OnSite® RF Rapid Test

REF R0310C CE

Instructions for Use

INTENDED USE

The *OnSite* RF Rapid Test is a lateral flow immunoassay for the qualitative detection of all subtypes of rheumatoid factor (RF) in human serum, plasma or whole blood at a sensitivity of 8 IU/mL. It is intended to be used by professionals as a preliminary test result to aid in the diagnosis of rheumatoid arthritis and Sjögren's syndrome.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

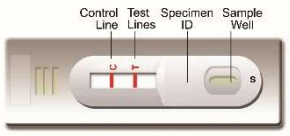
Rheumatoid factors are human auto-antibodies that bind to the Fc moieties of immunoglobulins leading to tissue damage. Elevated levels of RF are found in 70-90% of rheumatoid arthritis, 75-95% of Sjögren's syndrome, 95% of Felty's syndrome cases, as well as a number of connective tissue and inflammatory diseases including infectious mononucleosis, SLE, scleroderma and hepatitis[1,2].

The majority of RF are of the IgM subtype, but it can also be of IgG, IgA, IgE or IgD subtypes. IgM RF, IgA RF and IgG RF are seen in 92%, 65% and 66% of RA patients respectively[3]. Therefore, detection of all the isotypes of RF is necessary. Pathological values of RF vary with subtypes, test methods and laboratories. Levels of IgM RF are reported to be ≥ 3-15 IU/mL, IgA RF are ≥ 4-15 IU/mL and IgG RF ≥ 6-60 IU/mL[4-6].

RF is traditionally detected by agglutination assays[7]. In recent years, many laboratories have begun to use nephelometry and enzyme-linked immunosorbent assays (ELISA) for the quantitative detection of RF[8].

The *OnSite* RF Rapid Test is a lateral flow immunoassay for the qualitative detection of all subtypes of RF in serum, plasma or whole blood. The *OnSite* RF Rapid Test can be performed within 10 minutes by minimally skilled personnel and without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* RF Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a colored conjugate pad containing immunoglobulins conjugated with colloidal gold (Ig conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with another immunoglobulin for the detection of rheumatoid factor, and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. RF, if present in the specimen, will bind to the Ig conjugates. The immunocomplex is then captured on the membrane by the pre-coated immunoglobulin forming a colored T line, indicating a level of RF greater than or equal to 8 IU/mL. Absence of the T line suggests that the RF level in the specimen is lower than 8 IU/mL.

The test contains an internal control (C line) which should exhibit a colored line of the immunocomplex of the control antibodies regardless of any color development on the T line. If the C line does not develop, the test result is invalid, and the specimen must be re-tested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. 5 µL capillary tubes
3. Sample diluent (REF SB-R0310, 5 mL/bottle)
4. Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

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

MATERIALS MAY BE REQUIRED BUT NOT PROVIDED

1. RF Positive Control
2. RF Negative Control

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. Read these Instructions for Use completely before performing the test. Failure to follow the insert may lead to inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimens 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 biohazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The test results should be read 10-15 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside of the 10-15 minute window should be considered invalid and must be repeated.
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/Serum

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.
Step 2: To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.
Step 3: To make serum specimen, allow blood to clot, then centrifuge collected specimens and 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. The specimens can be stored at 2-8°C 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 samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Whole Blood

Step 1: Drops of whole blood can be obtained by either fingertip puncture or venipuncture. Collect blood specimen into a collection tube containing EDTA, citrate or heparin. 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 thawed, mix the specimen well prior to assay.

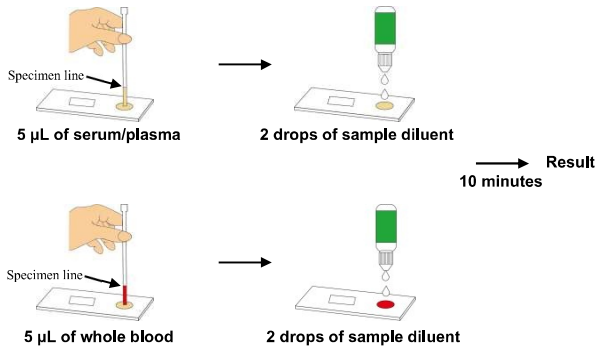
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.

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

Step 4: Using a squeezing motion, fill the capillary tube with specimen (about 5 µL) not to exceed the specimen line as shown in the image below. ***For better precision, transfer specimen using a pipette capable of delivering a volume of 5 µL.***

Holding the capillary tube vertically, dispense the entire amount of 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 the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Results should be read at 10 minutes. Positive results may be visible in as short as 1 minute. Negative results must be confirmed at the end of the 15 minutes only. ***Any results interpreted outside of the 10-15 minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local requirements governing the disposal of devices.***

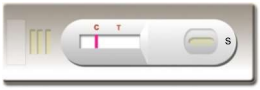
QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding the specimen and 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 the external controls, positive and negative, to ensure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit prior to performing the testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of kits is used.
   d. The temperature used during storage of the kit 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 level of rheumatoid factor is less than 8 IU/mL. The result is negative or non-reactive.



2. **POSITIVE RESULT:** If both the C and T lines develop, the test indicates that the level of rheumatoid factor is greater than or equal to 8 IU/mL. The result is positive or reactive.

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

3. **INVALID:** If no C line develops, the assay is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. **Analytic Sensitivity**

Defined as the 95% detection level, the limit of detection or sensitivity for the *OnSite* RF Rapid Test is 8 IU/mL. Serum RF levels greater than or equal to 8 IU/mL routinely test positive. Samples containing RF less than 8 IU/mL may also produce a very faint positive line.

