WHO Emergency Use Assessment SARS-CoV-2 IVDs
PUBLIC REPORT

Product: Abbott RealTim*e* SARS-CoV-2 assay
Manufacturer: Abbott Molecular Inc
EUL Number: EUL 0503-027-00
Outcome: Accepted.

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents.
- Product Dossier Review: assessment of the documentary evidence of safety and performance.

The Abbott RealTim*e* SARS-CoV-2 assay with product codes 09N77-090 and 09N77-080, CE-Mark regulatory version manufactured by Abbott Molecular Inc ,1300 East Touhy Avenue Des Plaines, IL 60018, United States of America**,** was listed as eligible for WHO procurement on 9 April 2020. The product was assessed for EUL renewal and met EUL renewal requirements on 13 June 2022. Therefore, the product is eligible for WHO procurement until further notice.

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the EUL product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	Update the package insert to: "- clarify the interpretation of results table to more closely align with how the m2000rt displays the results, - update the volume of internal control used from 750ul to 1200ul; and, - make other minor corrections to package insert."	16 September 2020
3.0	Transitioned from print on demand component labels to pre-printed component labels;	3 March 2022

	• to incorporate Global Harmonized System (GHS) symbology for translation free labelling, and • to modify the storage condition on the label for the Abbott RealTime SARS-CoV-2 Internal Control from "-10 °C or colder" to " -25 to -15 °C", to be consistent with the storage condition of Abbott RealTime SARS CoV-2 Amplification.	
4.0	Updated public report on post EUL commitments that were fulfilled and closed. Extension of product's eligibility for procurement after EUL renewal assessment until further notice.	13 June 2022

Intended use:

According to the claim of intended use from Abbott Molecular Inc, "*the Abbott RealTime SARS-CoV-2 assay is a real-time (rt) reverse transcriptase (RT) polymerase chain reaction (PCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal (NP) and oropharyngeal (OP) swabs from patients who are suspected of COVID-19 by their healthcare provider.*
Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal and oropharyngeal swabs 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.
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 Abbott RealTime SARS-CoV-2 assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures".

Specimen type(s) that were validated:

Nasopharyngeal (NP) and Oropharyngeal (OP) swabs

Test kit contents:

Component	**Number of tests and product code**
Abbott RealTime SARS-CoV-2 Amplification Reagent Kit	**96 tests (product code 09N77-090)**
Abbott RealTime SARS-CoV-2 Internal Control	4 vials x 1.2mL
Abbott RealTime SARS-CoV-2 Amplification Reagent Pack	4 packs X 24 tests/pack (9N77)

Items required but not provided:

Abbott RealTime SARS-CoV-2 Control Kit	**product code 09N77-080**
Abbott RealTime SARS-CoV-2 Negative Control	8 vials x 1.3mL/ vial
Abbott RealTime HCV Positive Control	8 vials x 1.3mL/vial

Component	**Product code and description**
Sample preparation area	
Abbott m2000sp instrument	m2000sp software version 8.1 or higher
Abbott *m*Sample Preparation System	06K12-24
Abbott m2000sp Operations Manual	09K20-009 or higher
Abbott RealTime SARS-CoV-2 Application Specification	09N77-001 or higher
Calibrated precision pipettes	20 µL to 1 000 µL
Aerosol barrier pipette tips	20 µL to 1 000µL
13 to 16 mm sample tubes	13 to 16 mm sample tubes
200 µL disposable tips	04J71-17
1000 µL disposable tips	04J71-1
200 mL Reagent Vessels	4J71-60
Vortex mixer	General lab equipment
Abbott Optical Adhesive Covers	04J71-75
Abbott Adhesive Cover Applicator	9K32-01
Abbott Splash-Free Support Base	09K31-01
5 mL Reaction Vessels	(12 x 75mm) 4J70-20
Amplification Reagent Pack caps (optional)	3N20-01
Transport tubes	04J71-81
Sample racks	General lab material
Biohazard bags	4J71-45
Master Mix Tube	04J71-80
Abbott 96-Deep-Well Plate	04J71-30
Abbott 96-Well Optical Reaction Plate	04J71-70
Waste bags	3N17-01
USP Grade 190-200 Proof ethanol (95-100% ethanol)	General lab material
Amplification area	
Abbott m2000rt	Software version 8.1 or higher
Abbott m2000rt Operations Manual	06N03-009 or higher

Abbott RealTime SARS-CoV-2 Application Specification	List No. 09N77-001 or higher
Abbott m2000rt Optical Calibration Kit	List No. 4J71-93
Other Materials	
Biological safety cabinet approved for working with infectious materials	General lab equipment
Sealable plastic bags	General lab material
RNase-free water (Eppendorf or equivalent) †	General lab material
1.7 mL molecular biology grade microcentrifuge tubes (Dot Scientific, Inc. or equivalent) †	
Cotton Tip Applicators (Puritan or equivalent) †	General lab material

Storage:
The test kit should be stored at -15 to -25 °C.

Shelf-life upon manufacture:
Reagent kit: 18 months, Control kit: 12 months. Shelf-life has been assigned based on similar products.

Warnings/limitations:

Refer to the instructions for use.

Product dossier assessment

Abbott Molecular Inc submitted a product dossier for Abbott RealTime SARS-CoV-2 assay as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid and Antigen (PQDx_0347)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external product evaluating committee (PEC) assessor appointed by WHO.

Post listing commitment for EUL

As a requirement to listing, the manufacturer is required to participate in the WHO collaborative study for the assessment of the suitability of an interim standard for SARS-CoV-2 virus nucleic acid amplification tests. Commitment was fulfilled. Issue closed.

Risk benefit assessment conclusion: Acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Abbott Molecular, Inc was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation by WHO staff and external technical experts (assessors), it was established that sufficient information was provided by Abbott Molecular Inc to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid, PQDx_ 347"*.

Quality management system assessment conclusion: Acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL status:
1. Notification to WHO of any planned changes to a EUL listed product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*); and
2. Post-market surveillance activities, in accordance with "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"*
(ISBN 978-92-4-001531-9).

Abbott Molecular Inc is also required to submit all categories of complaints in a summarized form. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents. The sales data will serve as denominator data to guide the frequency of re-inspection.

The manufacturer has committed to ensure that post-emergency use listing safety, quality and performance monitoring activities are in place which are in accordance with WHO guidance *"Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics*[1]*"*

Scope and duration of procurement eligibility

The Abbott RealTime SARS-CoV-2 assay with product codes 09N77-090 and 09N77-080 manufactured by Abbott Molecular Inc is considered to be eligible for WHO procurement until further notice. The assay may be used for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement, Abbott Molecular Inc must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Abbott Molecular Inc is

[1] Available on the web page https://www.who.int/publications/i/item/guidance-for-post-market-surveillanceand-market-surveillance-ofmedical-devices-including-in-vitro-diagnostics

required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days.

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality, performance during post-market surveillance activities, and if new data becomes available to WHO that changes the risk benefit balance.

Labelling

1. **Labels**
2. **Instructions for use**

1. Labels

1.1 Amplification reagent kit label

Abbott RealTi*me* SARS-CoV-2

Amplification Reagent Kit

REF 09N77-090

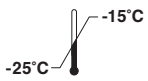
Σ 96

In Vitro Test

IVD

INTERNAL CONTROL 4 x 1.2 mL

AMPLIFICATION REAGENT PACK 4



CE

H317, H319, H315, H402, H412,
P261, P264, P272, P280, P273,
P302+P352, P333+P313, P362+P364,
P305+P351+P338, P337+P313,
P501

