DRG® RF IgM (Rheumatoid Factor) (EIA-2775)

CE IVD

As of 27 July 2010 (Vers. 1.1)

INTENDED USE

The DRG Rheumatoid Factor (RF) IgM ELISA test system is intended for the qualitative and semi-quantitative detection of RF IgM-class antibody in human serum. The test system is intended to be used as an aid in the diagnosis of rheumatoid arthritis. This test is for *in vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Rheumatoid arthritis (RA) is a chronic, usually progressive inflammatory disorder of the joints. RA is a highly variable disease that ranges from a mild illness of brief duration to a progressive, destructive polyarthritis associated with a systemic vasculitis (1). The disease has been recently estimated to occur in one to two percent of the general population (2), and two times more likely to occur in women than in men (1). Clinical features of the early disease include lymphadenopathy, anorexia, weakness, fatigue, and morning stiffness or generalized achiness (1,3). RA is associated with a number of attributes, which are measurable within the laboratory setting (4). The most common laboratory findings associated with RA include rheumatoid factor (RF), antinuclear antibodies (ANA), immune complexes, and characteristic complement levels (3). Measurement of serum RF IgM plays an important role in the diagnosis of RA, and more recently has been implicated with disease prognosis (6). RF belongs to a group of immunoglobulins typically defined as antibodies, which react to the Fc portion of human (and some other species of) IgG molecules (1,4). RF is a polyclonal antibody, reacting with a wide range of determinants on the IgG molecule (4). RF are of three major immunoglobulin classes; IgM, IgG, and IgA; however, IgE RF have also been described (5). IgM and IgG RF is the most common (1), with IgM RF being present in 75% of patients diagnosed with RA (4). RF has also been associated with some bacterial and viral infections such as hepatitis and infectious mononucleosis and some chronic infections such as tuberculosis, parasitic disease, subacute bacterial endocarditis, and cancer (1). Also, elevated levels of RF may be seen in 15% of the population greater than 5 years of age. (4). Historically, RF was measured using various agglutination tests such as sensitized sheep cell test, latex agglutination, and bentonite flocculation (1), but more recently, newer more sensitive methods such as nephelometry, RIA, and ELISA have been developed. ELISA provides a simple and accurate means to measure RF. Additionally, ELISA provides the distinct advantage of being class specific and non-susceptible to prozone, two common drawbacks of agglutination based test systems.

PRINCIPLE OF THE ELISA ASSAY

The DRG Rheumatoid Factor ELISA test system is designed to detect IgM class antibodies to RF in human sera. Wells of plastic microwell strips are sensitized by passive absorption with RF antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgM is added to the wells and the plate is incubated. The Conjugate will react with the antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

MATERIALS PROVIDED

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note that all the active reagents contain sodium azide as a preservative at a concentration of 0.1 % (w/v)

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1. Plate. 96 wells configured in twelve 1x8-well strips coated with affinity purified human IgG. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgM. Ready to use. One, 15 mL vial with a white cap. Preservative added.
3. Positive Control (Human Serum). One, 0.35 mL vial with a red cap. Preservative added.
4. Calibrator A (Human Serum). One, 0.35 mL vial with a white cap. Preservative added.
5. Calibrator B (Human Serum). One 0.35 mL vial with a yellow cap. Preservative added.
6. Calibrator C (Human Serum). One 0.35 mLvial with an orange cap. Preservative added.
7. Calibrator D (Human Serum) One 0.35 mL vial with a blue cap. Preservative added.
8. Negative Control (Human Serum). One, 0.35 mL vial with a green cap. Preservative added.
9. Sample diluent. One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Green solution, ready to use. **Note:** Shake Well Before Use. Preservative added.
10. TMB: One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO ≤. 15% (w).
11. Stop solution: One 15 mL bottle (red cap) containing 1M H_2S0_4, 0.7M HCI. Ready to use.
12. Wash buffer concentrate (10X): dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). Contains preservative NOTE: 1X solution will have a pH of 7.2 ±0.2.

Kit also contains:

- component list
- package insert providing instructions for use.

PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
4. The human serum controls are **POTENTIALLY BIOHAZARDOUS MATERIALS.** Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety In Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (9).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be allowed to reach room temperature (20-25°C) before starting the assay. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate- Do not allow the wells to dry out between incubations.
7. The human serum controls. Sample Diluent, Conjugate, and Wash Buffer concentrate contain sodium azide at a concentration of 0.1% (w/v). Sodium azide has been reported to form lead or copper azides in laboratory plumbing

which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
8. The Stop Solution is TOXIC. Causes burn. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB Solution is HARMFUL Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of TMB to be used.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or Incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at add pH should be neutralized before adding to bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

MATERIALS REQUIRED BUT NOT PROVIDED

- ELISA microwell reader capable of reading at a wavelength of 450nm.
- Pipettes capable of accurately delivering 10 to 200/JL
- Multichannel pipette capable of accurately delivering (50-200pL)
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system.
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.

- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant, (example: 10% household bleach, 0.5% sodium hypochlorite.)

STORAGE CONDITIONS

1. Store the unopened kit between 2° and 8-C.
2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. Conjugate: Store between 2° and 8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2° and 8°C.
5. TMB: Store between 2° and 8°C.
6. Wash Buffer concentrate (10X): Store between 2° and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25° C) for up to 7 days or for 30 days between 2° and 8°C.
7. Sample Diluent: Store between 2° and 8°C.
8. Stop Solution: Store between 2° and 25°C.

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (7, 8). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

GENERAL PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow seven Control/Calibrator determinations (one Blank, one Negative Control, four Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 2
B	Neg. Control	Patient 3
C	Calibrator A	Etc.
D	Calibrator B	
E	Calibrator C	
F	Calibrator D	
G	Pos. Control	
H	Patient 1	

3. Prepare a 1:21 dilution (e.g.: 10 µL of serum + 200 µL of Sample Diluent. **NOTE:** Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100 µL of. Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5X.

A. Manual Wash Procedure:

a. Vigorously shake out the liquid from the wells.
b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
c. Repeat steps a. and b. for a total of 5 washes.
d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

B. Automated Wash Procedure:

If using an automated microwell wash system, set the dispensing volume to 300-350 µL /well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100 µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100 µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50 µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

QUALITY CONTROL

1. Each time the assay is run, the positive control, negative control, calibrator A, B, C, and D must be included- Additionally, a reagent blank must be Included.
2. The specifications for the positive and negative controls are as follows;

Positive Control Must be > 15 IU/mL
Negative Control Must be < 5 IU/mL

a. The OD of the negative control divided by the OD of the positive control should be ≤ 0.9.
b. If the control values are not within the above ranges, the test should be considered invalid and the test should be repeated.
3. The positive control is intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.

4. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION OF RESULTS

1. Calibrator

Based upon testing of normal sera, disease state sera, and the World Health Organization (WHO) international standard, a maximum normal IU/mL value has been determined by DRG International, Inc., and correlated to the calibrators. The calibrators will allow you to determine the unit value for each of the test samples evaluated. The unit values are determined for each lot of kit produced, and are printed on the Component List included with each kit.

2. Quality Control

Refer to the specification sheet included with each kit. This sheet describes the lot specific specifications for each of the calibrators. If any of the calibrators are out of range, the results are considered invalid, and the patient results may not be reported.

3. Conversion of Optical Density to IU/mL

Optical densities of the specimens are determined from the standard curve generated from the calibrators. A standard curve should be generated using the paired data points for each of the four calibrators (0D on the Y axis and corresponding IU/mL value on the X axis). Using the best fit, point-to-point curve, and determine the IU/mL value for each of the specimens tested by extrapolation.

INTERPRETATION OF RESULTS

Using normal healthy individuals, disease-state specimens, and the WHO standard, DRG International, Inc., has established the following guidelines for interpretation of patient results:

< 6.0 IU/mL Negative
>=6.0 IU/mL Positive
>=25 IU/mL Strongly Reactive, Indicative of Rheumatoid Arthritis

LIMITATION OF THE ASSAY

1. A diagnosis should not be made solely on the basis of the ELISA result. Test results for RF IgM should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. A negative result does not exclude rheumatoid arthritis. Approximately 25% of patients with a diagnosed case of rheumatoid arthritis may present with a negative result for RF.
3. Certain non-rheumatoid conditions, connective tissue disorders and a variety of other disease states such as hepatitis may elicit a positive RF test.
4. RF exists in three major immunoglobulin classes: IgA, IgG, and IgM. This test system will only detect IgM class RF antibodies.
5. Reproducible results with an ELISA system require careful pipetting, strict adherence to incubation periods and temperatures requirements, as well as thorough washing of the test wells and thorough mixing of all solutions.
6. Hemolytic, icteric, or lipemic samples may interfere with this ELISA. Use of these types of specimens should be avoided.

