OnSite™ Rubella IgG/IgM Rapid Test

REF R0243C

INTENDED USE

The *OnSite* Rubella IgG/IgM Rapid Test is a lateral flow immunoassay for the semi-quantitative detection and differentiation of antibodies (IgG and IgM) to rubella virus in human serum, plasma or whole blood. It is intended to be used by professionals as a screening test and as an aid in the diagnosis of infection with rubella virus. Any reactive result with the *OnSite* Rubella IgG/IgM Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

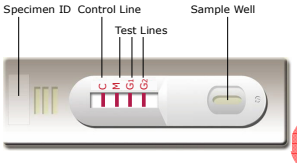
SUMMARY AND EXPLANATION OF THE TEST

An infection with rubella virus occurs most often during childhood. The infection usually leads to mild symptoms including maculopapular rash of head and trunk, fever, arthritis and lymphadenopathy[1]. However, if a rubella virus infection occurs during pregnancy, a group of birth defects collectively known as congenital rubella syndrome (CRS) may develop, including congenital eye defects, deafness, congenital heart diseases and mental retardation[1].

Clinical diagnosis of Rubella is unreliable and unspecific[1]. Therefore, laboratory diagnosis is essential to confirm an acute infection. During an acute infection with rubella virus, IgM anti-rubella virus can be detected 3-6 days after onset of symptoms and generally decrease to undetectable levels within 12-14 weeks[2]. IgG anti-rubella virus can be detected within 2-3 weeks post infection and levels may rise during the acute phase of the disease to levels above 200 IU/mL[2]. Protective immunity from an infection with rubella virus is indicated by an IgG anti-rubella virus level ≥10-15 IU/mL[3,4]. However, the presence of IgG anti-rubella virus ≥10-15 IU/mL does not necessarily ensure protection from future infection with rubella virus. A patient without protective levels of IgG anti-rubella virus (<10-15 IU/mL) is considered at risk of acquiring a rubella virus infection during pregnancy[3,4].

The *OnSite* Rubella IgG/IgM Rapid Test allows detection and differentiation of IgG and IgM anti-rubella virus in human serum, plasma and whole blood. The test allows differentiation of high titer IgG anti-rubella virus (≥250IU/mL) from low titer IgG anti-rubella virus (≥15IU/mL and <250 IU/mL). The test can be performed by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* Rubella IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing rubella virus antigens conjugated with colloidal gold (rubella conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing three test lines (M, G1, G2 lines) and a control line (C line). The M line is pre-coated with mouse anti-human IgM for detection of IgM anti-rubella virus. The G1 and G2 lines are pre-coated with mouse anti-human IgG for detection of different levels of IgG anti-rubella virus. The C line is pre-coated with a control line antibody.

When an adequate volume of test specimen and sample diluent are dispensed into the sample well, the specimen migrates by capillary action across the cassette. IgM anti-rubella virus, if present in the specimen, will bind to the rubella conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgM forming a burgundy colored M line, indicating an IgM anti-rubella virus positive test result.

IgG anti-rubella virus, if present in the specimen, will bind to the rubella conjugates. The immunocomplex is then captured on the membrane by the pre-coated mouse anti-human IgG forming burgundy colored G1 and/or G2 test lines, indicating an IgG anti-rubella virus positive test result. An IgG anti-rubella virus titer ≥15 IU/mL produces a burgundy colored G1 test line. An IgG anti-rubella virus titer ≥250 IU/mL produces burgundy colored G1 and G2 test lines. Absence of any test lines (M, G1 or G2) suggests a negative result.

The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the control antibodies, regardless of color development on the test lines (G1, G2 and M). If the C line does not develop, the test result is invalid, and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. 10 µL capillary tubes
3. Sample diluent (REF SB-R0243C, 5 mL/bottle)
4. One package insert (instruction for use)

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Positive control
2. Negative control

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer
2. Lancing device for whole blood test

WARNINGS AND PRECAUTIONS

For *in vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert may lead to inaccurate test results.
2. Do not open the sealed pouch until ready to conduct the assay.
3. Do not use expired devices or components.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use components from any other test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The test result should be read within 10 minutes after a specimen is applied to the sample well of the device. Reading the result after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°C. If stored at 2-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Plasma

Step 1: Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by venipuncture.
Step 2: Separate the plasma by centrifugation.
Step 3: Carefully withdraw the plasma into new pre-labeled tube.

Serum

Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
Step 2: Allow the blood to clot.
Step 3: Separate the serum by centrifugation.
Step 4: Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately, for up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Blood

Drops of whole blood can be obtained by either finger tip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®). Do not use hemolyzed blood for testing.