51-603323/R1

LOT

REF

Abbott RealTi*me* SARS-CoV-2

Amplification Reagent Kit

Abbott Molecular Inc.
1300 East Touhy Avenue
Des Plaines, IL 60018 USA

www.abbottmolecular.com

EC REP

Abbott GmbH
Max-Planck-Ring 2
65205 Wiesbaden, Germany

Abbott

1.2 Internal Control label

Top Edge

IC

Abbott RealTi*me*

SARS-CoV-2

INTERNAL CONTROL

REF 9N77Y

1.2 mL

51-603354/R2

-15°C

-25°C

Abbott Molecular Inc.
Des Plaines, IL 60018 USA

LOT

Colors: PMS 292
PMS 185
BLACK

DTP: Nick

1.4 Positive and Negative Control label

Abbott RealTi*me* SARS-CoV-2

Control Kit

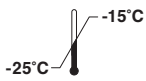
REF 09N77-080

CONTROL + 8 x 1.3 mL

CONTROL − 8 x 1.3 mL

In Vitro Test

IVD



CE

51-603324/R1

H318, H316, P280,
P305+P351+P338, P310,
P332+P313

LOT

REF

Abbott RealTi*me* SARS-CoV-2

Control Kit

Abbott Molecular Inc.
1300 East Touhy Avenue
Des Plaines, IL 60018 USA

www.abbottmolecular.com

EC REP Abbott GmbH
Max-Planck-Ring 2
65205 Wiesbaden, Germany

Abbott

1.5 CD Label

REF 09N77-001

LOT

Abbott RealTi*me* SARS-CoV-2

Application CD-ROM

Version 1.0

IVD

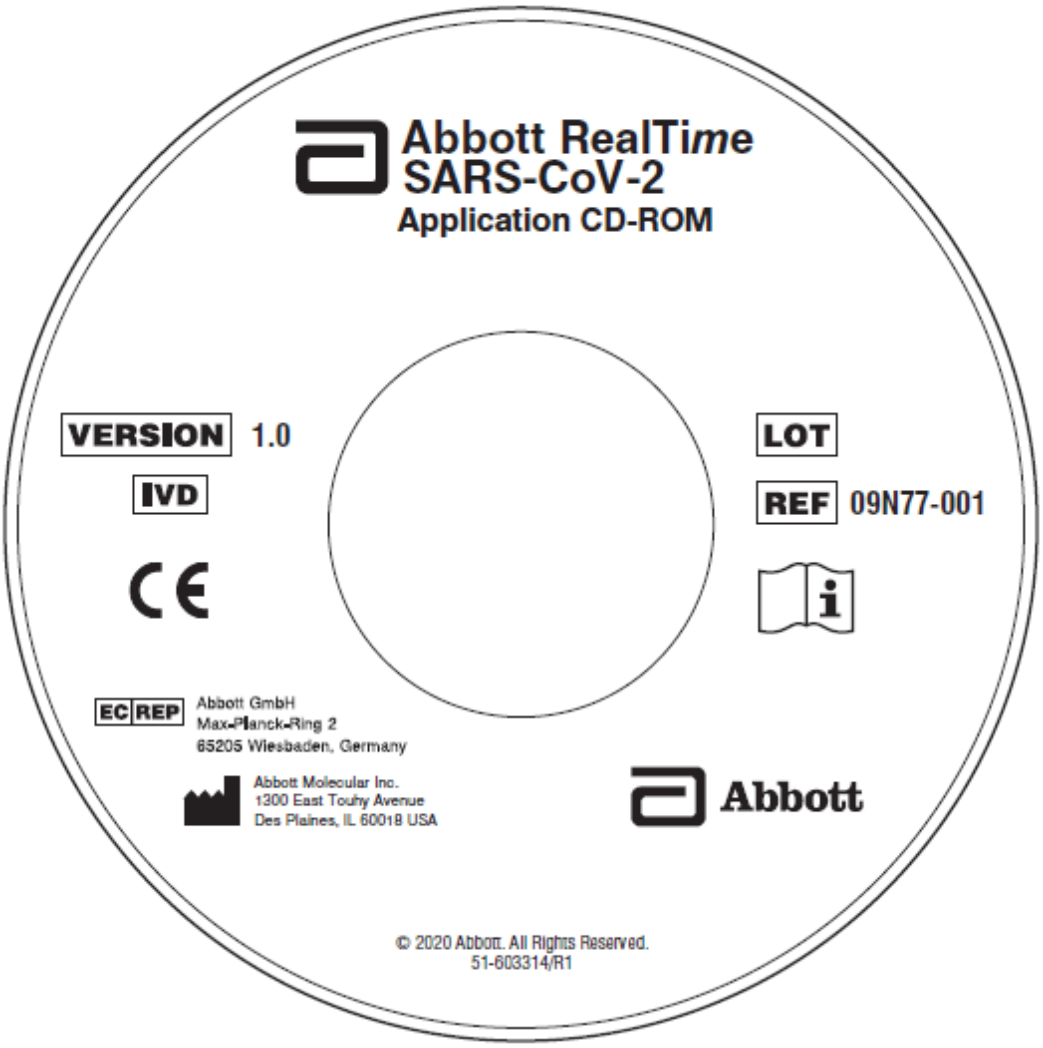
CE

EC REP Abbott GmbH
Max-Planck-Ring 2
65205 Wiesbaden, Germany

Abbott Molecular Inc.
1300 East Touhy Avenue
Des Plaines, IL 60018 USA

Abbott

51-603315/R1



2. Instructions for use[2]

[2] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

Abbott RealTi*me* SARS-CoV-2

Amplification Kit

en

REF 09N77-090

51-608442/R2

IVD

REF 09N77-090

51-608442/R2

NOTE: Changes highlighted

Instructions for Use

Key to Symbols Used	
REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Use By
	Temperature Limit
In Vitro Test	In Vitro Test
CONTROL –	Negative Control
CONTROL +	Positive Control
INTERNAL CONTROL	Internal Control
AMPLIFICATION REAGENT PACK	Amplification Reagent Pack
	Consult instructions for use
	Warning
	Corrosive
	Caution
	Contains Sufficient for <n> tests
EC REP	Authorized Representative in the European Community
	Manufacturer

INTRODUCTION

This instructions for use (IFU) must be read carefully prior to use. IFU instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this IFU.

NAME

Abbott RealTi*me* SARS-CoV-2

INTENDED USE

The Abbott RealTi*m*e SARS-CoV-2 assay is a real-time (rt) reverse transcriptase (RT) polymerase chain reaction (PCR) test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal (NP) and oropharyngeal (OP) swabs from patients who are suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal and oropharyngeal swabs 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.

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 Abbott RealTi*m*e SARS-CoV-2 assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

NOTICE TO USER

If a serious incident occurs in relation to this device, the incident should be reported to the manufacturer and to the appropriate competent authority of the member state in which the user and/or the patient is established. To report to the manufacturer, see the contact information provided in the technical assistance section of these instructions.

SUMMARY AND EXPLANATION OF THE TEST

The Abbott RealTi*m*e SARS-CoV-2 assay is real-time reverse transcription polymerase chain reaction (rRT-PCR) test on the Abbott *m*2000 System. The SARS-CoV-2 primer and probe sets are designed to detect RNA from SARS-CoV-2 in nasopharyngeal and oropharyngeal swabs from patients with signs and symptoms of infection who are suspected of COVID-19.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The Abbott RealTi*m*e SARS-CoV-2 assay consists of 2 reagent kits:

- Abbott RealTi*m*e SARS-CoV-2 Amplification Reagent Kit
- Abbott RealTi*m*e SARS-CoV-2 Control Kit

The Abbott RealTi*m*e SARS-CoV-2 assay is a dual target assay for the RdRp and N genes.

An RNA sequence that is unrelated to the SARS-CoV-2 target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence is simultaneously amplified by RT-PCR and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample.

The Abbott RealTi*m*e SARS-CoV-2 assay detects the SARS-CoV-2 virus and IC target sequences through the use of target-specific fluorescent-labeled oligonucleotide probes. The probes do not generate a signal unless they are specifically bound to the amplified product. The two SARS-CoV-2-specific probes are labeled with the same fluorophore and the IC-specific probe is labeled with a different fluorophore, thus allowing for simultaneous detection of both SARS-CoV-2 and IC amplified products in the same reaction well.

The Abbott RealTi*m*e SARS-CoV-2 assay is performed on the Abbott *m*2000 System consisting of a sample preparation unit, the Abbott *m*2000*sp*, and an amplification and detection unit, the Abbott *m*2000*rt*. Application parameters specific to the Abbott RealTi*m*e SARS-CoV-2 assay are contained on an assay-specific application specification file, distributed electronically, stored on portable media and loaded onto the Abbott *m*2000*sp* and Abbott *m*2000*rt* instruments.

