

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

k033802

B. Analyte:

Rheumatoid factor (RF)

C. Type of Test:

Homogeneous, microparticle immunoassay (flow-cytometry)

D. Applicant:

Zeus Scientific, Inc.

E. Proprietary and Established Names:

Zeus Scientific, Inc. AtheNA Multi-Lyte™ Rheumatoid Factor IgM Test System

F. Regulatory Information:

1. Regulation section:
21 CFR §866.5775 Rheumatoid Factor Immunological Test System
2. Classification:
Class II
3. Product Code:
DHR
4. Panel:
IM 82

G. Intended Use:

1. Intended use(s):
The Zeus Scientific, Inc. AtheNA Multi-Lyte™ Rheumatoid Factor IgM Test System is intended for the qualitative and/or quantitative detection of RF IgM class antibody. 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.
2. Indication(s) for use:
The Zeus Scientific, Inc. AtheNA Multi-Lyte™ Rheumatoid Factor IgM Test System is intended for the qualitative and quantitative detection of RF IgM class antibody in human serum. The test system is intended for the analysis of human serum for the presence of IgM RF. 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
3. Special condition for use statement(s):
The device is for prescription use only.

4. Special instrument Requirements:
AtheNA Multi-Lyte instrument

H. Device Description:

The AtheNA Multi-Lyte RF IgM assay consists of:

- multiplexed bead suspension containing 5.6 micron polystyrene beads conjugated with affinity-purified human IgG. The bead mix also contains one bead set designed to detect non-specific antibodies in the patient sample (if present) and four separate bead sets used for assay calibration;
- phycoerythrin conjugated goat anti-human IgM (μ -chain specific);
- human positive and negative serum controls; and
- sample diluent.

All these reagents are to be used on the AtheNA Multi-Lyte instrument.

I. Substantial Equivalence Information:

1. Predicate device name(s):
Zeus Scientific, Inc. RF IgM ELISA Test System
2. Predicate K number(s):
k961277
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	For the qualitative and quantitative detection of RF IgM class antibody in human serum as an aid in the diagnosis of rheumatoid arthritis	Same
Conjugate	Polyclonal goat IgM antibody	Same
Sample matrix	Serum	Same
Differences		
Item	Device	Predicate
Assay principle	Microparticle-based immunoassay (flow-cytometry)	ELISA
Solid phase	Polystyrene microsphere	Polystyrene microwell
Conjugate label	Phycoerythrin	Horse radish peroxidase
Conjugate signal	Fluorescence	Optical density
Detection range	0-238 IU/mL	Not furnished

J. Standard/Guidance Document Referenced (if applicable):

None referenced

K. Test Principle:

The Zeus Scientific, Inc. AtheNA Multi-Lyte™ RF test system is designed to detect rheumatoid factor IgM class antibodies in human sera. The test procedure involves two incubation steps: 1) test sera (properly diluted) are incubated in a vessel containing a multiplexed mixture of the bead suspension. The multiplexed bead suspension contains beads coated with human IgG. If present in the patient sera, RF IgM will bind to the immobilized antigen; 2) Phycoerythrin-conjugated goat anti-human IgM (μ -chain specific) is added to the vessel and the plate is incubated. The conjugate will react with RF IgM antibody immobilized on the solid phase in step 1. The bead suspension is then analyzed by the AtheNA Multi-Lyte instrument. The bead set(s) are sorted (identified) and the amount of reporter molecule (PE conjugate) is determined for each bead set. Using the *Intra-Well Calibration Technology™*, internal calibration bead sets are used to convert raw fluorescence into outcome (units). The test principle and performance of the microparticle-based immunoassay (flow-cytometry) for the AtheNA Multi-Lyte™ instrument was supported in k011244 and k021103.

L. Performance Characteristics (if/when applicable):1. Analytical performance:a. *Precision/Reproducibility:*

To evaluate both intra-assay and inter-assay reproducibility, six specimens were tested. On each day of testing, each sample was diluted twice and then loaded for four replicates resulting in a total of eight wells of each of the six samples. This protocol was followed for three days. These results were then used to calculate mean IU/mL values, standard deviation, and percent CV. Specimens were selected in such a way that resulted in two of them being clearly negative, two being clearly positive and two were selected that were weakly positive.

Intra-assay

Intra-assay %CVs for the two high positive samples ranged from 3.4 to 7.6%. For the low positive samples (near the cut-off) the %CVs ranged from 7.0 to 14.1%. The %CVs for the negative samples (close to 0 IU/mL) covered a wide range (0.0 to 52.9%) as would be expected.

