SARS-CoV-2 IgM(CLIA)

Severe Acute Respiratory Syndrome Coronavirus 2 IgM (CLIA)

Order Information

Catalog No.	Package Size
SARS-CoV-2 IgM121	2×50 tests (calibrators included)
SARS-CoV-2 IgM122	2×100 tests (calibrators included)
SARS-CoV-2 IgM111	2×50 tests (calibrators not included)
SARS-CoV-2 IgM112	2×100 tests (calibrators not included)

Intended Use

The Mindray SARS-CoV-2 IgM assay is a Chemiluminescent Immunoassay for the qualitative determination of SARS-CoV-2 IgM antibodies in human serum or plasma from suspected COVID-19 patients.

The SARS-CoV-2 IgM assay is only intended for the supplementary indicator for suspected cases of negative SARS-CoV-2 nucleic acid detection, or combination with nucleic acid detection in the diagnosis of suspected cases. Results from antibody testing should not be used as the sole basis to diagnosis or exclude SARS-CoV-2 infection. It is not intended for screening in general population.

Summary

Muptiple cases of unexplained pneumonia patients have been successively reported in Wuhan City, Hubei Province of China since December 2019. The pathogen was then identified as a new coronavirus, which was tentatively named as 2019-nCoV(2019 Novel Coronavirus) by the WHO and then formally designated as SARS-CoV-2(Severe Acute Respiratory Syndrome Coronavirus 2) by International Committee on Taxonomy of Viruses(ICTV) on Febury 11, 2020. The disease caused by the pathogen was named as COVID-19(Coronavirus Disease 2019) by the WHO at the same day.

SARS-CoV-2 belongs to β-coronaviruses and is an enveloped positive-sense single-stranded RNA virus[1]. It is spread by human-to-human transmission via droplets or direct contact.

Many patients with confirmed COVID-19 have developed fever and/or symptoms of acute respiratory illness (e.g., cough, difficulty breathing). However, limited information is currently available to characterize the full spectrum of clinical illness associated with COVID-19.

Although coronaviruses usually infect the upper or lower respiratory tract, viral shedding in plasma or serum is common.

Currently, virus nucleic acid Real-Time Reverse Transcription PCR(Real-Time RT-PCR), CT imaging and some hematology parameters are the primary tools for clinical diagnosis of the infection. The virus nucleic acid RT-PCR test has become as the current standard diagnostic method for COVID-19[2].

Testing of specific antibodies of SARS-CoV-2 in patient blood is a good choice for rapid, simple, highly sensitive diagnosis of COVID-19. It is widely accepted that IgM is the first antibody elicited in an immune response following immunization or infection, prior to the generation of adaptive, high affinity IgG responses that are important for long term immunity and immunological memory. Therefore, detection of IgM antibodies tends to indicate a recent exposure to SARS-CoV-2.[3]

Assay Principles

The CL-series SARS-CoV-2 IgM assay is a two step assay to qualitatively detect IgM antibodies to SARS-CoV-2.

In the first step, sample, sample treatment solution, paramagnetic microparticles coated with SARS-CoV-2 antigens are added into a reaction vessel. After incubation, SARS-CoV-2 IgM antibodies in the sample will bind to SARS-CoV-2 antigen coated microparticles. Afterwards, microparticles are magnetically captured while other unbound substances are removed by washing.

In the second step, diluent solution, ALP labeled anti-human IgM monoclonal antibody are added to the reaction vessel. After incubation, ALP labeled anti-huamn IgM monoclonal antibody will form sandwich with microparticle captured SARS-CoV-2 IgM antibodies. Afterwards, microparticles are magnetically captured while other unbound substances are removed by washing.

In the third step, the substrate solution is added to the reaction vessel. It is catalyzed by anti-human IgM antibody-ALP conjugate in the immune-complex retained on the microparticles. The resulting chemiluminescent reaction is measured as relative light units (RLUs) by a photomultiplier built into the system.

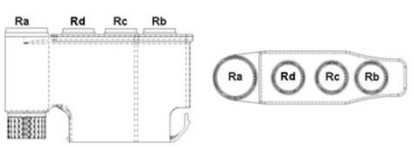
A direct relationship exists between the amount of SARS-CoV-2 IgM antibodies in the sample and the RLUs generated during the reaction. The presence or absence of analytes in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from calibration. A cutoff index (COI) is calculated by Sample RLU/Cutoff RLU.