Sample Preparation

The Abbott *m*2000*sp* provides automated sample preparation using a magnetic microparticle-based protocol and reagents (Abbott *m*Sample Preparation System$_{DNA}$) to process 0.5 mL samples from nasopharyngeal and oropharyngeal swabs.

During the sample preparation protocol, SARS-CoV-2 virions are disrupted by guanidine isothiocyanate, nucleic acids are captured on the magnetic microparticles, and inhibitors and unbound sample components are removed by washing steps. The bound nucleic acids are eluted off the microparticles with buffer and transferred to a 96 deep-well plate. The nucleic acids are then ready for amplification. The Internal Control (IC) is introduced into each specimen at the beginning of the sample preparation process to demonstrate that the process was completed correctly for each specimen and control.

A positive control and a negative control are processed from the start of sample preparation for each test order to evaluate run validity.

The purpose of sample preparation is to extract and concentrate the target nucleic acids (DNA and RNA) molecules to make the target accessible for amplification, and to remove potential inhibitors of amplification from the extract.

The Abbott *m*Sample Preparation System$_{DNA}$ uses magnetic particle technology to capture nucleic acids and washes the particles to remove unbound sample components. The bound nucleic acids are eluted and transferred to a 96 deep-well plate. The nucleic acids are then ready for amplification. The IC is taken through the entire sample preparation procedure along with the controls and specimens.

The Abbott *m*2000*sp* automated instrument system is used to prepare samples for the Abbott RealTi*m*e SARS-CoV-2 assay. The Abbott *m*2000*sp* provides automated sample eluate transfer and reaction assembly in the Abbott 96-Well Optical Reaction Plate.

Reagent Preparation and Reaction Plate Assembly

The Abbott *m*2000*sp* combines the Abbott RealTi*m*e SARS-CoV-2 assay amplification reagent components (SARS-CoV-2 Oligonucleotide Reagent, Thermostable rTth Polymerase Enzyme, and Activation Reagent). The Abbott *m*2000*sp* dispenses the resulting master mix to the Abbott 96-Well Optical Reaction Plate along with aliquots of the nucleic acid samples prepared by the Abbott *m*2000*sp*. The plate is ready, after manual application of the optical seal, for transfer to the Abbott *m*2000*rt*.

Amplification

During the amplification reaction on the Abbott *m*2000*rt*, the target RNA is converted to cDNA by the reverse transcriptase activity of the thermostable rTth DNA polymerase. First, the SARS-CoV-2 and IC reverse primers anneal to their respective targets and are extended during a prolonged incubation period. After a denaturation step, in which the temperature of the reaction is raised above the melting point of the double-stranded cDNA:RNA product, a second primer anneals to the cDNA strand and is extended by the DNA polymerase activity of the rTth enzyme to create a double-stranded DNA product.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences. Amplification of the three targets (SARS-CoV-2 RdRp, SARS-CoV-2 N, and IC) takes place simultaneously in the same reaction.

The target sequences for the Abbott RealTi*m*e SARS-CoV-2 assay are in the SARS-CoV-2 RdRp and N genes of the SARS-CoV-2 genome. The selected target sequences are highly conserved and also specific to this strain of coronavirus.

The IC target sequence is derived from the hydroxypyruvate reductase gene from the pumpkin plant, *Cucurbita pepo*, and is delivered in an Armored RNA® particle that has been diluted in negative human plasma.

Detection

During the read cycles of amplification on the Abbott *m*2000*rt*, the temperature is lowered further to allow fluorescent detection of amplification products as the SARS-CoV-2 and IC probes anneal to their targets (real-time fluorescence detection). The SARS-CoV-2 probes have a fluorescent moiety that is covalently linked to the 5′ end and has a quencher molecule at its 3′ end. In the absence of target sequences, the probes adopt a conformation that brings the quencher close enough to the excited fluorophore to absorb its energy before it can be fluorescently emitted. When the probe binds to its complementary sequence in the target, the fluorophore and the quencher are held apart, allowing fluorescent emission and detection. The IC probe is a single-stranded DNA oligonucleotide with a fluorophore at the 5′ end and a quencher at the 3′ end. In the absence of IC target sequences, probe fluorescence is quenched. In the presence of IC target sequences, probe hybridization to complementary sequences separates the fluorophore and the quencher and allows fluorescent emission and detection. The SARS-CoV-2 and IC specific probes are each labeled with a different fluorophore, thus allowing for simultaneous detection of both amplified products.

PREVENTION OF NUCLEIC ACID CONTAMINATION

The possibility of nucleic acid contamination is minimized because:

- Reverse transcription, PCR amplification, and oligonucleotide hybridization occur in a sealed Abbott 96-Well Optical Reaction Plate.
- Detection is carried out automatically without the need to open the Abbott 96-Well Optical Reaction Plate.
- Pipettes with aerosol barrier tips or disposable transfer pipettes are used for all pipetting. The disposable pipettes or pipette tips are discarded after use.
- Separate, dedicated areas are used to perform the Abbott RealTi*m*e SARS-CoV-2 assay. Refer to the **SPECIAL PRECAUTIONS** section of this IFU.

REAGENTS

Abbott RealTi*m*e SARS-CoV-2 Amplification Reagent Kit (List No. 09N77-090)

1. Abbott RealTi*m*e SARS-CoV-2 Internal Control
   (4 vials, 1.2 mL per vial)

   Less than 0.01% noninfectious Armored RNA with internal control sequences in negative human plasma. Negative human plasma tested and found to be non-reactive by appropriate FDA-licensed, approved, or cleared tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, HIV-1 Ag, HBsAg, and Syphilis. The material is also tested and found to be negative by appropriate FDA-licensed, approved, or cleared PCR methods for HIV RNA, HCV RNA, and HBV DNA. Preservatives: 0.1% ProClin® 300 and 0.15% ProClin 950.
2. Abbott RealTi*m*e SARS-CoV-2 Amplification Reagent Pack (List No. 9N77)
   (4 packs, 24 tests/pack)
   - 1 bottle (0.141 mL) Thermostable rTth Polymerase Enzyme (2.9 to 3.5 Units/µL) in buffered solution.
   - 1 bottle (1.0 mL) SARS-CoV-2 Amplification Reagent containing synthetic oligonucleotides (6 primers and 3 probes), and dNTPs in a buffered solution with a reference dye. Preservative: 0.10% ProClin 300 and 0.15% ProClin 950.
   - 1 bottle (0.400 mL) Activation Reagent. 30 mM manganese chloride solution. Preservatives: 0.10% ProClin 300 and 0.15% ProClin 950.

Abbott RealTi*m*e SARS-CoV-2 Control Kit (List No. 09N77-080)

1. Abbott RealTi*m*e SARS-CoV-2 Negative Control
   (8 vials, 1.3 mL per vial) Contains 1.0% ammonium sulfate and 7.9% detergent in a buffer solution.
2. Abbott RealTi*m*e SARS-CoV-2 Positive Control
   (8 vials, 1.3 mL per vial) Contains non-infectious, recombinant Sindbis virus containing SARS-CoV-2 RNA sequences, 1.0% ammonium sulfate, and 7.9% detergent in a buffer solution.

WARNINGS AND PRECAUTIONS

IVD

In Vitro Diagnostic Medical Device

Safety Precautions

Refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Hazard Section, for instructions on safety precautions.

Important information regarding the safe handling, transport and disposal of this product is contained in the Safety Data Sheet.

CAUTION: This preparation contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive by appropriate FDA-licensed, approved, or cleared tests for antibody to HCV, antibody to HIV-1, antibody to HIV-2, HIV-1 Ag, HBsAg, and Syphilis. The material is also tested and found to be negative by appropriate FDA-licensed, approved, or cleared PCR methods for HIV RNA, HCV RNA, and HBV DNA. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using laboratory safety procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[1] OSHA Standards on Bloodborne Pathogens,[2] CLSI Document M29-A4,[3] and other appropriate biosafety practices.[4] Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- 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 a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[1]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[4]

Components of the Abbott RealTi*m*e SARS-CoV-2 Internal Control, Oligonucleotide Reagent, and Activation Reagent contain the following components:

Potassium Hydroxide

2-Methyl-4-isothiazol-3-one:

- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one (EC no. 247-500-7) and 2-methyl-2H-isothiazol-3-one (EC no. 220-239-6)(3:1)
- Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one (EC no. 247-500-7) and 2-methyl-4-isothiazolin-3-one (EC no. 220-239-6)(3:1)

The following warnings apply:

Warning	
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H315	Causes skin irritation.
H402	Harmful to aquatic life.*
H412	Harmful to aquatic life with long lasting effects.
P261	Avoid breathing mist/vapors/spray.
P264	Wash hands thoroughly after handling.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/eye protection.
P273	Avoid release to the environment,
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash before reuse.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice/attention.
P501	Dispose of contents/container in accordance with local regulations.