Inter-assay

Inter-assay assay %CVs for the two high positive samples were 5.9 and 8.0%. For the low positive samples (close to the cut-off) the %CVs were 9.7 and 11.7%. The two negative samples showed high %CVs (32.6 and 53.4%) as would be expected.

b. *Linearity/assay reportable range:*

The reportable range studied was 0 to 238 IU/mL. Linearity was assessed by assaying two-fold serial dilutions of a strong positive serum. When actual concentrations were plotted against expected concentrations, non-linearity at the upper end of the assay range was observed ($y = 1.7424x + 17.937$, $r^2 = 0.9722$) and was as expected

for assay of this type in which the amount of fluorescence changes with the patient antibody concentration but the change is not directly proportional to the quantity of autoantibody present on the bead i.e. doubling of antibody concentration does not double reactivity.

- c. *Traceability (controls, calibrators, or method):*
The assay was calibrated against WHO 64/2, a standard provided by the World Health Organization (WHO) that has a defined value of 25 IU/mL. When analyzed using the AtheNA Multi-Lyte RF IgM test system, a result of 24.25 IU/mL was obtained.
- d. *Detection limit:*
Not furnished but not relevant for this assay.
- e. *Analytical specificity:*
The Multi-Lyte™ Rheumatoid Factor IgM Test System was evaluated for potential cross reactivity to other antibodies and interference from serum components. For this study, a total of 38 specimens were evaluated. Eighteen specimens which were positive for various autoimmune and infectious disease antibodies were tested on the AtheNA RF test system. Of the 18 evaluated, 2 were reactive on the Multi-Lyte™ RF IgM assay. One of the 2 was also reactive for RF IgM by ELISA. There were a total of 20 specimens evaluated which contained potentially interfering substances. These 20 specimens contained either abnormal levels of hemolysis (n=5), bilirubin (n=5), above normal IgG concentration (n=5), or above normal lipid levels (n=5). One hemolyzed and 1 sample with elevated bilirubin were positive.
- f. *Assay cut-off:*
The study included 150 specimens from normal blood donors. It was assumed that this group should, in large part, be disease free and therefore display a low incidence of RF IgM antibody. The samples were tested and the mean fluorescence and standard deviation were determined for this population. The cut-off was set equal to the mean plus three times the standard deviation. The investigation also include 150 specimens from patients all diagnosed with rheumatoid arthritis. Since RF IgM antibody is a marker for this disease, it was presumed that a significant number of these patients should demonstrate the RF IgM antibody. In the normal blood donor group, 2 of the specimens were invalid on the assay, reducing the total to 148 normal specimens. Of the 148 remaining specimens, 128/148 (86.5%) were negative, 18/148 (12.2%) were positive and 2/148 (1.4%) were strong positive. In the clinical specimens (those diagnosed with rheumatoid arthritis), 1/150 (0.7%) was negative, 149/150 (99.3%) were positive.

2. Comparison studies:

- a. *Method comparison with predicate device:*
There were a total of 450 specimens tested in the comparative study. Samples were categorized according to the following: normal blood

donors (n=150), specimens sent to a serology laboratory for routine RF testing (n=150) and specimens obtained from patients diagnosed with rheumatoid arthritis (n=150). The side-by-side comparison yielded an overall agreement of 97.5% (437/448) with two samples omitted for invalid results on the instrument.

b. Matrix comparison:

Serum is the only recommended matrix for both assays.

3. Clinical studies:

a. Clinical sensitivity:

Clinical sensitivity for the new assay was determined by testing 150 clinically defined serum samples from patients diagnosed with rheumatoid arthritis. In this group, 149/150 (99.3%) were positive for RF IgM antibody. Expressed as a 95% confidence interval, the clinical sensitivity was determined to be 0.963 - 0.999.

b. Clinical specificity:

Clinical specificity of the new assay was evaluated using 150 normal blood donors. Using this group, 128/148 (2 invalid samples) were negative for RF IgM antibody. The clinical specificity of the test system was determined to be 128/148 or 86.5%. Expressed as a 95% confidence interval, the clinical specificity was determined to be 0.799 to 0.916.

c. Other clinical supportive data (when a and b are not applicable)

Not applicable.

4. Clinical cut-off:

See assay cut-off.

5. Expected values/Reference range:

The expected value in the normal population is negative. According to published literature, the incidence in this group is around 5-12% depending on age.

M. Conclusion:

The Zeus Scientific, Inc. AtheNA Multi-Lyte™ Rheumatoid Factor IgM Test System is substantially equivalent to other devices regulated under 21 CFR §866.5775, product code DHR, Class II.