Reagent Components

The reagent kit is composed of four components: Ra, Rb, Rc, and Rd. The component cannot be exchanged, and the detailed information of each component is listed below:

Ra	Paramagnetic microparticles coated with SARS-CoV-2 specific antigens inMES buffer with preservative.
Rb	ALP labeled anti-human IgM monoclonal antibody(mouse IgG) in MES buffer with preservative.
Rc	Sample diluents in TRIS buffer with preservative.
Rd	Blockers in TRIS buffer with preservative.
Calibrators C0 and C1 (optional)	IgM antibodies to SARS-CoV-2 of different levels in buffer with preservative

The position of each component is shown in the figure below（front view on the left and top view on the right）:



Storage and Stability

The unopened SARS-CoV-2 IgM reagent kit is stable up to the expiration date as indicated on the label when stored at 2~8 °C. The actual expiration date is stated on the label.

The SARS-CoV-2 IgM reagent kit can be stored onboard at 2~8 °C and used for a maximum of 7 days after opening for use.

Reagent Preparation

Ra: Ready to use

Rb: Ready to use

Rc: Ready to use

Rd: Ready to use

C0：Ready to use

C1：Ready to use

Materials Required but not Provided

Mindray CL-series Chemiluminescence Immunoassay Analyzer

Cat. No. CS511: Substrate Solution, 4×115mL

Cat. No. WB411: Wash Buffer

Reaction Vessel

Instrument System

Mindray CL-series Chemiluminescence Immunoassay Analyzer

Specimen Collection and Preparation

Human serum, heparin plasma or EDTA plasma is suitable for the test. Human serum is recommended.

Specimens must be separated from clots or red blood cells using centrifugation as recommended by the tube manufacturer after clot formation is complete. Specimens should be tested as soon as possible after sample collection and pre-analytical treatment.

If testing is not completed within 24 hours, transfer the supernatant into tubes for storage. Specimens tightly capped are stable for 7 days refrigerated at 2~8°C. If testing will be delayed for more than 7 days, specimens should be frozen at -20°C or below. The specimen can be stored at -20°C for as long as 10 days.

Avoid repeated freeze and thaw cycles, which may cause sample deterioration. Specimen can be used after a maximum of five cycles of freeze and thaw.

Do not use specimen with the following conditions:

- grossly hemolyzed
- obvious microbial contamination
- visible fibrin or other debris

Assay Procedure

For optimal performance of this assay, operators should read the related system operation manual carefully to get sufficient information such as operation instructions, sample preservation and management, safety precautions, and maintenance. Prepare all required materials for the assay as well.

Before loading the SARS-CoV-2 IgM reagent kit on the instrument for the first time, invert unopened reagent bottle gently for at least 30 times to resuspend the microparticles, which have settled during shipment or storage. Visually inspect the bottle to ensure the microparticles have been well mixed. If the microparticles remain adhered to the bottle, continue inverting until the microparticles have been completely mixed. If the microparticles cannot be homogenized, it is recommended not to use this bottle of reagent. Contact Mindray Customer Service for help. Do not invert opened reagent bottle.

This assay requires 10 μL of sample volume for a single test. This volume does not include the dead volume of the sample container. Additional volume is required when performing additional tests from the same sample. Operators should refer to the system operation manual and specific requirement of the assay to determine the minimum sample volume.

Calibration

The calibrators are traceable to Mindray internal SARS-CoV-2 IgM reference.

The calibration information is stored in the barcode attached in the reagent and calibrator pack. When performing the calibration, scan the information from the barcodes into the system first, and then test the calibrators of two levels. A valid calibration is required before any SARS-CoV-2 IgM test. Recalibration is recommended every 7 days, or when a new reagent lot is used, or when the quality controls are out of specified ranges. For detailed instruction of calibration, refer to the system operation manual.

Control

Users can prepare quality controls with clinical samples or use third-party controls. Reference ranges can be established according to protocol approved by individual laboratories.

It is recommended that quality controls should be run once every 24 hours if the tests are in use, or after every calibration. The quality control frequency should be adapted to each laboratory's individual requirements.

Quality control results should be within the acceptable ranges. If a control is out of its specified range, the associated test results are invalid and the samples must be retested. Recalibration may be required. Refer to the system operation manual to check up the system. If the quality control results are still out of the specified ranges, please contact Mindray Customer Service for help.

Calculation

The CL-series analyzer calculates the mean RLU signals of both calibrator C1 and C0.

The CL-series analyzer calculates SARS-CoV-2 IgM result based on the ratio of the sample RLU to the cutoff RLU (Cutoff Index, COI) for each specimen and control.

Cutoff RLU = [(Mean RLU of C1 − Mean RLU of C0) x Calibration Coefficient] + Mean RLU of C0

Calibration Coefficient is specific for each reagent and calibrator lot.

