mix** to each well.
2. Add 5 µl of every RNA sample to be tested except CV Positive Control and NTC.
3. Add 5 µl of **CV Positive Control** to one dedicated well in each plate.
4. Add 5 µl of extracted **No Template Control** (NTC) to one dedicated well in each plate.

Example plate layout for a Quick SARS-CoV-2 Multiplex Kit assay in 384-well format is illustrated below:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	S1	S17	S33	S49	S65	S81	S97	S113	S129	S145	S161	S177	S193	S209	S225	S241	S257	S273	S289	S305	S321	S337	S353	S369
B	S2	S18	S34	S50	S66	S82	S98	S114	S130	S146	S162	S178	S194	S210	S226	S242	S258	S274	S290	S306	S322	S338	S354	S370
C	S3	S19	S35	S51	S67	S83	S99	S115	S131	S147	S163	S179	S195	S211	S227	S243	S259	S275	S291	S307	S323	S339	S355	S371
D	S4	S20	S36	S52	S68	S84	S100	S116	S132	S148	S164	S180	S196	S212	S228	S244	S260	S276	S292	S308	S324	S340	S356	S372
E	S5	S21	S37	S53	S69	S85	S101	S117	S133	S149	S165	S181	S197	S213	S229	S245	S261	S277	S293	S309	S325	S341	S357	S373
F	S6	S22	S38	S54	S70	S86	S102	S118	S134	S150	S166	S182	S198	S214	S230	S246	S262	S278	S294	S310	S326	S342	S358	S374
G	S7	S23	S39	S55	S71	S87	S103	S119	S135	S151	S167	S183	S199	S215	S231	S247	S263	S279	S295	S311	S327	S343	S359	S375
H	S8	S24	S40	S56	S72	S88	S104	S120	S136	S152	S168	S184	S200	S216	S232	S248	S264	S280	S296	S312	S328	S344	S360	S376
I	S9	S25	S41	S57	S73	S89	S105	S121	S137	S153	S169	S185	S201	S217	S233	S249	S265	S281	S297	S313	S329	S345	S361	S377
K	S10	S26	S42	S58	S74	S90	S106	S122	S138	S154	S170	S186	S202	S218	S234	S250	S266	S282	S298	S314	S330	S346	S362	S378
L	S11	S27	S43	S59	S75	S91	S107	S123	S139	S155	S171	S187	S203	S219	S235	S251	S267	S283	S299	S315	S331	S347	S363	S379
M	S12	S28	S44	S60	S76	S92	S108	S124	S140	S156	S172	S188	S204	S220	S236	S252	S268	S284	S300	S316	S332	S348	S364	S380
N	S13	S29	S45	S61	S77	S93	S109	S125	S141	S157	S173	S189	S205	S221	S237	S253	S269	S285	S301	S317	S333	S349	S365	S381
O	S14	S30	S46	S62	S78	S94	S110	S126	S142	S158	S174	S190	S206	S222	S238	S254	S270	S286	S302	S318	S334	S350	S366	S382
P	S15	S31	S47	S63	S79	S95	S111	S127	S143	S159	S175	S191	S207	S223	S239	S255	S271	S287	S303	S319	S335	S351	S367	NTC
Q	S16	S32	S48	S64	S80	S96	S112	S128	S144	S160	S176	S192	S208	S224	S240	S256	S272	S288	S304	S320	S336	S352	S368	PC

S# = Test Sample
NTC = No Template Control
PC = CV Positive Control

5. Firmly seal the PCR plate with an optical sealing film.
6. Briefly vortex the PCR plate and centrifuge to eliminate bubbles and bring any droplets to the bottom of the well.
7. Place the PCR plate on ice.

II. Real-Time PCR Instrument Setup

Steps of '**Real-Time PCR Machine Set Up**' are to be performed in an area designated specifically for amplification reactions in order to avoid contamination of the sample processing area.

1. Using the Real-Time PCR software (Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 software for the CFX96 or CFX384 Touch™ Real-Time PCR Detection System), create the following PCR protocol using the following parameters:

Step	Temperature	Time (min:sec)	
1	55 °C	15:00	
2	95 °C	10:00	
3	95 °C	0:05	45 Cycles (Steps 3-6)
4	72 °C	0:30	
5	57 °C	0:30	
6	**Plate Read**		
7	**END**		

2. Assign each well the corresponding sample names, targets, and fluorophores. 2X CV Mix contains two different fluorophores.