* Not applicable where regulation EC 1272/2008 (CLP) has been implemented.

Safety Data Sheet Statement: 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 your Abbott Representative.

SPECIAL PRECAUTIONS

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

- For in vitro diagnostic use.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories,[1] in the CLSI Document M29-A4,[3] and other appropriate biosafety practices.[4] Only personnel proficient in handling infectious materials and the use of the Abbott RealTi*m*e SARS-CoV-2 assay and the Abbott *m*2000 System should perform this procedure.

Handling Precautions for Specimens

- The Abbott RealTi*m*e SARS-CoV-2 assay is only for use with nasopharyngeal and oropharyngeal swabs that have been handled and stored as described in the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section.
- 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. Refer to CLSI MM13-A[5] as an appropriate resource.
- During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of ribonucleases (RNases) into samples during and after the extraction procedure.
- Proper aseptic technique should always be used when working with RNA.
- 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 *m*2000*sp* and the *m*2000*rt* instruments may be operated in the same location. The use of 2 dedicated areas (Sample Preparation Area and Amplification Area) within the laboratory is recommended when performing the Abbott RealTi*m*e SARS-CoV-2 assay.

The **Sample Preparation Area** is dedicated to processing samples (specimens and Abbott RealTi*m*e SARS-CoV-2 Controls) and to adding processed samples and controls to the 96-Well Optical Reaction Plate. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips, and vortexers used in the Sample Preparation Area must remain in this area and not be moved to the Amplification Area. 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.

- Components contained within a kit are intended to be used together. Do not mix components from different kit lots. For example, do not use the negative control from control kit lot X with the positive controls from control kit lot Y.
- Do not use kits or reagents after the expiration dates shown on kit labels.
- 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, negative control, positive controls, or specimens. Refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals for instrument cleaning procedures.
- If the Abbott *m*2000*sp* instrument run is aborted, dispose of all commodities and reagents according to the Abbott *m*2000*sp* Operations Manual.
- If the Abbott *m*2000*sp* master mix addition protocol is aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*sp* Operations Manual, Hazards section, along with the gloves used to handle the plate.
- If the Abbott *m*2000*rt* instrument run is interrupted or aborted, seal the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.
- Decontaminate and dispose of all potentially biohazardous materials in accordance with local, state, and federal regulations.[4] All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

NOTE: Autoclaving the sealed Reaction Plate will not degrade the amplified product and may contribute to the release of the amplified product by opening the sealed plate. The laboratory area can become contaminated with amplified product if the waste materials are not carefully handled and contained.

Aerosol Containment

To reduce the risk of nucleic acid contamination due to aerosols formed during manual pipetting, aerosol barrier pipette tips must be used for all manual pipetting. The pipette tips must be used only 1 time. Clean and disinfect spills of specimens and reagents as stated in the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals.

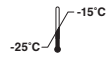
Contamination and Inhibition

The following precautions should be observed to minimize the risks of RNase contamination, cross-contamination between samples, and inhibition:

- Wear appropriate personal protective equipment at all times.
- Use powder-free gloves.
- Change gloves after having contact with potential contaminants (such as specimens, eluates, and/or amplified product).
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting, pipettes with aerosol barrier tips must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Change aerosol barrier pipette tips between ALL manual liquid transfers.
- The Abbott *m*Sample Preparation System$_{DNA}$ reagents are single use only. Use new reagent troughs or vessels, reaction vessels, and newly opened reagents for every new Abbott RealTi*m*e SARS-CoV-2 assay run. At the end of each run, discard all remaining reagents from the worktable as stated in the Abbott *m*2000*sp* Operations Manual and the Abbott *m*Sample Preparation System$_{DNA}$ product information sheet.

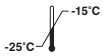
STORAGE INSTRUCTIONS

Abbott RealTi*m*e SARS-CoV-2 Amplification Reagent Kit (List No. 09N77-090)



- Abbott RealTi*m*e SARS-CoV-2 Amplification Reagent Packs and Internal Control (IC) vials must be stored at – 25 to – 15°C when not in use. Care must be taken to separate the Abbott RealTi*m*e SARS-CoV-2 Amplification Reagent Pack that is in use from direct contact with samples and controls.

Abbott RealTi*m*e SARS-CoV-2 Control Kit (List No. 09N77-080)



- The Abbott RealTi*m*e SARS-CoV-2 Negative and Positive Controls must be stored at – 25 to – 15°C.

SHIPPING CONDITIONS

- Abbott RealTi*m*e SARS-CoV-2 Amplification Reagent Kit: Ship on dry ice.
- Abbott RealTi*m*e SARS-CoV-2 Control Kit: Ship on dry ice.

If you receive reagents that are in a condition contrary to label recommendation, or that are damaged, contact your Abbott Representative.

INDICATION OF INSTABILITY OR DETERIORATION OF REAGENTS

When a positive or negative control value is out of the expected range, it may indicate deterioration of the reagents. Associated test results are invalid and samples must be retested.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE

Specimen Collection and Storage

Human nasopharyngeal and oropharyngeal swab specimens may be used with the Abbott RealTi*m*e SARS-CoV-2 assay. Refer to the European Centre for Disease Prevention and Control at ecdc.europa.eu/en/novel-coronavirus/laboratory-support.[6]

Specimen Transport

For domestic and international shipments, specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.

INSTRUMENT PROCEDURE

The Abbott RealTi*m*e SARS-CoV-2 application specification files must be installed on the Abbott *m*2000*sp* and Abbott *m*2000*rt* instruments from the Abbott RealTi*m*e SARS-CoV-2 Application Specification (List No. 09N77-001 or higher) prior to performing the assay. For a detailed description of how to perform an Abbott *m*2000*sp* instrument and Abbott *m*2000*rt* instrument protocol, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions sections.

ABBOTT REALTI*ME* SARS-COV-2 ASSAY PROCEDURE

This IFU contains instructions for running the Abbott RealTi*m*e SARS-CoV-2 assay.

Materials Provided

- Abbott RealTi*m*e SARS-CoV-2 Amplification Reagent Kit (List No. 09N77-090)

Materials Required But Not Provided

- Abbott *m*Sample Preparation System$_{DNA}$ (List No. 06K12-24)
- Abbott RealTi*m*e SARS-CoV-2 Application Specification (List No. 09N77-001 or higher)
- Abbott RealTime SARS-CoV-2 Control Kit (List No. 09N77-080)

Sample Preparation Area

- Abbott *m*2000*sp* Instrument (*m*2000*sp* software version 8.1 or higher)
- Abbott *m*2000*sp* Operations Manual (List No. 09K20-009 or higher)
- Abbott *m*Sample Preparation System$_{DNA}$ (List No. 06K12-24)
- Abbott RealTi*m*e SARS-CoV-2 Application Specification (List No. 09N77-001 or higher)
- 5 mL Reaction Vessels (12 x 75 mm) (List No. 4J71-20)
- Master Mix Tubes (List No. 04J71-80)
- Amplification Reagent Pack Caps (List No. 3N20-01) (Optional)
- Transport Tubes (List No. 04J71-81)
- 200 mL Reagent Vessels (List No. 4J71-60)
- Abbott 96-Well Optical Reaction Plate (List No. 04J71-70)
- Abbott 96-Deep-Well Plate (List No. 04J71-30)
- Abbott Splash-Free Support Base (List No. 09K31-01)
- 200 µL and 1000 µL Disposable Tips for Abbott *m*2000*sp* (List No. 4J71-17 and 4J71-10)

- Abbott Optical Adhesive Cover (List No. 04J71-75)
- Abbott Adhesive Cover Applicator (List No. 9K32-01)
- Biohazard bags (List No. 4J71-45)
- Sample racks
- Vortex mixer
- USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol).
  Do not use ethanol that contains denaturants.
- Calibrated precision pipettes capable of delivering 20 µL to 1000 µL
- 20 µL to 1000 µL aerosol barrier pipette tips for precision pipettes.