EXPECTED RESULTS

In a study conducted by DRG, 162 normal donor sera from Southeastern United States were evaluated for RF IgM antibodies. Of the 162 tested, 150 (92.6%) were found to be negative, six (3.7%) were positive, and six (3.7%) were strongly reactive.

PERFORMANCE CHARACTERISTICS

Comparative Study

An in-house comparative study was performed to demonstrate the equivalence of the DRG RF IgM ELISA test system to another commercially available RF IgM-ELISA test system. Performance was evaluated using 232 specimens; 182 assorted normal donor sera, 25 specimens previously tested and found to be RF IgM positive, and 25 disease-state specimens from clinically diagnosed rheumatoid arthritis patients. The results of the investigation have been summarized in Table 1 below.

Table 1: Calculation of Relative Sensitivity, Specificity, and Agreement

		DRG RF IgM ELISA Result:			
		-	+	Strong +	Totals:
Commercial ELISA Test System	-	160	4	0	164
	±*	3	2	0	5
	+	1	9	53	63
	Totals:	164	15	53	232

*Five samples were equivocal on the Commercial assay. These equivocal specimens were excluded from all calculations.

Relative Sensitivity = 62/63 = 98.5% — 95% Confidence Interval** = 95.3 to 100%
Relative Specificity = 160/164 = 97.6% — 95% Confidence Interval** = 95.2 to 99.9%
Relative Agreement = 222/227 = 97.8% — 95% Confidence Interval** = 95.9 to 99.7%

** 95% confidence intervals calculated using the exact method.

REPRODUCIBILITY

To evaluate both infra-assay and inter-assay reproducibility, eight specimens were tested; eight replicates each, on each of three days. These results were then used to calculate mean IU/mL values, standard deviations, and percent CV. One of the eight specimens was negative, three of the specimens were near the cut off zone, and four of the specimens were strongly reactive. The results of the study have been summarized below.

Table 2. DRG RF IgM ELISA; Results of Precision Testing

Day 1. Infra-Assay Reproducibility

Specimen	Mean Result (IU/mL)	Standard Deviation	Percent CV	Replicates
1	37.4	3.00	8.0%	8
2	0.3	0.05	20.0%	8
3	5.6	0.15	2.7%	8
4	9.4	0.40	4.3%	8
5	34.3	1.40	4.1%	8
6	29.2	0.54	1.8%	8
7	28.8	1.50	5.2%	8
8	8.2	0.50	6.1%	8

DAY 2. INFRA-ASSAY REPRODUCIBILITY

Specimen	Mean Result (IU/mL)	Standard Deviation	Percent CV	Replicates
1	45.4	3.70	8.1%	8
2	0.1	0.10	200.0%	8
3	6.2	0.44	7.1%	8
4	7.5	0.43	5.7%	8
5	31.8	2.60	8.2%	8
6	26.0	2.00	7.7%	8
7	31.9	3.30	10.3%	8
8	6.8	0.33	4.9%	8

DAY 3. INFRA-ASSAY REPRODUCIBILITY

Specimen	Mean Result (IU/mL)	Standard Deviation	Percent CV	Replicates
1	38.1	3.47	9.1%	8
2	0.1	0.10	113.3%	8
3	7.2	0.63	8.8%	8
4	7.3	0.50	6-8%	8
5	35.5	2.46	6.9%	8
6	31.2	2.90	9.3%	8
7	33.1	2.14	6.5%	8
8	7.1	0.50	7.0%	8

INTER-ASSAY REPRODUCIBILITY; ALL DAYS COMBINED

Specimen	Mean Result (IU/mL)	Standard Deviation	Percent CV	Replicates
1	40.3	4.95	12.3%	24
2	0.1	0.12	95.4%	24
3	6.3	0.80	12.7%	24
4	8.0	1.05	13.1%	24
5	33.2	2.37	7.1%	24
6	28.8	2.94	10.2%	24
7	31.3	3.00	9.6%	24
8	7.4	0.73	9.9%	24

CE IVD

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ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21
2. Add diluted serum to microwell 100 uL/Well
3. ⟶ Incubate 20 to 30 minutes.
4. Wash
5. Add Conjugate – 100 uL/Well
6. ⟶ Incubate 20 to 30 minutes
7. Wash
8. Add TMB 100 uL/Well
9. ⟶ Incubate 10 to 15 minutes
10. Add Stop Solution 50 uL/Well-Mix
11. READ