Target	Fluorophores	Ex.	Em.
SARS-CoV-2	HEX™	535 nm	556 nm
RNase P	Quasar® 670	647 nm	670 nm

3. Enable all filters required to detect both fluorophores.

4. Load the PCR plate into the CFX96 or CFX384 Touch™ Real-Time PCR Detection System (Bio-Rad) and start the PCR protocol

III. Data Analysis (Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 software)

The Data Analysis steps described below are for the CFX96 and CFX384 Touch™ Real-Time PCR Detection System (Bio-Rad) with the CFX Maestro Software 1.1 Version 4.1.2433.1219. Adjustments to the procedure may be necessary to perform the reaction using a different real-time PCR instrument and software.

1. Visually inspect the plate for any issues that occurred during PCR (e.g. evaporation) and take note of any problems.

2. Adjust the Baseline Threshold value to 50 RFU for each fluorophore. Under "Settings", click on "Baseline Threshold", select "User Defined" and enter 50 into the user defined field.

3. Under "Settings", click on "Baseline Setting", and select "Apply Fluorescence Drift Correction."

4. Note and record any samples, PC, or NTC with amplification in the HEX™ or Quasar® 670 channel before 10 cycles. This step is necessary to identify samples with Ct values < 10 which may be excluded by adjustments made in Step 5.

5. Exclude the first 10 cycles of the PCR by going to "Settings", clicking on "Cycles to Analyze". Manually enter the range as 10 to 45.

6. If aberrant amplification curves are observed, please refer to the Appendix on page 10 for examples and detailed explanations.

IV. Interpretation of Results

Guidelines for the Interpretation of Controls written in this section are based upon results obtained using a CFX96 or CFX384 Touch™ Real-Time PCR Detection System (Bio-Rad). Ct values provided in this section are representative and may vary between different real-time PCR instruments.

All controls should be examined prior to the interpretation of the test samples:

CV Positive Control (PC): The CV Positive Control will show amplification signals in both the HEX™ and Quasar® 670 channels, which detect the presence of SARS-CoV-2 and RNase P, respectively. Signals generated from the CV Positive Control are considered valid if the cycle threshold (Ct) value is ≤ 40 for the virus target (HEX™ channel) and ≤ 30 for RNase P (Quasar® 670 channel) when using the recommended systems settings. If signals for the CV Positive Control are detected after 40 amplification cycles (Ct > 40) for the virus target or after 30 amplification cycles (Ct > 30) for RNase P, the control must be replaced with a new aliquot. If this problem is not resolved, the whole kit must be replaced with a new one.

No Template Control (NTC): The No Template Control sample will show no amplification signal for either the virus target (HEX™ channel) or RNase P (Quasar 670® channel). The No Template Control sample will be considered valid if no amplification occurs in either the HEX™ or Quasar® 670 channels. If amplification occurs in either channel, contamination of extraction and/or rRT-PCR reagents may have occurred, and reagents must be replaced.

Sample	SARS-CoV-2 (HEX™)	RNase P (Quasar® 670)	Interpretation
No Template Control	No amplification	No amplification	Valid
CV Positive Control	Ct ≤ 40	Ct ≤ 30	Valid
Test Sample	Ct ≤ 40	Any amplification	Positive
	No amplification or Ct > 40	Ct < 40	Negative
	No amplification	No amplification or Ct ≥ 40	Invalid

Appendices

Examples of Normal and Aberrant Amplification Curves

Note that the first 10 cycles of PCR have been excluded from the graphs, the baseline threshold for both fluorophores have been adjusted to 50 RFU, and fluorescence drift correction has been applied as described on page 8-9.

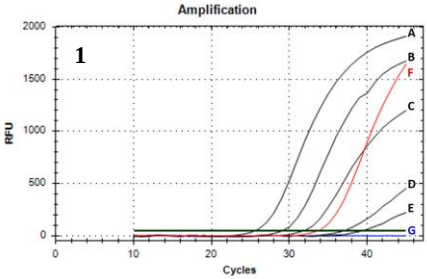


Figure 1. Curves A through E are examples of normal amplification curves for the SARS-CoV-2 target. Curve F (red) represents the SARS-CoV-2 signal in the CV Positive Control. Curve G (blue) represents the No Template Control.