Other Materials

- Biological safety cabinet approved for working with infectious materials
- Sealable plastic bags
- RNase-free water (Eppendorf or equivalent)†
- 1.7 mL molecular biology grade microcentrifuge tubes (Dot Scientific, Inc. or equivalent)†
- Cotton Tip Applicators (Puritan or equivalent)†

† **Note: These 3 items are used in the procedure for Monitoring the Laboratory for the Presence of Contamination**.
Refer to the **QUALITY CONTROL PROCEDURES** section of this IFU.

Amplification Area

- Abbott *m*2000*rt* Instrument (*m*2000*rt* software version 8.1 or higher)
- Abbott *m*2000*rt* Operations Manual (List No. 06N03-009 or higher)
- Abbott RealTi*m*e SARS-CoV-2 Application Specification (List No. 09N77-001 or higher)
- Abbott *m*2000*rt* Optical Calibration Kit (List No. 4J71-93)

Other Materials

- Sealable plastic bags

Procedural Precautions

- Read the instructions in this IFU carefully before processing samples.
- The Abbott RealTi*m*e SARS-CoV-2 Negative Control and Positive Control vials are intended for single-use only and should be discarded after use.
- Use aerosol barrier pipette tips or disposable pipettes only one time when pipetting specimens or IC. To prevent contamination to the pipette barrel while pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Monitoring procedures for the presence of amplification product can be found in the **QUALITY CONTROL PROCEDURES** section in this IFU.
- To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.
- The Abbott RealTi*m*e SARS-CoV-2 Controls must be prepared in conjunction with the specimens to be tested. The use of the Abbott RealTi*m*e SARS-CoV-2 Controls is integral to the performance of the Abbott RealTi*m*e SARS-CoV-2 assay. Refer to the **QUALITY CONTROL PROCEDURES** section of this IFU for details.

ASSAY PROTOCOL

For a detailed description of how to perform an Abbott *m*2000*sp* instrument and Abbott *m*2000*rt* instrument protocol, refer to the Abbott *m*2000*sp* and Abbott *m*2000*rt* Operations Manuals, Operating Instructions sections.

Laboratory personnel must be trained to operate the Abbott *m*2000*sp* and Abbott *m*2000*rt* instruments. The operator must have a thorough knowledge of the applications run on the instruments and must follow good laboratory practices.

Sample Preparation Area

1. Thaw assay controls and IC at 15 to 30°C or at 2 to 8°C.
   - Once thawed, assay controls and IC can be stored at 2 to 8°C for up to 24 hours before use.
   - Vortex each assay control 3 times for 2 to 3 seconds before use. Ensure that the contents of each vial are at the bottom after vortexing by tapping the vials on the bench to bring liquid to the bottom of the vial. **NOTE: Avoid excessive foaming.**
2. Select amplification reagent packs to be used in the run. Refer to the Abbott *m*2000*sp* Operations Manual (List No. 09K20 version 9 or higher), Operating Instructions section, for instructions pertaining to amplification reagent pack inventory management. All amplification reagent packs used in runs of greater than 24 reactions must have the same lot number. Thaw amplification reagents at 15 to 30°C or at 2 to 8°C and store at 2 to 8°C until required for the amplification master mix procedure. Once thawed, the amplification reagents can be stored at 2 to 8°C for up to 24 hours if not used immediately.

The following table shows the number of sample preparation reagents and internal control vials needed based on the number of reactions.

Sample Preparation Reagents and Internal Control Requirements				
Reagent	**1 to 24 Reactions**	**25 to 48 Reactions**	**49 to 72 Reactions**	**73 to 96 Reactions**
*m*Microparticles	1 bottle	1 bottle	1 bottle	1 bottle
*m*Lysis	1 bottle	2 bottles	3 bottles	4 bottles
*m*Wash 1	1 bottle	1 bottle	2 bottles	2 bottles
*m*Wash 2	1 bottle	1 bottle	2 bottles	2 bottles
*m*Elution Buffer	1 bottle	1 bottle	1 bottle	1 bottle
Internal Control	1 vial	2 vials	3 vials	4 vials

Abbott *m*2000*sp* Procedure

3. Gently invert the Abbott *m*Sample Preparation bottles to ensure a homogeneous solution. If crystals are observed in any of the reagent bottles upon opening, allow the reagent to equilibrate at room temperature until the crystals disappear. Do not use the reagents until the crystals have dissolved. Add USP Grade 190 to 200 Proof Ethanol (95 to 100% Ethanol) to the *m*Lysis$_{DNA}$, *m*Wash1$_{DNA}$, and *m*Wash2$_{DNA}$ bottles as indicated below. Do not use ethanol that contains denaturants.
   - Add 35 mL ethanol to each bottle of *m*Lysis$_{DNA}$ being used.

- Add 23 mL ethanol to each bottle of *m*Wash1$_{DNA}$ being used.
- Add 70 mL ethanol to each bottle of *m*Wash2$_{DNA}$ being used.

4. Vortex each IC 3 times for 2 to 3 seconds before use.
5. Use a calibrated precision **PIPETTE DEDICATED FOR INTERNAL CONTROL USE ONLY** to add 1200µL of IC to each bottle of *m*Lysis Buffer. Mix by gently inverting the container 5 to 10 times to minimize foaming and pour the contents into the appropriate reagent vessels per the table above. When pouring in 2 bottles of the *m*Lysis$_{DNA}$ with the Ethanol and IC, fill reagent vessel no higher than the fill line where the top of the reagent label is placed. Ensure bubbles or foam are not generated in the reagent vessels; if present, remove with a sterile pipette tip, using a new tip for each reagent vessel.
6. Gently pour in remaining Abbott *m*Sample Preparation bottles into the reagent vessels per the table above except for *m*Microparticles$_{DNA}$ which will be loaded later.
   A total of 96 samples can be processed in each run. A negative control and a positive control are included in each run, therefore allowing a maximum of 94 specimens to be processed per run.
   - The Abbott RealTi*m*e SARS-CoV-2 assay minimum sample volume and associated rack requirements on the Abbott *m*2000*sp* are:

Rack	Tube Diameter[a]	Minimum Volume
13 mm	11.5 - 14.0 mm	1.3 mL
16 mm	14.5 - 16.0 mm	1.3 mL

[a] Refers to sample tube outer diameter. Minimum sample volume varies with tube geometry and size. Refer to the Abbott *m*2000*sp* Operations Manual and **QUICK REFERENCE GUIDE FOR SAMPLE TUBE SIZES AND VOLUMES** for recommended sample input volume.

   - If frozen, thaw specimens at 15 to 30°C or at 2 to 8°C. Once thawed, specimens can be stored at 2 to 8°C for up to 6 hours if not processed immediately.

   NOTE: For every stored specimen, if centrifugation is needed, the following actions must be done in the order described: vortex the specimen first and follow with centrifugation of nasopharnygeal and oropharyneal swab specimens. If these actions are not performed in this order, then invalid results may occur.

   - Vortex each specimen 3 times for 2 to 3 seconds.
   - If needed, centrifuge nasopharyngeal and oropharyngeal swab specimens only at 2000 *g* for 5 minutes before loading onto the Abbott *m*2000*sp* worktable. Aliquot each specimen into clean tubes or vials if necessary. Refer to the Abbott *m*2000*sp* Operations Manual for tube sizes. Avoid touching the inside of the cap when opening tubes.

