Ref: 0501

IMMUNOQUICK® RF

Rheumatoid factor Test

IMMUNOQUICK® RF Human Ig (equivalent to Latex Agglutination Test) and IMMUNOQUICK® RF Animal Ig (equivalent to Waaler Rose)

Rapid test for detection of rheumatoid factor in human serum
(Human and animal immunoglobulins)

INTENDED USE

IMMUNOQUICK RF® Test Devices are immunochromatographic tests for the rapid qualitative and semi-quantitative detection of rheumatoid factor in serum. Each test is used to obtain a visual result. The IMMUNOQUICK RF® Test Devices can be used as an aid to initiate or attend therapeutic treatments by physicians. Each device is designed for professional and in-vitro-diagnostic.

SUMMARY

The rheumatoid factors (RF) are immunoglobulins which are directed against Fc fragments of IgG. Quantification of RF makes it possible to differentiate the rheumatoid arthritis from other articular diseases. Indeed, RF is found in a majority of patients suffering rheumatoid arthritis whereas they are seldom present among patients with other articular diseases.
According to ARA's criteria (American Rhumatism Association), one of the main serological indicators for the detection of rheumatoid arthritis is the presence of RF of IgM type in the human serum. RFs of IgM type have the capacity to form immune complexes with IgG, which activate molecules of the complement system which cause inflammatory reaction.
High RF IgM rates are in general a bad signal: the disease progresses more quickly, the articulations and the bones are touched and the incidence of nodules is higher. However the concentration of RF is not always in correlation with the state of the disease: for example, the presence of RF IgM can precede the beginning of rheumatoid arthritis from several years.
Although the presence of RF IgM is not specific of rheumatoid arthritis (one also finds them in bacterial infections, hyperglobulinemia or in elderly people), the rheumatoid arthritis is more severe in patients with high and old seropositivity. RF is present in the serum of 70 to 80% of t patients with rheumatoid arthritis. That means that negative result does not mean necessarily the absence of rheumatoid arthritis. It also should be noted, in case of a recent rheumatoid arthritis, that the RF IgM synthesis can have a latency time of 4 weeks or more.

TEST PRINCIPLE

IMMUNOQUICK® RF is based on immunochromatographic method for detection of RF in the human serum. It is based upon a combination human or rabbit immunoglobulins fixed on the membrane and human or rabbit immunoglobulins marked with latex microparticles.
An initial reaction between a diluted sample of serum and the immunoglobulin marked with latex takes place in liquid phase. Then the formed complex will migrate along the membrane. The immobilized immunoglobulins on the level of the "T" zone will capture the marked complex which leads to the presence of a blue "T" band (human) or red (animal) according to the concentration of RF in the sample. An internal control line on the level of the "C" zone makes it possible to validate a correct procedure.

MATERIALS PROVIDED

IMMUNOQUICK® RF Ig Human kit (Ref N° 0501_K50H)

- 50 blue strips using human immunoglobulins.
- Human immunoglobulins conjugate 1 bottle of 3 ml (blue).
- 1 bottle of diluent buffer (30 mL).
- 1 instruction for use.

IMMUNOQUICK® RF Ig Animal kit (Ref N° 0501_K50A)

- 50 red strips using animal immunoglobulins.
- Animal immunoglobulins conjugate 1 bottle of 3 ml (red).
- 1 bottle of dilution buffer (30 mL).
- 1 instruction for use.

MATERIAL REQUIRED BUT NOT PROVIDED

- Blood collection tubes.
- Plastic tubes.
- Centrifugal machine.
- Timer.
- Precision pipettes.

STORAGE

- The complete kit must be stored at refrigerated temperature **(2-8°C).** The vials of conjugate and dilution buffer must be stored at **2-8°C.**
- The aluminium pouches containing the reactive strips can be preserved separately at room temperature or cooled **(2-30°C).**

PRECAUTIONS

- For *in vitro* diagnostic use only. Not to use after the expiry date.
- Not to eat, drink or smoke during the handling of the samples and reagents.
- The vials of conjugate and dilution buffer contain sodium azide used as preservative. It can react with copper and lead from water pipe and form explosive components. Flush the water system abundantly with water to avoid formation of azide within the pipes. Sodium azide is toxic and should be handled carefully to avoid ingestion and skin contact.
- The samples of serum must be regarded as potentially infectious. When operating the test, precautions shall be taken as for handling of infectious products. Treat the various elements of the test and samples according to the procedure of potentially infectious waste.
- Not to interchange the reagents of various batches.
- Wear blouse, gloves and ocular protection during the realization of the test.
- Humidity and temperature can affect the result of the test.
- The reactive strip is stable until the expiry date indicated on the aluminium pouch.
- The strip must be preserved in its pouch until use.
- **NOT TO FREEZE.**

- Not to use beyond the expiry date.