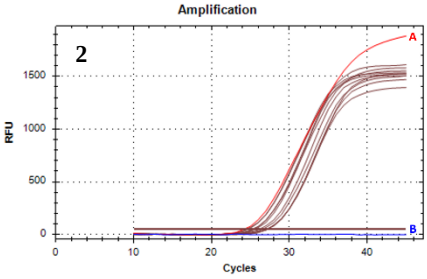


Figure 2. Examples of samples with normal amplification curves for the IC (RNase P) are shown in brown. The IC curve for the CV Positive Control is indicated in red (A) ant the No Template Control in blue (B).

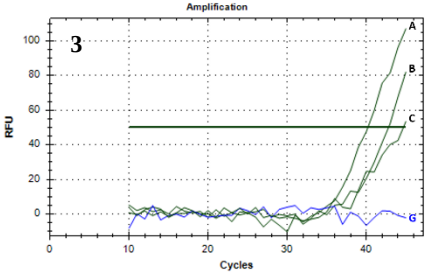


Figure 3. Curves A, B, and C are examples of weak signals for the SARS-CoV-2 target that would not be considered a positive test result (Ct > 40). For these samples, a repeat rRT-PCR may be performed to confirm the results. Note that the Y axis scale has been adjusted (magnified) to better visualize the difference between curves.

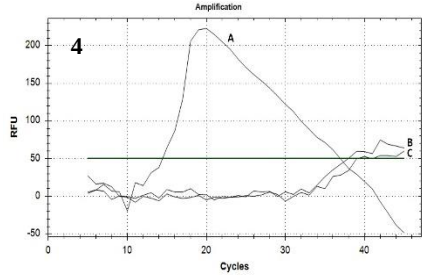


Figure 4. Curve A is an example of an aberrant amplification for the SARS-CoV-2 target. Curves B and C are examples of aberrant or weak positive amplification. In cases like these, results should be confirmed by repeat testing. The Y axis scale has been adjusted (magnified) to better visualize details of the curves.

Troubleshooting Guide

Problem	Possible Causes and Suggested Actions
Reduction in volume observed in the wells after rRT-PCR.	• **Cause:** Evaporation of rRT-PCR reaction mixture due to improper sealing of the PCR plate. • **Action:** Record the wells with reduced volume and proceed with normal analysis. Repeat rRT-PCR on samples experiencing evaporation if results are invalid.
Aberrant amplification after pre-analytical steps have been executed (page 8-9). Please see examples of aberrant and normal amplification curves on page 10.	• **Cause:** Presence of air bubbles in the reaction, poor quality sample, incorrect setup, or compromised reagents. • **Action:** Record the wells and fluorophores that show aberrant amplification. Samples that exhibit aberrant amplification should be repeated to determine a conclusive result. Re-extraction of the sample or use of new reagents may be necessary if re-testing the sample does not produce a clear signal.
SARS-Cov-2 signal in CV Positive Control detected after 40 Cycles.	• **Cause:** Incorrect rRT-PCR set-up or the *Quick* SARS-CoV-2 Multiplex Kit reagents may have been compromised (e.g. improper storage or more than 5 freeze-thaw cycles). • **Action:** Replace the control. If the problem persists, the entire kit should be replaced.
RNase P signal in CV Positive Control detected after 30 Cycles.	• **Cause:** Incorrect rRT-PCR setup or the *Quick* SARS-CoV-2 Multiplex Kit reagents may have been compromised (e.g. improper storage or more than 5 freeze-thaw cycles). • **Action:** Replace the control. If the problem persists, the entire kit should be replaced.
SARS-CoV-2 and/or RNase P amplification in the No Template Control (NTC)	• **Cause:** Contamination from the environment, contamination of extraction and/or rRT-PCR reagents, or well-to-well cross contamination. • **Action:** Clean and decontaminate all surfaces and instruments. Ensure that filter tips are used during the procedure and changed between samples. Ensure that extraction and rRT-PCR setup is properly executed. Repeat extraction and rRT-PCR with new reagents including all required controls.

Limitations of Procedures

Testing is limited to laboratories certified to perform high complexity tests.

Optimum performance of this kit requires upper and lower respiratory tract specimens collected in DNA/RNA Shield™ (Zymo Research, Cat. R1107, R1109, R1124, R1210), extracted using the *Quick*-DNA/RNA Viral MagBead Kit (Zymo Research, Cat. R2140, R2141), both manually or automated (using the KingFisher Flex; Thermo Fisher Scientific), and analyzed using the Bio-Rad CFX96 Touch™ and the CFX384 Touch™ Real-Time PCR Detection Systems and the Bio-Rad CFX Maestro™ 1.1 Version 4.1.2433.1219 software (Bio-Rad). Variations in any component of the validation workflow indicated above may results in changes in performance characteristics of the *Quick* SARS-CoV-2 Multiplex Kit.

Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g. presence of symptoms), and/or stage of infection.

False-negative results may arise from improper collection, shipping, and/ or storage of specimen.

Pooled sample testing may decrease the sensitivity of the *Quick* SARS-CoV-2 Multiplex Kit since it lowers the volume of each individual sample that is analyzed.

Performance of the test was established in sputum. A shift in Ct was observed for all targets at high mucin concentrations. Therefore, high mucin concentrations at or above 0.1% (≥ 1 mg/ml) may result in invalid results.

The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

As with any molecular test, mutations within the target regions detected by the *Quick* SARS-CoV-2 Multiplex Kit could affect primer and/or probe binding resulting in failure to detect the presence of virus.

The performance of this SARS-CoV-2 assay was established using sputum. Nasal, nasopharyngeal, oropharyngeal and mid-turbinate swabs, BAL and tracheal aspirates are also considered acceptable specimen types for use with the SARS-CoV-2 Assay but performance has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected at a healthcare site or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.

Negative results do not preclude infection with the SARS-CoV-2 virus and should not be the sole basis of a patient treatment/management or public health decision. Follow up testing should be performed according to the current Health authorities' recommendations.

- Members of the Infectious disease laboratory will be trained to perform this assay and competency will be assessed and documented.
- A false negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization for the Laboratory

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A. Laboratories using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

C. Laboratories will collect information on the performance of your product and report to Health Authorities and Zymo Research any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

D. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

Performance Characteristics

This insert contains instructions for the *Quick* SARS-CoV-2 Multiplex Kit. The *Quick* SARS-CoV-2 Multiplex Kit has been validated using upper and lower respiratory tract specimens collected in DNA/RNA Shield™ (Zymo Research, cat. R1107-E, R1109-E, R1210-E). All samples were extracted using the *Quick*-DNA/RNA Viral Magbead (Zymo Research, cat. R2140-E, R2141-E) extraction kit by both manual and automated procedures. Automated extraction was performed on the KingFisher Flex Purification System (Thermo Fisher Scientific). Real-time RT-PCR was performed using the CFX96 and CFX384 Touch Real-Time PCR Detection System (Bio-Rad) with CFX Maestro™ 1.1 Version 4.1.2433.1219 software.

Deviation from the validated workflow described above may results in changes to the performance of the *Quick* SARS-CoV-2 Multiplex Kit.

Detailed information about the validation workflow and protocols are available upon request at tech@zymoresearch.com

I. Limit of Detection (LoD) – Analytical Sensitivity

The LoD of the *Quick* SARS-CoV-2 Multiplex Kit utilizing the entire test system from sample preparation to detection, has been determined. A preliminary LoD, was determined using heat-inactivated SARS-CoV-2, strain USA-WA1/2020 (VR-1986HK, ATCC) spiked into sputum collected using the DNA/RNA Shield™ Saliva/Sputum Collection Kit. Heat-inactivated virus was quantified by ATCC using quantitative RT-PCR and reported as copies per milliliter (copies/ml). The preliminary LoD study was performed using automated nucleic acid extraction of sputum samples containing 10-fold dilutions of virus from 8.33×10^4 to 0 copies/ml. All samples were processed using the *Quick*-DNA/RNA Viral Magbead (Zymo Research Corp.) extraction kit automated on the KingFisher Flex Purification System (Thermo Fisher Scientific). Real-time RT-PCR was performed using the Bio-Rad CFX96 Touch Real-Time PCR Detection System. The preliminary LoD was determined to be 83 copies/ml (5 copies/rxn), the lowest concentration for which 5/5 independent replicates tested positive for SARS-CoV-2 (**Table 1**).

Table 1: Preliminary LoD study in sputum specimens using automated extraction

Concentration in Dilution Tested [copies/ml]	Concentration in Dilution Tested [copies/rxn]	Replicate 1 C_t	Replicate 2 C_t	Replicate 3 C_t	Replicate 4 C_t	Replicate 5 C_t	Call Rate	Average Ct	Lowest Concentration with Uniform Positivity	Preliminary Limit of Detection (LoD)
8.33×10^4	5,000	25.55	25.68	25.63	25.82	25.59	5/5	25.68	83 copies/ml (5 copies/rxn)	83 GEC/ml (5 GEC/rxn)
8.33×10^3	500	29.11	28.97	29.32	28.87	29.10	5/5	29.06		
8.33×10^2	50	31.87	32.57	32.05	32.12	32.88	5/5	32.40		
8.3×10^1	5	37.07	36.22	35.58	35.05	37.71	5/5	36.14		
8.3×10^0	0.5	NA*	NA	39.35	38.65	NA	2/5	39.00		