   Refer to the Abbott *m*2000*sp* Operations Manual for tube sizes.
7. Remove cap. Avoid touching the inside of the cap when opening tubes. Remove swab if present.
8. Place the positive and negative controls, if applicable, and the patient specimens into the Abbott *m*2000*sp* sample rack. If used, bar codes on tube labels must face right for scanning.
9. Place the 5 mL Reaction Vessels into the Abbott *m*2000*sp* 1 mL subsystem carrier.
10. Immediately prior to initiation of the sample extraction protocol, vigorously mix or vortex the *m*Microparticles$_{DNA}$ until they are fully resuspended and pour the *m*Microparticles$_{DNA}$ into the appropriate 200 mL reagent vessel.
11. Load the Abbott *m*Sample Preparation System$_{DNA}$ reagents and the Abbott 96 Deep-Well Plate on the Abbott *m*2000*sp* worktable as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
12. From the Protocol screen, select the appropriate application file and initiate the sample extraction protocol as described in the Abbott *m*2000*sp* Operations Manual, Operating Instruction section.
    - **The application specification file m2000 SARS_CoV-2 is required for nasopharnygeal and oropharyngeal swab specimens.**
    - **The Abbott *m*2000*sp* Master Mix Addition protocol (step 14) must be initiated within 1 hour after completion of Sample Preparation.**

      NOTE: Change gloves before handling the amplification reagents.
13. Load the amplification reagents and the master mix tube on the Abbott *m*2000*sp* worktable after sample preparation is completed.
    The following table shows the number of amplification reagent packs needed based on the number of reactions.

Amplification Reagent Pack Requirements			
1 to 24 Reactions	**25 to 48 Reactions**	**49 to 72 Reactions**	**73 to 96 Reactions**
1 pack	2 packs	3 packs	4 packs

    - All amplification reagent packs used in runs of greater than 24 reactions must have the same lot number.
    - Ensure that the contents of amplification reagent packs are at the bottom of the vials prior to opening the amplification reagents by tapping the vials in an upright position on the bench 5 to 10 times.
    - **Ensure that amplification reagent packs are firmly seated on the instrument.**
14. Select the appropriate deep-well plate that matches the corresponding sample preparation extraction. Initiate the Abbott *m*2000*sp* Master Mix Addition protocol. Follow the instructions as described in the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.

    NOTE: The operator should not manually fill any empty/unfilled wells in the Abbott 96-Well Optical Reaction Plate.

    - **The Abbott *m*2000*rt* protocol (step 18) must be started within 50 minutes of the initiation of the Master Mix Addition protocol (step 14).**

      NOTE: If the run is aborted for any reason subsequent to step 14, a new 96-well PCR plate must be used if the Abbott *m*2000*sp* Master Mix Addition Protocol (step 14) will be repeated.

Amplification Area

15. Switch on and initialize the Abbott *m*2000*rt* instrument in the Amplification Area.

    NOTE: The Abbott *m*2000*rt* requires 15 minutes to warm-up.

    NOTE: Remove gloves before returning to the sample preparation area.
16. Seal the Abbott 96-Well Optical Reaction Plate according to the Abbott *m*2000*sp* Operations Manual, Operating Instructions section.
17. Place the sealed optical reaction plate into the Abbott Splash-Free Support Base for transfer to the Abbott *m*2000*rt* instrument. Export the completed PCR plate results to a CD (or directly to a mapped Abbott *m*2000*rt* via a network connection).

Abbott *m*2000*rt* Procedures

For a detailed description of how to perform the Abbott *m*2000*rt* SARS-CoV-2 assay protocol, refer to the Operating Instructions section in the Abbott *m*2000*rt* Operations Manual.

18. Place the Abbott 96-Well Optical Reaction Plate in the Abbott *m*2000*rt* instrument. Initiate the Abbott RealTi*m*e SARS-CoV-2 assay protocol (***m*2000 SARS-CoV-2)**, as described in the Abbott *m*2000*rt* Operations Manual, Operating Instructions section.

    NOTE: Test order transfer through the use of CD-ROM or network connection with export and import features of the *m*2000*sp* and *m*2000*rt* software is recommended. If creating the Abbott *m*2000*rt* test order manually, enter sample IDs in the corresponding PCR tray locations according to the "Wells for Selected Plate" grid, found on the detail screen of the "PCR Plate Results" on the Abbott *m*2000*sp*. See Section 5 of the Abbott *m*2000*sp* Operations Manual.

POST PROCESSING PROCEDURES

1. Remove the Abbott 96 Deep-Well Plate from the worktable and dispose of according to the Abbott *m*2000*sp* Operations Manual.
2. Place the Abbott 96-Well Optical Reaction Plate in a sealable plastic bag and dispose according to the Abbott *m*2000*rt* Operations Manual along with the gloves used to handle the plate.
3. Clean the Abbott Splash-Free Support Base before next use, according to the Abbott *m*2000*rt* Operations Manual.

QUALITY CONTROL PROCEDURES

Abbott *m*2000*rt* Optical Calibration

Refer to the Calibration Procedures section in the Abbott *m*2000*rt* Operations Manual for a detailed description of when and how to perform an Abbott *m*2000*rt* Optical Calibration.

Optical calibration of the Abbott *m*2000*rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the Abbott RealTi*m*e SARS-CoV-2 assay.

The following Abbott *m*2000*rt* Optical Calibration Plates are used to calibrate the Abbott *m*2000*rt* instrument for the Abbott RealTi*m*e SARS-CoV-2 assay:

- FAM™ Plate (Carboxyfluorescein)
- ROX™ Plate (Carboxy-X-rhodamine)
- VIC® Plate (Proprietary dye)

Detection of Inhibition

A defined, consistent quantity of IC nucleic acid is introduced into each specimen and control at the beginning of sample preparation and measured on the Abbott *m*2000*rt* to demonstrate proper specimen processing and assay validity. The IC is comprised of a RNA sequence unrelated to the SARS-CoV-2 virus target sequences. An IC CN validity range is defined within the Abbott RealTi*m*e SARS-CoV-2 Assay Application File.

An error code or flag is displayed when a specimen or control fails to meet the IC specification. Refer to **INTERPRETATION OF RESULTS** section of this IFU and the Abbott *m*2000*rt* System Operations Manual for a list of error codes and flags.

Negative and Positive Controls

A negative control and a positive control are included in each test order to evaluate run validity in order to generate a valid result.

The Abbott *m*2000*rt* instrument automatically reports the control results on the Abbott *m*2000*rt* workstation. An error control flag is displayed when a control result is out of range. Refer to the Abbott *m*2000*rt* Operations Manual for an explanation of the corrective actions for the error control flag. If negative or positive controls are out of range, all of the specimens and controls from that run must be reprocessed, beginning with sample preparation.

The presence of the SARS-CoV-2 virus must not be detected in the negative control. SARS-CoV-2 virus detected in the negative control is indicative of contamination by other samples or by amplified product introduced during sample preparation or during preparation of the Abbott 96-Well Optical Reaction Plate. To avoid contamination, clean the Abbott *m*2000*sp* instrument and the Abbott *m*2000*rt* instrument and repeat sample processing for controls and specimens following the **Procedural Precautions**. If negative controls are persistently reactive, contact your Abbott representative.

Monitoring the Laboratory for the Presence of Contamination

It is recommended that this test be done at least once a month to monitor laboratory surfaces and equipment for contamination by amplification product. It is very important to test all areas that may have been exposed to processed specimens, controls, and/or amplification product. This includes routinely handled objects such as pipettes, the Abbott *m*2000*sp* and Abbott *m*2000*rt* function keys, laboratory bench surfaces, microcentrifuges, and centrifuge adaptors.

1. Add 0.8 mL RNase-free water to a 1.7 mL molecular biology grade microcentrifuge tube.
2. Saturate the cotton tip of an applicator (Puritan or equivalent) in the RNase-free water from the microcentrifuge tube.
3. Using the saturated cotton tip of the applicator, wipe the area to be monitored using a sweeping motion. Place the applicator into the microcentrifuge tube.
4. Swirl the cotton tip in RNase-free water 10 times, and then press the applicator along the inside of the tube so that the liquid drains back into the solution at the bottom of the microcentrifuge tube. Discard the applicator.
5. Pipette 0.5 mL of *m*Wash 1 buffer to a clean tube using the pipette dedicated for Internal Control use.
6. Add 20 µL of the *m*Wash 1 buffer to each microcentrifuge tube.
7. Cap the microcentrifuge tube.
8. Test this sample according to the assay procedure section of this study brochure.
    - Transfer liquid from the microcentrifuge tube to a 5 mL Reaction Vessel.
    - Bring the volume to 1.5 mL with RNase-free water.
9. The presence of contamination is indicated by the detection of SARS-CoV-2 nucleic acid in the swab samples.
10. If SARS-CoV-2 nucleic acid is detected on equipment, follow the cleaning and decontaminating guidelines given in that equipment's operations manual.
    If SARS-CoV-2 nucleic acid is detected on surfaces, clean the contaminated areas with 1.0% (v/v) sodium hypochlorite solution, followed by 70% ethanol or water.