COI = Sample RLU/Cutoff RLU

Specimen Dilution

Specimens cannot be diluted for Mindray SARS-CoV-2 IgM assay.

Interpretation of Results

Specimens with a COI<1.00 are negative for IgM antibodies to SARS-CoV-2. A negative result can not rule out COVID-19.

Specimens with a COI≥1.00 are considered positive for IgM antibodies to SARS-CoV-2, suggesting recent infection. Positive test results need further confirmation.

The assay results should not be used solely for confirmation or exclusion of COVID-19. Clinical decisions should be made in conjunction with other evidences, such as symptoms, clinical history, results of nucleic acid test, etc.

Limitation of Measurement

The SARS-CoV-2 IgM result of a given specimen can vary, depending on the assays from different manufacturers, which have differences in assay methods, calibration, and reagent specificity.

If the SARS-CoV-2 IgM results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

As with all tests containing monoclonal mouse antibodies, anomalous results maybe obtained from specimens taken from patients who have received monoclonal mouse antibodies for diagnosis or therapy in rare cases[4,5].

IgM rheumatoid factor (RF) in combination with SARS-CoV-2 specific IgG can lead to false reactive results in IgM detecting assays. The pretreatment of samples in the Mindray SARS-CoV-2 IgM assay minimizes RF interference, however, in rare cases interference caused by high concentrations of RFs

and SARS-CoV-2 specific IgG cannot be excluded.

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.[6] Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

Results may differ between laboratories due to the variations in population. It is recommended that each laboratory establish its own reference range.

Performance Characteristics

Precision

The CL-series SARS-COV-2 IgM assay is designed to have a precision of ≤10% (within-device CV). Precision was determined by following National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP15-A3[7]. Three levels of samples were tested one run per day, five replicates per run, for a total of 5 days, using a single lot of reagents and a single calibration. Results are shown in the table below*.

Sample	Average (COI)	SD(COI)	%CV
1	0.05	0.00	/
2	1.14	0.03	2.62%
3	2.24	0.07	3.18%

**Representative data; results in individual laboratories may vary.*

Interference

The test results of Mindray SARS-CoV-2 IgM do not be interfered with the endogenous substances at the following concentrations(criterion: recovery within ± 10% of initial value)*.

Substance	Concentration
Bilirubin	20 mg/dL
Hemoglobin	500mg/dL
Triglyceride	3000mg/dL
Total protein	10g/dL
Mucoprotein	200mg/dL
Total IgG	4g/dL
Total IgM	0.5g/dL
Rheumatoid Factor(RF)	1500IU/mL
Antinuclear Antibody(ANA)	Not available
Anti-mitochondrial Antibody(AMA)	Not available
Human Anti-mouse Antibody(HAMA)	Not available

**Representative data; results in individual laboratories may vary.*

The test results of Mindray SARS-CoV-2 IgM do not be interfered with the exogenous substances at the following concentrations(criterion: recovery within ± 10% of initial value)*.

Substance	Concentration
Zanamivir	0.6mg/dL
Ribavirin	30mg/dL
Osteltamivir	4.5mg/dL
Peramivir	36mg/dL
Lopinavir	24mg/dL
Ritonavir	6mg/dL
Arbidol Hydrochloride	12mg/dL
Levofloxacin	36mg/dL
Azithromycin	30mg/dL
Ceftriaxone Sodium	120mg/dL
Meropenem	30mg/dL
Tobramycin	1.2mg/dL
Histamine Hydrochloride	0.03mg/dL
Phenylephrine	0.3mg/dL
Oxymetazoline	0.06mg/dL
Beclomethasone	0.06mg/dL
Dexamethasone	1.2mg/dL
Flunisolide Hemihydrate	0.06mg/dL
Triamcinolone Acetonide	0.06mg/dL
Budesonide	0.016mg/dL
Mometasone	0.024mg/dL
Fluticasone Propionate	0.06mg/dL

**Representative data; results in individual laboratories may vary.*

Mindray SARS-CoV-2 IgM assay was evaluated for potential cross-reactivity with substances from individuals with medical conditions unrelated to SARS-CoV-2 infection. Results are shown in the table below.*