*NA: No amplification

The LoD of the assay was confirmed for both manual and automated nucleic acid extraction methods using sputa as a clinical matrix. Heat-inactivated SARS-CoV-2 was spiked into negative sputa samples and 20 replicates were independently processed for LoD determination. The lowest concentration for which all 5 replicates were positive in the preliminary LoD evaluation (i.e. 83 copies/ml) was used as a starting point for the confirmatory LoD study. Testing of 83 copies/mL could not confirm the tentative LoD; therefore, a concentration 2-fold above 83 copies/mL was also tested. The final LoD for which ≥ 19/20 replicates tested positive for both manual and automated nucleic acid extraction methods was determined to be 167 copies/ml (10 copies/rxn) (**Table 2**). Results are summarized in **Table 3**.

Table 2: Confirmatory LoD for sputum specimens

	Automated Extraction					Manual Extraction			
Replicate	167 copies/ml (10 copies/rxn)	83 copies/ml (5 copies/rxn)	42 copies/ml (2.5 copies/rxn)	Lowest Concentration with at least 19/20 (95%) Positive	Confirmatory Limit of Detection (LoD)	Replicate	167 copies/ml (10 copies/rxn)	Lowest Concentration with at least 19/20 (95%) Positive	Confirmatory Limit of Detection (LoD)
	Ct	Ct	Ct				Ct		
1	34.30	40.24	38.71			1	35.05		
2	35.24	39.55	36.09			2	36.22		
3	36.05	36.02	37.10			3	35.04		
4	36.24	36.67	38.25			4	33.75		
5	35.57	38.22	40.26			5	35.82		
6	35.10	36.58	37.83			6	35.57		
7	33.85	35.01	36.92			7	35.40		
8	37.78	37.71	37.71			8	34.76		
9	35.20	NA	36.69			9	36.08		
10	36.59	36.26	NA	167 copies/ml (10 copies/rxn)	167 copies/ml (10 copies/rxn)	10	35.73	167 copies/ml (10 copies/rxn)	167 copies/ml (10 copies/rxn)
11	34.58	35.18	37.93			11	38.07		
12	33.41	37.72	37.95			12	35.75		
13	34.59	35.28	37.45			13	34.85		
14	34.61	35.92	NA			14	35.08		
15	34.45	35.45	37.92			15	35.24		
16	35.37	35.27	36.98			16	35.77		
17	35.34	35.33	37.65			17	35.60		
18	34.35	39.84	39.50			18	34.99		
19	34.55	35.68	36.12			19	34.20		
20	33.95	NA	38.94			20	34.48		
Call Rate	20/20	17/20	17/20			Call Rate	20/20		

Table 3: Confirmatory LoD Study - Summary

Target Level	Extraction Method	Valid results	SARS-CoV-2			Internal Control		
			Positive			Positive		
			n	Average Ct	Detection Rate	n	Average Ct	Detection Rate
167 copies/ml (10 copies/rxn)	Automated	20	*20*	*35.06*	*100%*	*20*	*23.47*	*100%*
83 copies/ml (5 copies/rxn)	Automated	20	*17*	*36.77*	*85%*	*20*	*23.35*	*100%*
42 copies/ml (2.5 copies/rxn)	Automated	20	*17*	*37.78*	*85%*	*20*	*23.41*	*100%*
167 copies/ml (10 copies/rxn)	Manual	20	*20*	*35.37*	*100%*	*20*	*23.18*	*100%*

II. Clinical Evaluation

A clinical evaluation was performed using 30 SARS-CoV-2 positive and 30 negative sputum specimens collected using the DNA/RNA Shield™ Saliva/Sputum Collection Kit. Specimens were collected from patients experiencing one or more symptoms of respiratory infection under the supervision of a healthcare provider. SARS-CoV-2 status was determined using the *Quick* SARS-CoV-2 Multiplex Kit and a comparator rRT-PCR assay authorized by the FDA for emergency use. Results are summarized in **Table 4.**