    NOTE: Chlorine solutions may pit equipment and metal. Use sufficient amounts or repeated applications of 70% ethanol or water until chlorine residue is no longer visible.
11. Repeat testing of the contaminated area by following steps 1 through 10.

INTERPRETATION OF RESULTS

The Abbott *m*2000*rt* instrument automatically reports the results and interpretations on the Abbott *m*2000*rt* workstation. An error is displayed when a result is invalid. Assay results and interpretations will look similar to the following examples:

Location	Sample ID	Sample Type	Assay	Result	Interpretation	Flags	Error Code
A1	CoV-2_NEG	Control	SARS-CoV-2	Not Detected			
A1	CoV-2_NEG	Control	SARS-CoV-2				XXXX[1]
B1	CoV-2_POS	Control	SARS-CoV-2	XX.XX CN			
B1	CoV-2_POS	Control	SARS-CoV-2				XXXX[2]
C1	Sample 1		SARS-CoV-2	XX.XX CN	Positive		
D1	Sample 2		SARS-CoV-2	Not Detected	Negative		
E1	Sample 3		SARS-CoV-2	XX.XX CN	Positive	IC[3]	
F1	Sample 4		SARS-CoV-2				XXXX[4]
G1	Sample 5		SARS-CoV-2	Not Detected	Negative	-QC, +QC[5]	
H1	Sample 6		SARS-CoV-2	XX.XX CN	Positive	-QC, +QC[5]	

[1] Error code generated due to negative control failure.

[2] Error code generated due to positive control failure

[3] Patient sample with positive amplification of target but failed internal control will produce valid result with a flag for internal control failure.

[4] Error code generated due to no amplification of target and internal control failure.

[5] Indicates a failed control, invalidating all results in the run. Users are instructed to rerun the samples starting at sample preparation.

For more information about error codes and flags, refer to the Abbott *m*2000*rt* Operations Manual.

LIMITATIONS OF THE PROCEDURE

- This assay is for in vitro diagnostic use.
- Use of the Abbott RealTi*m*e SARS-CoV-2 assay is limited to personnel who have been trained in the procedures of a molecular diagnostic assay and the Abbott *m*2000 System.
- Laboratories are required to report all positive results to the appropriate public health authorities.
- The instruments and assay procedures reduce the risk of contamination by amplification product. However, nucleic acid contamination from the positive controls or specimens must be controlled by good laboratory practices and careful adherence to the procedures specified in this IFU.
- Optimal performance of this test requires appropriate specimen collection, storage, and transport to the test site (refer to the **SPECIMEN COLLECTION, STORAGE, AND TRANSPORT TO THE TEST SITE** section of this IFU).
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (eg, presence of symptoms), and/or stage of infection.
- False-negative results may arise from degradation of the viral RNA during shipping/storage.
- 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 of Abbott RealTi*m*e SARS-CoV-2 assay could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.
- Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
- 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. Viral detection tests should assist in the decision on when to discontinue additional precautions for hospitalised patients.

SPECIFIC PERFORMANCE CHARACTERISTICS

Limit of Detection (Analytical Sensitivity)

Limit of Detection (LOD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater than or equal to 95% of all (true positive) replicates test positive.

To determine the LOD, a recombinant virus containing SARS-CoV-2 RNA (Seracare, AccuPlex COVID-19, 1.3E+07 copies/mL as determined by digital PCR) was serially diluted in simulated nasal matrix (SNM). The initial LOD was determined by testing 4 levels at target concentrations of 900, 300, 100, and 33 copies/mL. Each panel member was tested in replicates of 3. The final LOD was confirmed by testing 4 panel members with target concentrations at 400, 300, 200, and 100 copies/mL tested in replicates of 21.

The results are summarized in **Table 1.** The lowest concentration level with observed positive rates ≥ 95% was 100 virus copies/mL.

Table 1. LOD Determination Using Recombinant Virus Containing SARS-CoV-2

Virus Copies/mL	GE/Reaction[a]	Total Valid Replicates	Positive Replicates	Positive Rate (%)
400	12.5	21	21	100
300	9.4	21	21	100
200	6.2	21	21	100
100	3.1	21	20	95.2

[a] Genome equivalent per reaction (GE/reaction) was determined from calibration curve established using genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WA1/2020 (BEI Resources, Catalog No. NR-52285).

Inclusivity

Inclusivity was demonstrated by comparing the Abbott RealTi*m*e SARS-CoV-2 assay primers and probes to an alignment of all SARS-CoV-2 sequences available in Genbank as of March 5, 2020. The MUSCLE alignment was generated by NCBI (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/virus?SeqType_s=Nucleotide&VirusLineage_ss=SARS-CoV-2,%20taxid:2697049) and viewed in Bioedit.

The regions of the test's primers and probes were compared by in silico analysis to verify sequence homology with circulating SARS-CoV-2 strains. A total of 78 sequences from 10 countries (Australia, Belgium, Brazil, China, Finland, Nepal, South Korea, Sweden, Taiwan, and USA) had sequence coverage of at least one of the test's primers or probes for the comparison. Amongst the 78 sequences, there were also 6 strains without any country information listed in Genbank. All of the primers and probes in the test had 100% homology to all of the available circulating SARS-CoV-2 sequences.

Cross-reactivity

In Silico Analysis

Related pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen have been evaluated in silico to identify the % homology between the selected probe/primer sequences and the sequence present in the microorganism.

The conclusion of this analysis is that there is limited opportunity for cross-reactivity to allow for false-positive reporting or affect performance of SARS-CoV-2 virus detection based upon the following:

- For many organisms, only one primer (forward or reverse) has >80% homology, making an amplified product unlikely.
- The probe is unlikely to bind for any of the hits (< 80% homology)
- Mismatches in the 3′ end of primers makes extension unlikely
- For the N amplicon, two organisms with forward and reverse primers having >80% homology (LS483366.1, CP040804.1) have both primer binding sites on the same plus-sense strand and will not result in amplification.
- For the N amplicon, the remaining two organisms that may potentially give rise to amplicons due to both forward and reverse primers having >80% homology on opposite strands (CP000262.1, CP002888.1) have primer binding sites separated by >100,000 nucleotides in the bacterial chromosome, making amplification unlikely.

Overall, the results of this analysis predict no significant cross-reactivity or microbial interference.

Laboratory Testing

Cross reactivity performance of Abbott RealTi*m*e SARS-CoV-2 assay was evaluated by testing whole organisms or appropriate representative samples listed below in **Table 2**.

No cross-reactivity of the Abbott RealTi*m*e SARS-CoV-2 assay with the selected microorganisms was observed at the concentrations tested. The results are summarized in **Table 2**.