Whole blood specimens should be stored in refrigeration (2-8°C) if not tested immediately. The specimens must be tested within 24 hours of collection.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay.

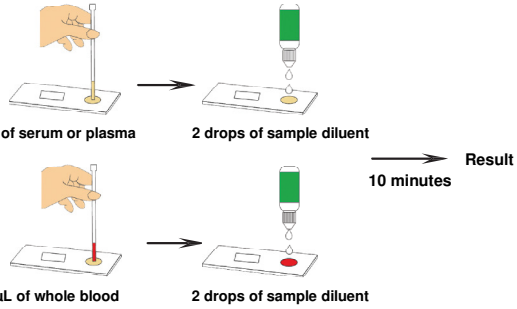
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with the specimen's ID number.

Step 4: Fill the capillary tube with specimen not exceeding the specimen line as shown in the images below. The volume of specimen is approximately 10 µL. **For better precision, transfer specimen using a pipette capable of delivering a 10 µL volume.**

Holding the capillary tube vertically, dispense the entire specimen into the center of the sample well making sure that there are no air bubbles.

Immediately add 2 drops (about 60-80 µL) of Sample Diluent to the sample well with bottle positioned vertically.



Step 5: Set up the timer.

Step 6: Result should be read in 10 minutes. Positive results may be visible in as soon as 1 minute.

Do not read the result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

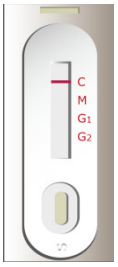
1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.

2. **External Control**: Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. New operator uses the kit, prior to performing the testing of the specimens.
   b. A new lot of test kits is used.
   c. A new shipment of test kits is used.
   d. The temperature used during storage of the kits falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.

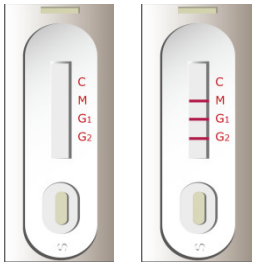
g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. NEGATIVE RESULT:
If only the C line develops, the test indicates that the levels of IgM and IgG anti-rubella virus in the specimen are below the detection limits of the assay. The result is negative or non-reactive.

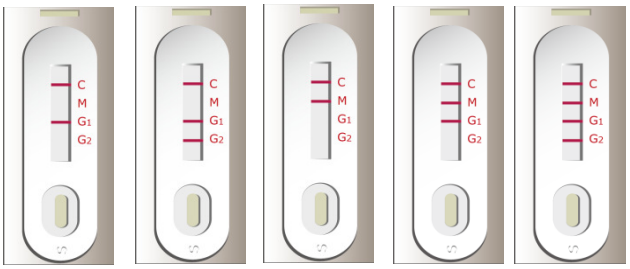


2. INVALID:
If no C line is developed, the assay is invalid regardless of color development on any of the test lines (M, G1, G2). Repeat the assay with a new device.



3. POSITIVE RESULT:

IgM Negative IgG 15-250 IU/mL	IgM Negative IgG ≥ 250 IU/mL	IgM Positive IgG <15IU/mL	IgM Positive, IgG 15-250 IU/mL	IgM Positive, IgG ≥ 250 IU/mL



Specimens with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis is made.

PERFORMANCE CHARACTERISTICS

1. Analytic Sensitivity of IgG Detection
Twelve groups of matrix were spiked with IgG anti-rubella virus to the WHO 1st International Standard (RUBI-1-94) concentrations of 0, 5, 10, 15, 20, 30, 60, 100, 160, 200, 250, and 300 IU/mL. The specimens were run on the *OnSite* Rubella IgG/IgM Rapid Test. Defined as the 95% detection level, the limit of detection or sensitivity for the *OnSite* Rubella IgG/IgM Rapid Test G1 and G2 test lines is 15 IU/mL and 250 IU/mL, respectively.

LOD for G1 test line

IgG IU/mL	0	5	10	15	20	30	60
Number Positive	0	2	13	19	20	20	20
Number Negative	20	18	7	1	0	0	0

N=20, Analytic sensitivity at 15 IU/mL = 19/20 x 100 = 95%

LOD for G2 test line

IgG IU/mL	30	60	100	160	200	250	300
Number Positive	0	4	11	13	18	20	20
Number Negative	20	16	9	7	2	0	0

N=20, Analytic sensitivity at 250 IU/mL = 20/20 x 100 = 100%

2. Accuracy of IgG Detection
A total of 214 specimens were collected and tested with the *OnSite* Rubella IgG/IgM Rapid Test and by a commercial IgG anti-rubella virus ELISA with positive cut off level at 10 IU/mL. Comparison for all subjects is shown in the following table:

	OnSite Rubella IgG/IgM Rapid Test		
Reference	**Positive**	**Negative**	**Total**
Positive	171	3	174
Negative	2	38	40
Total	173	41	214

Relative Sensitivity: 98.3%, Relative Specificity: 95.0%, Overall Agreement: 97.7%

Among the 214 specimens, 3 specimens were detected to have IgG levels higher than 250 IU/mL. These specimens were all detected as positive on the *OnSite* Rubella IgG/IgM Rapid Test G1 and G2 test line.