SPECIMEN COLLECTION AND HANDLING

- IMMUNOQUICK® RF must be only used on serum.
- Not to use haemolysed or contaminated serums. Turbid samples must be centrifuged before performing the test.
- The test must be used immediately after collection and centrifugation of the sample; if not, sample can be stored 24 hours at 2-8°C to the maximum. For a prolonged conservation, the serum must be frozen at – 20°C.
- The frozen samples must be brought back to room temperature before performing the test and mixed in a homogeneous way. The samples should not undergo repeated freezing cycles.
- In the event of samples shipment, conform to regulation relating to the shipment of products of human origin.
- For lyophilized preparations, follow the instructions relating to the reconstitution of the sample and wait until the preparation returned at room temperature before carrying out the test.

TEST PROCEDURE

Keep the various components of the kit and serums to return at room temperature (15-30°C) before starting the test.

Qualitative procedure

1. Take the strips out of the aluminium pouch and proceed as soon as possible.
2. Dilute the serum 1/20th in the dilution buffer **(25 µl of serum in 475 µl dilution buffer).**
3. Carefully shake the bottle of conjugate and mix in a clean and dry **tube one drop of conjugate with 30 µl of diluted serum (1/20th)**
4. Slightly agitate the mixture and **let rest 5 minutes at room temperature.**
5. Dip the strip arrows directed downwards in the mixture.
6. **Read the result after 5 minutes.**

Important: The procedure is identical for human and animal RF. For human RF, the blue human strips must be used with blue human conjugate. For animal RF, the red strips must be used with red animal conjugate.

Note:

- The test line can be positive before the fifth minute notably in the presence of a high rate of rheumatoid factor
- A low and non-significant titer of rheumatoid factor can lead in approximately 5% of the cases in the presence of a test line of low intensity beyond the deadline of 5 minutes. It is thus important to carry out the reading at the fifth minute of migration.

Semi-quantitative procedure

In case of positive result, with the serum diluted 1/20, a semi quantitative assay can be run in a dilution serial with dilution buffer.

Prepare the dilutions as follows:

D1 = 25 µl pure serum + 475 µl dilution buffer = Dilution D1 used in qualitative procedure

D2 = 50 µl dilution buffer + 50 µl D1 = Dilution ½

D4 = 50 µl dilution buffer + 50 µl D2 = Dilution ¼

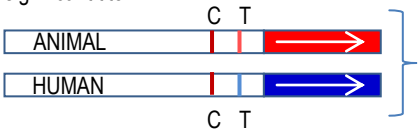
D8 = 50 µl dilution buffer + 50 µl D4 = Dilution 1/8

Then, proceed according to points 3 to 6 of qualitative procedure.

INTERPRETATION OF RESULTS

Positive Result:

Presence of Test band: Presence of rheumatoid factor at a clinically significant titer.



Note: The color intensity of the test result line regions may vary depending on the concentration of aimed substances present in the specimen. Therefore, any shade of color in the test region should be considered positive. Besides, the concentration of RF cannot be determined by this qualitative test.

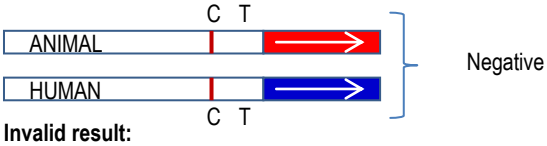
Semi-quantitative procedure: The titer is comprised between the inverse of last positive dilution and first negative dilution multiplied by the detection threshold of the test.

Ex: If a serum is positive up to ¼ dilution, the titer is between 4 x 12 IU/ml (i.e. 48 IU/ml) and 8 x 12 IU/ml (i.e. 96 IU/ml).

Note: This is only a semi quantitative approach that cannot replace quantitative measurement (Elisa, turbidimetry, nephelemetry).

Negative Result:

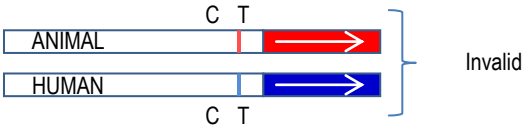
Absence of Test band on both the human and animal strips: Rheumatoid factor is not present at clinically significant rate.