Table 2. Abbott RealTi*m*e SARS-CoV-2 Cross-reactivity Summary

Microorganism	Concentration	Result (No. Positive/No. Tested)	Final Result
Human coronavirus 229E	1.00 x 10^5 Copies/mL	0/4	Negative
Human coronavirus OC43	1.00 x 10^5 Copies/mL	0/4	Negative
Human coronavirus HKU1	Clinical Isolates	0/2	Negative
Human coronavirus NL63	1.00 x 10^5 Copies/mL	0/4	Negative
SARS-coronavirus	25-28 (Ct range)	0/4	Negative
MERS-coronavirus	25-28 (Ct range)	0/4	Negative
Adenovirus (Ad. 71)	1.00 x 10^5 TCID50/mL	0/4	Negative
Human Metapneumovirus (hMPV)	Clinical Isolates	0/3	Negative
Parainfluenza virus 1	1.00 x 10^5 TCID50/mL	0/4	Negative
Parainfluenza virus 2	1.00 x 10^5 Copies/mL	0/4	Negative
Parainfluenza virus 3	5.00 x 10^5 TCID50/mL	0/4	Negative
Parainfluenza virus 4	Clinical Isolates	0/4	Negative
Influenza A (H1N1)	1.00 x 10^5 Copies/mL	0/4	Negative
Influenza A /(H3N2)	1.00 x 10^5 Copies/mL	0/4	Negative
Influenza B	1.00 x 10^5 Copies/mL	0/4	Negative
Enterovirus Type 71	1.00 x 10^5 TCID50/mL	0/4	Negative
Respiratory syncytial virus	1.00 x 10^5 Copies/mL	0/4	Negative
Rhinovirus	1.00 x 10^5 Copies/mL	0/4	Negative
Chlamydia pneumoniae	1.00 x 10^6 IFU/mL	0/4	Negative
Haemophilus influenzae	1.00 x 10^6 CFU/mL	0/4	Negative
Legionella pneumophila	1.00 x 10^6 CFU/mL	0/3	Negative
Mycobacterium tuberculosis	1.00 x 10^6 CFU/mL	0/4	Negative
Streptococcus pneumoniae	1.00 x 10^6 CFU/mL	0/4	Negative

Table 2. Abbott RealTi*m*e SARS-CoV-2 Cross-reactivity Summary

Microorganism	Concentration	Result (No. Positive/No. Tested)	Final Result
Streptococcus pyogenes	1.00 x 10^6 CFU/mL	0/4	Negative
Bordetella pertussis	1.00 x 10^6 CFU/mL	0/3	Negative
Mycoplasma pneumoniae	1.00 x 10^6 CFU/mL	0/4	Negative
Pneumocystis jirovecii (PJP)	23-25 (Ct range)	0/4	Negative
Candida albicans	1.00 x 10^5 CFU/mL	0/4	Negative
Pseudomonas aeruginosa	1.00 x 10^6 CFU/mL	0/4	Negative
Staphylococcus epidermis	1.00 x 10^6 CFU/mL	0/4	Negative
S. salivarius	1.00 x 10^6 CFU/mL	0/4	Negative

Clinical Performance Evaluation

A clinical evaluation study was performed to evaluate the performance of the Abbott RealTi*m*e SARS-CoV-2 Assay using nasopharyngeal swab specimens. A total of 61 contrived positive specimens at approximately 1X to 2X LOD and 20x LOD were tested. Samples were contrived by spiking known concentrations of recombinant virus containing SARS-CoV-2 RNA sequences into negative patient specimens. In addition to the contrived positive specimens, 34 negative specimens were tested.

There were 21 total samples tested at the 1X to 2X LOD level with 20 results valid and included in the analysis. One result was invalid and excluded from the analysis. There were 40 total samples tested at 20x LOD with 40 results valid and included in the analysis. There were 34 total samples tested for the negative level with 31 results valid and included in the analysis and 3 results invalid and excluded from the analysis.

Table 3. Clinical Evaluation of the Abbott RealTi*m*e SARS-CoV-2 Assay

SARS-CoV-2 Concentration	Number Tested	Number Detected	% Detection
1X to 2X LOD[a]	20	20	100 (N=20/20)
20X LOD	40	40	100 (N=40/40)
Negative[b]	31	0	0 (N=0/31)

[a] One replicate was invalid and excluded from the analysis.

[b] Three replicates were invalid and excluded from the analysis.

	N	Agreement	95% CI
PPA	60	100%	(94.0, 100.0)
NPA	31	100%	(88.8, 100.0)

PPA – Positive Percent Agreement

NPA – Negative Percent Agreement

BIBLIOGRAPHY

1. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. *Type*> www.cdc.gov, *search*>BMBL>look up sections III and IV.]
2. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
3. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Fourth Edition*. CLSI Document M29-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
4. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.
5. Clinical and Laboratory Standards Institute. *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline.* CLSI Document MM13-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
6. European Centre for Disease Prevention and Control (ECDC). *Laboratory support by specialised laboratories in the EU/EEA*. At: ecdc.europa.eu/en/novel-coronavirus/laboratory; updated 8 February 2020. Accessed 19 March 2020.

IN VITRO DIAGNOSTIC MEDICAL DEVICE

TECHNICAL ASSISTANCE

For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042 in the US and from outside the US at +49-6122-580 or email molecularsupport@abbott.com, or visit the Abbott Molecular website at http://www.abbottmolecular.com.

Abbott Molecular Inc. is the legal manufacturer of the:

Abbott RealTi*m*e SARS-CoV-2 Amplification Reagent Kit (List No. 09N77-090)

Abbott RealTi*m*e SARS-CoV-2 Control Kit (List No. 09N77-080)

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Abbott

Abbott RealTi*me* SARS-CoV-2

Control Kit

en

REF 09N77-080

51-608443/R2

IVD

REF 09N77-080

51-608443/R2

Note:Changes Highlighted

Key to Symbols used	
REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
In Vitro Test	In Vitro Test
	Use By
CONTROL –	Negative Control
CONTROL +	Positive Control
	Temperature limit
	Consult instructions for use
	Corrosive
	Caution
EC REP	Authorized Representative in the European Community
	Manufacturer

Instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from these instructions.

Intended Use

The Abbott RealTi*me* SARS-CoV-2 controls are used to establish run validity of the Abbott RealTi*me* SARS-CoV-2 assay when used for the direct, qualitative detection of SARS-CoV-2 RNA.

Contents

1. CONTROL – **Abbott RealTi*me* SARS-CoV-2 Negative Control (8 vials, 1.3 mL per vial)** Contains 1.0% ammonium sulfate and 7.9% detergent in a buffer solution.
2. CONTROL + **Abbott RealTi*me* SARS-CoV-2 Positive Control (8 vials, 1.3 mL per vial)** Contains non-infectious, recombinant Sindbis virus containing SARS-CoV-2 RNA sequences, 1.0% ammonium sulfate, and 7.9% detergent in a buffer solution.

- The Abbott RealTi*me* SARS-CoV-2 controls are intended for single-use only and unused reagents should be discarded.
- The Abbott RealTi*me* SARS-CoV-2 Control Kit must only be used with the Abbott RealTi*me* SARS-CoV-2 assay (List No. 09N77).

Warnings and Precautions

- For In Vitro Diagnostic Use.
- Do not use beyond expiration date.

CAUTION: This preparation contains human sourced and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inactivated micro-organisms will not transmit infection. These reagents and human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories, [1] OSHA Standards on Bloodborne Pathogens, [2] CLSI Document M29-A4,[3] and other appropriate biosafety practices. [4] Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- 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 a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[1]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state and federal regulations.[4]

Components of the Abbott RealTi*me* SARS-CoV-2 Control Kit (List No. 09N77-80) contain the following components:

- Abbott RealTi*me* SARS-CoV-2 Negative Control
- Abbott RealTi*me* SARS-CoV-2 Positive Control

The following warnings apply:

Danger: Hazard-determining components of labeling:
Lithium dodecyl sulphate
Lithium hydroxide monohydrate

H318	Causes serious eye damage.
H316	Causes mild skin irritation.*
P280	Wear protective gloves / protective clothing / eye protection.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor physician.
P332+P313	If skin irritation occurs: Get medical advice attention.*

* Not applicable where regulation EC 1272/2008 (CLP) or OSHA Hazard Communication 29 CFR 1910.1200 (HCS) 2012 have been implemented.

Safety Data Sheet Statement: Important information regarding the safe handling, transport and disposal of this product is contained in the Safety Data Sheet.

Consult the Abbott RealTi*me* SARS-CoV-2 instructions for use.

-15°C -25°C **The Abbott RealTi*me* SARS-CoV-2 Negative and Positive Controls must be stored at -25°C to -15°C**

Shipping Conditions

Abbott RealTi*me* SARS-CoV-2 Control Kit: Ship on dry ice. If you receive reagents that are in a condition contrary to label recommendation, or that are damaged, contact your Abbott Representative.

BIBLIOGRAPHY

1. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. *Type*> www.cdc.gov, *search*>BMBL>*look up* sections III and IV.]
2. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
3. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Fourth Edition*. CLSI Document M29-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
4. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.

For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042, email molecularsupport@abbott.com, or visit the Abbott Molecular website at http://www.abbottmolecular.com.

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