Table 4: Clinical Evaluation Summary

Comparator Result	*Quick* SARS-CoV-2 Multiplex Kit		Total	Percent Agreement
	Positive	Negative		95% CI
Positive	30	0	30	PPA: 100% (30/30)
				95% CI: 88.7% - 100%
Negative	0	30	30	NPA: 100% (30/30)
				95% CI: 88.7% - 100%
Total	30	30	60	

III. Transport Medium Study

Additional transport medias commonly used for SARS-CoV-2 testing were evaluated for their compatibility for use with *Quick* SARS-CoV-2 Multiplex Kit compared to DNA/RNA Shield™. Performance was evaluated using sputum spiked into each transport medium (listed in **Table 5**) to a final concentration of 20%, which represents a complex clinical matrix equivalent to a swab specimen. SARS-CoV-2 low positive samples were created by spiking in 334 copies/ml (2X LoD) of heat-inactivated SARS-CoV-2 into the clinical matrix. All samples were extracted using an automated method. Ct values for both SARS-CoV-2 and the internal control for each transport medium are shown in **Table 6**. The average Ct values for each transport medium were within 1 Ct value of DNA/RNA Shield™, indicating that all transport medias tested are compatible for use with the *Quick* SARS-CoV-2 Multiplex Kit.

Table 5. List of transport medias tested

Transport Medium	Manufacturer
DNA/RNA Shield™	Zymo Research
Viral Transport Medium (VTM)	Prepared in-house according CDC instructions (https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf)
Saline	Prepared in-house (0.9% NaCl in nuclease-free water)
PBS	Gibco
Liquid Amies	Copan
PrimeStore MTM	Longhorn

Table 6. Results from SARS-CoV-2 negative and low positive samples collected in different transport medias

		SARS-CoV-2							Internal Control						
SARS-CoV-2	Transport Media	Replicate 1 Ct	Replicate 2 Ct	Replicate 3 Ct	Replicate 4 Ct	Replicate 5 Ct	Average Ct	Std Deviation	Replicate 1 Ct	Replicate 2 Ct	Replicate 3 Ct	Replicate 4 Ct	Replicate 5 Ct	Average Ct	Std Deviation
Negative	DNA/RNA Shield	NA	NA	NA	NA	NA	NA	NA	25.35	25.24	25.31	25.26	25.41	25.31	0.06
	VTM	NA	NA	NA	NA	NA	NA	NA	27.15	27.33	27.18	27.10	27.18	27.19	0.07
	Saline	NA	NA	NA	NA	NA	NA	NA	25.00	24.67	24.68	24.95	24.72	24.80	0.14
	PBS	NA	NA	NA	NA	NA	NA	NA	24.80	24.73	24.70	24.69	24.86	24.75	0.06
	Liquid Amies	NA	NA	NA	NA	NA	NA	NA	25.35	25.39	25.16	25.44	25.42	25.35	0.10
	PrimeStore MTM	NA	NA	NA	NA	NA	NA	NA	26.52	26.47	26.57	26.56	26.69	26.56	0.07
2X LoD (334 copies/ ml)	DNA/RNA Shield	33.32	33.72	33.27	34.00	33.36	33.53	0.28	25.56	25.51	25.36	25.23	25.29	25.39	0.13
	VTM	32.81	33.15	34.23	32.90	33.29	33.28	0.51	27.14	26.80	27.00	27.01	26.82	26.95	0.13
	Saline	34.15	33.83	34.16	33.17	33.08	33.68	0.47	24.61	24.51	24.55	24.47	24.44	24.52	0.06
	PBS	33.67	33.45	33.52	33.69	33.63	33.59	0.09	25.22	24.77	24.77	24.81	25.09	24.93	0.19
	Liquid Amies	33.95	33.84	33.54	33.33	34.02	33.74	0.26	25.33	25.41	25.27	25.12	25.14	25.25	0.11
	PrimeStore MTM	34.03	33.17	33.46	33.49	33.57	33.54	0.28	26.73	26.56	26.56	26.50	26.35	26.54	0.12

VI. Analytical Sensitivity and Specificity

The studies below were performed during validation of the *Quick* SARS-CoV-2 rRT-PCR Kit (Zymo Research, cat. R3011), which contains components and primer and probe sequences identical to the *Quick* SARS-CoV-2 Multiplex Kit (cat. R3013). The primers and probes used in both kits are listed above in **Table 7**. Data shown in **Tables 8** – **16** include results from only the primers and probe which are included in the *Quick* SARS-CoV-2 Multiplex Kit.