3. Positive Rate on the Random Clinical Specimens
The positive rate of the *OnSite* Rubella IgG/IgM Rapid Test was evaluated with 10,000 clinical specimens. M, G1 and G2 positive rates were 0.3%, 87% and 7%, respectively.

4. Boston Biomedica Inc (BBI) Mixed Titer Performance Panel
The performance of the *OnSite* Rubella IgG/IgM Rapid Test and a commercially available IgM anti-rubella virus Rapid Test were evaluated using BBI Mixed Titer Performance Panel PTR-201. The results are shown in the following table:

BBI Reference Panel: Abbott EIA-Rubella	Number	*OnSite* Rubella IgG/IgM Rapid Test		
		M Positive	G1 Positive	G2 Positive
IgM Positive	5	4	0	0
IgG < 15 IU/mL	2	0	0	0
15 IU/mL ≤ IgG < 250 IU/mL	14	0	14	0
IgG ≥ 250 IU/mL	9	0	9	6
Negative	20	0	0	0

5. Cross-Reactivity
No false positive IgG and IgM anti-rubella test results were observed with 4-10 specimens from the following disease stages or special conditions, respectively:

HAV	HBV	HCV	HIV	Syphilis	TB
Dengue	*H. pylori*	CMV	HSV-1	HSV-2	Toxoplasma
ANA	HAMA	RF (up to 2,500 IU/mL)			

All positive anti-rubella IgG results were confirmed with ELISA

6. Interference
Common substances (such as pain and fever medication and blood components) may affect the performance of the *OnSite* Rubella IgG/IgM Rapid Test. This was studied by spiking these substances into negative, IgG positive and IgM positive specimens, respectively. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the *OnSite* Rubella IgG/IgM Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Albumin	60 g/L	6. Hemoglobin	2 g/L
2. Bilirubin	20 mg/dL	7. Heparin	3,000 U/L
3. Creatinine	442 µmol/L	8. Salicylic acid	4.34 mmol/L
4. EDTA	3.4 µmol/L	9. Sodium citrate	3.8%
5. Glucose	55 mmol/L		

EXPECTED VALUES

IgM and IgG anti-rubella virus positive rates vary depending on the age of the population studied, the local vaccination programs. The reported IgG anti-rubella positive rates at ≥10-15 IU/mL and >200 IU/mL are 89-94% and 3.4%, respectively[5-8]. The reported IgM anti-rubella virus positive rate is 0.3-1.7%[7,8].

STANDARDIZATION

The *OnSite* Rubella IgM/IgG Rapid Test has been calibrated against the World Health Organization 1st International Standard for anti-Rubella immonglobulin (RUBI-1-94).

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to rubella virus in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The *OnSite* Rubella IgG/IgM Rapid Test is limited to the qualitative detection of antibodies to rubella virus in human serum, plasma or whole blood. The intensity of the test line does not have linear correlation with the titer of rubella antibody in the specimen.
3. A negative or non-reactive test result does not preclude the possibility of exposure to or infection with rubella virus. A negative or non-reactive result can occur if the titer of rubella virus antibody present in the specimen is below the level detectable by the assay or if rubella virus antibody was not present during the stage of disease in which the sample was collected.
4. Infection may progress rapidly. If the symptom persists, while the result from *OnSite* Rubella IgG/IgM Rapid Test is negative or non-reactive, it is recommended to re-test the patient a few days later or test with an alternative test method.
5. The *OnSite* Rubella IgG/IgM Rapid Test has not been validated on specimens from neonates.
6. Specimens from patients with infectious mononucleosis or high titers of heterophile antibodies, rheumatoid factor (>2,500IU/mL) may affect expected results.
7. The *OnSite* Rubella IgG/IgM Rapid Test does not differentiate antibodies generated by vaccine from that by infection.
8. Results obtained with the *OnSite* Rubella IgG/IgM Rapid Test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	Σ N	Tests per kit
2°C 30°C	Store between 2-30°C		Do not reuse		
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
10110 Mesa Rim Road
San Diego, CA 92121, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

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English Version

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