Invalid result:

In case of absence of control band ©, the result is INVALID. An insufficient sample volume and/or a wrong procedure are the two most frequent causes of non-significant result.

The test must be repeated with a new strip.



QUALITY CONTROL

An internal control of procedure is integrated into the test (control line **C**). This makes it possible to control that the volume of serum is sufficient and that the procedure was followed correctly.

LIMITATIONS OF PROCEDURE

- **IMMUNOQUICK® RF** is intended for screening of rheumatoid factor in serum. This test is for *in vitro* diagnosis use only. The quantification of rheumatoid factor and the follow-up of kinetics are not realizable with IMMUNOQUICK® RF. Any positive result will have to be confirmed by a quantitative method (ELISA, nephelemetry or turbidimetry.)
- **IMMUNOQUICK® RF** indicates only the presence or the absence of rheumatoid factor in serum and should not be used alone for the diagnostic of rheumatoid arthritis.
- Results obtained with human and animal strips are not 100% identical. Discrepant results may be due to different reactivity. Thus, presence of anti-rabbit IgM in patient serum can lead in 2% of cases in false positive anti animal Ig result.
- As for any *in vitro* diagnostic test, the result must be interpreted by the clinician based on all other biological and clinical information available.

PERFORMANCE

A. Sensitivity – Specificity

An internal study was carried out on 152 samples compared to a quantitative immunoenzymatic method (ELISA). The positivity limit of the immunoenzymatic technic was fixed at 12 IU/ml .

- Human test

HUMAN		
ELISA / IMMUNOQUICK® RF Ig Human	Positive	Negative
Positive	40	4
Negative	0	108

Sensitivity : 100% - Specificity : 96%

- Animal test

ANIMAL		
ELISA / IMMUNOQUICK® RF Ig Animal	Positive	Negative
Positive	27	6
Negative	5	114

Sensitivity : 84% - Specificity : 95%

B. Detection limit

A study carried out on a dilution serial of WHO standard provided by NIBSC (Ref. 64/002) allowed to determine the detection limit of IMMUNOQUICK® RF Ig HUMAN at **12 IU/ml.**

C. Intra lot and Inter lot reproducibility

IMMUNOQUICK® RF was evaluated in triplicate with two different batches on a panel of negative, slightly positive and strongly positive samples. The results obtained are as follows:

	IMMUNOQUICK® RF	ELISA
Number of samples tested	148	148
Number of positive results	40	36
Number of negative results	108	112

This study shows an inter lot and intra lot reproducibility of 100%.

Literature

1. Harris, EA. Rheumatoid arthritis: Pathophysiology and Implications for Therapy, New England Newspaper off Medicine, 322:1277 – 1289 (1990).
2. Linker, JB III, and Williams, RC, Jr. Tests for Detection off Rheumatoid Factors, Manual off Clinical Immunology Laboratory, 3rd edition, Rose, NR, Friedman, H, and Fahey, JL, eds., ASM, Washington, cd., pp. 759-761 (1986).
3. Jackson, G. Immunodeficiencies and Autoimmune Disorders, In: Clinical Medicine Laboratory, Tilton, R, et al., eds., Mosby Year Book, Inc., St Louis, MO, CH. 36, pp. 485-504 (1992).
4. Richardson, C, Emery, P. Laboratory Markers off Disease Activity, Newspaper off Rheumatology, 23 (suppl. 44): pp. 23-30 (1996).
5. Sager, D, Wernick, RM, and Davey, MP. Assays for Rheumatoid Factor: In Review off Their Utility and Limitations in Clinical Practice, Medicine Laboratory, 23 (1): 15-18 (1992).
6. Carson, DA. Rheumatoid Factor, In: Textbook off Rheumatology, Kelley, WN, Harris, ED, and Sledge, RS, eds., Philadelphia, W.B. Saunders, pp. 664-679 (1985).
7. Truesdsson, L and Sturfelt G. False positive Waaler-Rose test due to anti-rabbit IgM antibodies in IgA deficiency. Clin. Exp. Rheumatol. 1983 Oct-Dec ; 1(4) : 307-10.

Symbols

	Attention, see instructions for use
IVD	For *in vitro* diagnostic use only
30°C 2°C	Store between 2-8°C
Σ	Content
	Expiry date
LOT	Lot Number
	Manufacturer
2	Do not re-use
REF	Catalog number

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