Inclusivity (Analytical Sensitivity)

In silico inclusivity analysis of the oligo sets for SARS-CoV-2 (taxonomy ID 2697049) was performed using multiple sequence alignment of the individual SARS-CoV-2 primers and probe against all SARS-CoV-2 N gene sequences found in the NCBI database (1,354 in total as of April 24, 2020). The primers and probe correspond to CDC's primers and probe with unmodified sequence. Each primer and probe was found to have single nucleotide mismatches within a small number (≤ 0.74%) of the N gene sequences analyzed (**Table 8**). None of the mismatches occurred within the last five 3' nucleotides of any of the primers, reducing the potential detrimental effect of those mismatches on PCR efficiency. Overall, the number of sequences with unfavorable mismatches is too small to impact the inclusivity (analytical sensitivity) of the test.

Table 7. Primers and probes used in the *Quick* SARS-CoV-2 rRT-PCR Kit and *Quick* SARS-CoV-2 Multiplex Kit. All primers and probes with the same name have identical sequence.

Quick SARS-CoV-2 rRT-PCR Kit (cat. R3011)	Quick SARS-CoV-2 Multiplex Kit (cat. R3013)
CV Mix 1 (SARS-CoV-2)	**2X CV Mix (SARS-CoV-2 and IC)**
SARS-CoV-2 Target 1	**SARS-CoV-2 Target**
CV1_Fw	CV1_Fw
CV1_Rv	CV1_Rv
CV1_PB (HEX)	CV1_PB (HEX)
SARS-CoV-2 Target 2	**IC Target (RNase P)**
CV2_Fw	RP-Fw
CV2_Rv	RP_Rv
CV2_PB (HEX)	RP_PB (Quasar 670)
SARS-CoV-2 Target 3	
CV3_Fw	
CV3_Rv	
CV3_PB (HEX)	
CV Mix 2 (IC, RNase P)	
RP-Fw	
RP_Rv	
RP_PB (Quasar 670)	

Table 8. Primer and probe single nucleotide mismatches to SARS-CoV-2 N gene sequences. SARS-CoV-2 N gene accession numbers in bold have single nucleotide mismatches that occur at the same position within the primer/probe.

Primer/Probe	Total Number of SARS-CoV-2 N Gene Sequences Analyzed	Number of N Gene Sequences with 1 Mismatch	% N Gene Sequences with 1 Mismatch	Number of N Gene Sequences Having > 1 Mismatch	NCBI Accession Numbers for N Genes with 1 Mismatch
CV1_Fw	1,354	2	0.15	0	MT293178, MT350243
CV1_Rv	1,354	1	0.07	0	MT293178
CV1_PB	1,354	10	0.74	0	MT371038, MT326026, **MT372482**, **MT372481**, **MT372480**, **MT344946**, **MT304476**, **MT304475**, **MT304474**, **MT293161**

Cross-reactivity (Analytical Specificity)

Cross reactivity studies were performed using the NCBI Basic Alignment Search Tool (BLAST) to identify the largest regions of homology between the primers and probe to the genomes indicated (**Table 9**). Homology ≥ 80% was found for one primer with *Neisseria meningitidis*. A microbial interference study using samples containing 1 x 10^6 *Neisseria meningitis* cells with and without 3X LoD (750 GEC/ml) of whole genome SARS-CoV-2 RNA showed no interference (false positive or false negative result) from this microorganism with the test (**Table 10**).

In silico analysis showed homology of one primer and probe to the closely related SARS-CoV; however, since one primer has only 40% homology to SARS-CoV, the primers and probe used in this test are not expected to detect SARS-CoV. The primers and probe used in this test were found to have little cross-reactivity with endemic coronaviruses (229E, NL63, HKU1, and OC43) or MERS-CoV.

Table 9. *In silico* cross-reactivity analysis of primers and probe

Pathogen	Strain	GenBank Accession #	% Homology		
			CV1_Fw	CV1_Rv	CV1_PB
Human coronavirus	229E	NC_002645.1	50	46	38
	OC43	NC_006213.1	50	38	42
	HKU1	NC_006577.2	50	42	42
	NL63	NC_005831.2	45	42	42
SARS coronavirus	SARS	NC_004718.3	40	92	92
MERS coronavirus	MERS	NC_019843.3	50	42	38
Human adenovirus	Human adenovirus 1	AC_000017.1	45	38	38
Human metapneumovirus	00-1	NC_039199.1	45	42	33
Human respirovirus 1	Human parainfluenza virus 1	NC_003461.1	55	38	38
Human rubulavirus 2	Human parainfluenza virus 2	NC_003443.1	50	38	33
Human respirovirus 3	Human parainfluenza virus 3	EU326526.1	50	38	38
Human rubulavirus 4	Human parainfluenza virus 4	NC_021928.1	40	38	33
Influenza A virus	A/California/07/2009	NC_026431.1	35	42	29
		NC_026432.1	40	0	29
		NC_026433.1	40	38	29
		NC_026434.1	45	33	0
		NC_026435.1	35	33	33
		NC_026436.1	35	29	38
		NC_026437.1	35	29	33
		NC_026438.1	40	33	33