Potential Interfering Disease States	N of samples tesed	N of samples positive
Human Coronavirus OC43(HCoV-OC43)	2	0
Human Coronavirus 229E(HCoV-229E)	3	0
Influenza A virus (unclassified)	3	0
Novel H1N1 Subtype Influenza A virus（2009）	1	0
Seasonal H1N1 Flu Virus	1	0
H3N2 Flu Virus	4	0
H7N9 Flu Virus	1	0
Influenza B Virus(unclassified)	5	0
Influenza B virus Yamagata	1	0
Influenza B Virus Victoria	1	0
Respiratory Syncytial Virus	6	0
Rhinovirus(unclassified)	4	0
Adenovirus(unclassified)	6	0
Enterovirus(unclassified)	3	0
EB virus	9	0
Measles Virus	1	0
Cytomegalovirus	1	0
Measles Virus	1	0
Varicella-zoster Virus	1	0
Mycoplasma Pneumoniae	10	0
Total	64	0

**Representative data; results in individual laboratories may vary.*

Clinical Performance

405 specimens from confirmed COVID-19 cases (Real-Time PCR positive) were tested with Mindray SARS-CoV-2 IgM assay and 331 were detected as positive, with a Positive Percent Agreement (PPA) of 81.72%. 1912 specimens not related to COVID-19 were tested with Mindray SARS-CoV-2 IgM assay and 1753 were detected as negative, with a Negative Percent Agreement (NPA) of 91.68%. The results are summarized in the table below*.

/		Real-Time PCR		Subtotal
		Pos**	Neg	
Mindray SARS-CoV-2 IgM assay	Pos	331	159	490
	Neg	74	1753	1827
Subtotal		405	1912	2317

**Representative data; results in individual laboratories may vary.*
***Pos=positive; Neg=negative.*

SARS-CoV-2 IgM results of the 405 specimens from confirmed COVID-19 cases are classified with time from first symtom to sampling. Data from this study are summarized in the table below*.

Time**	Negative	Positive
≤7 days	8(57.14%)	6(42.86%)
>7 days and ≤14 days	9(50.00%)	9(50.00%)
>14 days	57(16.09%)	316(83.91%)
Total	74(18.52%)	331(81.48%)

**Representative data; results in individual laboratories may vary.*
**Time=time from first symtom to sampling*

The 405 specimens from confirmed COVID-19 cases were also tested with Mindray SARS-CoV-2 IgG assay. The combination results from SARS-CoV-2 IgG and IgM assays are shown in the table below*.

IgG/IgM	Number(percentage)
IgM-/IgG-	7(1.73%)
IgM-/IgG+	67(16.54%)
IgM+/IgG-	65(16.05%)
IgM+/IgG+	266(65.68%)
Total	405(100%)

**Representative data; results in individual laboratories may vary.*

1912 specimens not related to COVID-19 were also tested with Mindray SARS-CoV-2 IgG assay. The combination results from SARS-CoV-2 IgG and IgM assays of the 1912 specimens are shown in the table below*.

IgG/IgM	Number (percentage)
IgM-/IgG-	1675 (87.60%)
IgM-/IgG+	78(4.08%)
IgM+/IgG-	156(8.16%)
IgM+/IgG+	3(0.16%)
Total	1912(100%)

**Representative data; results in individual laboratories may vary.*

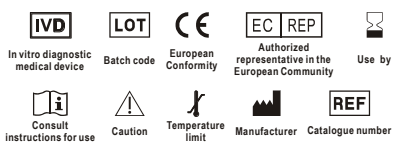
28 specimens from highly suspected cases with CT image features of chest but negative Real-Time PCR results were tested with Mindray SARS-CoV-2 IgM. These cases were followed up and finally confirmed with positive Real-Time PCR results. 12 of the 28 specimens were detected as positive with Mindray SARS-CoV-2 IgM.

147 specimens from suspected cases, but which were finally excluded from COVID-19 were tested with Mindray SARS-CoV-2 IgM assay. 138 specimens were detected as negative, with a relative agreement of 93.88%.

Warnings and Precautions

1. For in vitro diagnostic use only.
2. Follow all the rules in handling laboratory reagents and take necessary safety precautions.
3. Due to the differences in methodology and antibody specificity, test results of the same sample may be different when using reagent kits from different manufacturers on Mindray system, or using Mindray reagent kits on other systems.
4. Do not use reagent kits beyond the expiration date.
5. Do not use reagents mixed from different reagent lots.
6. Always keep the reagent pack in the upright position to ensure no microparticle has been lost prior to use.
7. Reagent pack opened for more than 7 days is not recommended for use.
8. Reliability of assay results cannot be guaranteed if the instructions in this package insert are not followed.
9. All the specimen and reaction wastes should be considered potentially biohazard. Specimens and reaction wastes should be handled in accordance with the local regulations and guidelines.
10. The Material Safety Data Sheet (MSDS) is available upon request.

Graphical Symbols



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