The WHO RF standard (2nd revision, W1066) was reconstituted and diluted into RF negative matrix at concentrations of 0, 2, 4, 6, 8, 9, 10, 15, and 20 IU/mL. 20 samples of each concentration were tested with the *OnSite* RF Rapid Test. The results are presented in Table 1 below.

Table 1:

RF (IU/mL)	0	2	4	6	8	9	10	15	20
Number Positive	0	0	6	13	19	20	20	20	20
Number Negative	20	20	14	7	1	0	0	0	0

N=20, Analytic Sensitivity at 8 IU/mL = 19/20 x 100% = 95%

2. **Specificity**

Specificity of the *OnSite* RF Rapid Test was determined from studies with specimens from various disease stages. The results are presented in Table 2; no cross-reactivity was observed except with the high level HAMA specimens (Ref. **Limitations of Test 7**).

Table 2:

Specimen	Size	*OnSite* RF Rapid Test	Specimen	Size	*OnSite* RF Rapid Test
HBsAg Positive	10	Negative	*H. pylori* Positive	10	Negative
HAV Positive	10	Negative	Malaria Positive	10	Negative
HCV Positive	10	Negative	Typhi Positive	5	Negative
Dengue Positive	10	Negative	HAMA Positive	6	2 Positive
TB Positive	10	Negative	ANA Positive	6	Negative

3. **Accuracy**

3.1 Comparison with Nephelometry

The accuracy of the *OnSite* RF Rapid Test was determined by comparing with a nephelometry reference test. A total of 188 specimens, including 43 at a level of RF ≥ 20 IU/mL, 45 at a level 8 IU/mL ≤ RF < 20 IU/mL and 100 at a level of RF < 8 IU/mL, were tested (Table 3).

Table 3:

	OnSite RF Rapid Test		
Nephelometry Test	Positive	Negative	Total
RF ≥ 20 IU//mL	40	3	43
8 IU/mL ≤ RF < 20 IU/mL	43	2	45
RF < 8 IU/mL	0	100	100
Total	83	105	188

For RF ≥ 20 IU/mL, the detection agreement is 93% (95% CI: 80.9-98.5%). For 8 IU/mL ≤ RF < 20 IU/mL, the detection agreement is 95.6% (95% CI: 84.9-99.5%). For RF < 8 IU/mL, the detection agreement is 100% (95% CI: 96.4-100%). Positive Predictive Value: 100%, Negative Predictive Value: 95%

3.2 Comparison with Latex Agglutination

The accuracy of the *OnSite* RF Rapid Test was also compared with a latex agglutination test against 60 clinical specimens. The *OnSite* RF Rapid Test detected all of the specimens accurately. The latex agglutination test detected 95% of positive specimens and 100% of negative specimens (Table 4).

Table 4:

Specimen	Number	Latex Agglutination	*OnSite* RF Rapid Test
RF ≥ 20 IU/mL	22	95.5% Positive	100% Positive
8 IU/mL ≤ RF < 20 IU/mL	18	100% Positive	100% Positive
RF < 8 IU/mL	20	100% Negative	100% Negative

4. **Reactivity in Random Clinical Specimens**

A total of 300 fresh, random, clinical specimens were tested with the *OnSite* RF Rapid Test, and the positive rate was on average 6.1%.

5. **Hook Effect**

Specimens with RF values as high as 8,000 IU/mL were tested with the *OnSite* RF Rapid Test was tested; no hook effect was observed.

6. **Interference**

No interference in test performance was observed when spiking three levels of RF standard controls (negative, positive and strong positive) with 50 g/L albumin, 20 mg/dL bilirubin, 442 µmol/L creatinine, 3.4 µmol/L EDTA, 55 mmol/L glucose, 3,000 U/L heparin, 1,000 mg/dL human IgG, 4.34 mmol/L salicylic acid or 3.8% sodium citrate.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of RF in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate results.
2. The *OnSite* RF Rapid Test is limited to the qualitative detection of rheumatoid factor in human serum, plasma or whole blood.
3. A negative RF test result does not rule out RA or Sjögren's syndrome. In these patients, RF may be persistently absent or the RF concentration may be below the limit of detection of the assay. A negative result might also be caused by 'hidden' RFs which are complexed to serum IgG so their reaction sites are not exposed to the antibodies used in the test device.
4. If the symptoms persist when the result from *OnSite* RF Rapid Test is negative or non-reactive, it is recommended to re-test the patient a few months later or to test with an alternative test method.
5. Specimens with RF concentrations higher than 8,000 IU/mL have not been tested with the *OnSite* RF Rapid Test and may produce a negative result due to the hook effect. If the patient's clinical condition does not correlate with the result of the *OnSite* RF Rapid Test, dilute the specimen and repeat the test.
6. A decrease in serum RF levels has been reported in association with response to treatment such as during therapeutic intervention using the drug, Rituximab[9].
7. It is not uncommon for HAMA positive specimens to produce a RF positive test result.
8. The results obtained with the *OnSite* RF Rapid Test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

EXPECTED VALUES

RF can be found in 4-5% of apparently healthy persons and may be present in 10-25% of the population over the age of 65 years. Over 70% of patients with rheumatoid arthritis and over 60% of patients with Sjögren's syndrome have RF levels ≥20 IU/mL[10,11]. RF may be present in 5% of children who have juvenile idiopathic arthritis[1], and other connective tissue or inflammatory diseases including infectious mononucleosis, SLE, scleroderma and hepatitis[1-2].

STANDARDIZATION

The *OnSite* RF Rapid Test has been calibrated against the World Health Organization 2nd revision of Rheumatoid Arthritis Serum, Human reference reagent (2nd revision, W1066).

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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	N	Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		