Influenza B virus	B/Lee/1940	NC_002204.1	40	38	33
		NC_002205.1	35	50	33
		NC_002206.1	40	29	29
		NC_002207.1	40	33	29
		NC_002208.1	35	33	38
		NC_002209.1	0	33	29
		NC_002210.1	0	33	29
		NC_002211.1	35	29	33
Enterovirus D68	Fermon	NC_038308.1	40	38	38
Respiratory syncytial virus	Strain not indicated	NC_001803.1	45	38	38
Rhinovirus A	ATCC VR-1559	NC_038311.1	45	38	38
Chlamydia pneumoniae	TW-183	NC_005043.1	60	58	50
Haemophilus influenzae	NCTC8143	NZ_LN831035.1	60	45	50
Legionella pneumophila	NCTC12273	NZ_LR134380.1	65	54	67
Mycobacterium tuberculosis	H37Rv	NC_018143.2	60	50	50
Streptococus pneumoniae	NCTC7465	NZ_LN831051.1	65	67	54
Streptococcus pyogenes	NCTC8198	NZ_LN831034.1	75	54	50
Bordetella pertussis	18323	NC_018518.1	65	50	46
Mycoplasma pneumoniae	FH	NZ_CP010546.1	60	46	54
Influenza C virus	C/Ann Arbor/1/50	NC_006312.2	0	0	0
		NC_006311.1	0	0	0
		NC_006310.2	0	0	0
		NC_006309.2	45	29	29
		NC_006308.2	45	33	29
		NC_006307.2	40	38	29
		NC_006306.2	40	29	29

Parechovirus	Human parechovirus 1	NC_038319.1	40	42	29
Candida albicans	SC5314	NC_032096.1	60	75	50
		NC_032095.1	60	50	50
		NC_032094.1	65	46	50
		NC_032093.1	60	50	50
		NC_032092.1	60	50	50
		NC_032091.1	60	79	50
		NC_032090.1	65	58	63
		NC_032089.1	60	50	54
Corynebacterium diphtheriae	NCTC11397	NZ_LN831026.1	70	54	54
Legionella longbeachae	NSW150	NC_013861.1	70	54	58
Bacillus anthrasis (Anthrax)	Vollum	NZ_CP007666.1	60	63	50
Moraxella catarrhalis	BBH18	NC_014147.1	70	54	63
Neisseria elongata	ATCC 29315	NZ_CP007726.1	70	50	50
Neisseria meningitidis	NCTC10025	NZ_LR134525.1	85	54	50
Pseudomonas aeruginosa	PAO1	NC_002516.2	75	54	54
Staphylococcus epidermidis	ATCC 14990	NZ_CP035288.1	60	50	50
Streptococcus salivarius	NCTC8618	NZ_LR134274.1	60	54	54
Leptospira interrogans	FMAS AW1	NZ_CP039283.1	65	54	50
Chlamydia psittaci	6BC	NC_017287.1	65	46	54

Table 10: Microbial Interference Testing. Ct values are for SARS-CoV-2.

Organism	SARS-CoV-2	Replicate 1 Ct	Replicate 2 Ct	Replicate 3 Ct	Average Ct
None	750 GEC/ml (3X LoD)	*33.01*	*33.63*	*33.57*	*33.40*
Neisseria meningitidis	750 GEC/ml (3X LoD)	*33.80*	*33.73*	*34.86*	*34.13*
None	Negative	*NA**	*NA*	*NA*	*NA*
Neisseria meningitidis	Negative	*NA*	*NA*	*NA*	*NA*

* NA: No Amplification

Symbols Legend


Collect sample by

Catalog number


Manufacturer


Storage instructions

Lot number


Do not reuse


Keep away from sunlight


See instruction manual

Contains sufficient for <n>tests

Package contains

Reagent

Positive Control

IN VITRO DIAGNOSTIC MEDICAL DEVICE

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