

Summary of Safety and Effectiveness Information  
MPO EIA Test Kit

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II. Description of Device

The MPO EIA test is an enzyme-linked immunosorbent assay (EIA) for the detection and semi-quantitation of antibodies to myeloperoxidase in human sera. The assay is to be used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid to the diagnosis of microscopic polyangiitis. FOR IN VITRO DIAGNOSTIC USE.

The MPO EIA test is an enzyme linked immunosorbent assay to detect IgG, IgM, and IgA antibodies to myeloperoxidase. Purified MPO is attached to a solid phase microtiter well. Diluted test sera is added to each well. If the antibodies are present that recognize the antigen they will bind to the antigen in the well. After incubation the wells are washed to remove unbound antibody. An enzyme labeled anti-human IgG, M, A is added to each well. If antibody is present it will bind to the antibody attached to the antigen on the well. After incubation the wells are washed to remove unbound conjugate. A substrate solution is added to each well. If enzyme is present the substrate will undergo a color change. After an incubation period the reaction is stopped and the color intensity is measured photometrically, producing an indirect measurement of specific antibody in the patient specimen.

III. Predicate Device

The MPO EIA test is substantially equivalent to IFA. Equivalence is demonstrated by the following comparative results

## Performance Characteristics

1. Sensitivity and Specificity - The MPO ELISA kit was evaluated relative to IFA for ANCA. Forty sera were from patients diagnosed with Wegener's granulomatosis. Forty sera were from patients diagnosed with microscopic polyangiitis. One hundred and fifty five sera were from normals with various ages, gender, and geographical areas. The data in Table 1 summarizes the data. Note: the sensitivity and specificity relative to IFA will not be as high if random IFA positive sera are selected due to other disease states causing ANCA patterns not associated with MPO.

**Table 1**  
**Sensitivity and Specificity of the MPO ELISA Kit Relative to IFA**

		MPO ELISA			Total
		Positive Index $\geq 1.10$	Equivocal 0.91-1.09	Negative $\leq 90$	
IFA	Positive	19*	0	1**	20
	Negative	0	0	213***	213
	Total	19	0	214	233

Relative Sensitivity =  $19/20 = 95.0\%$     95% Confidence interval = 85.3% - 100%  
 Relative Specificity =  $213/213 = 100\%$     95% Confidence interval = 98.6% - 100%  
 Relative Agreement =  $232/233 = 99.6\%$     95% Confidence interval = 98.7% - 100%

The 95% confidence intervals were calculated using the normal method.  
 The 95% confidence interval for Specificity was calculated assuming one false positive

\* Thirteen sera were patients diagnosed with microscopic polyangiitis with a p-ANCA pattern. One sera was from a patient diagnosed with Wegeners granulomatosis with a p-ANCA pattern. One sera was from a patient diagnosed with Wegeners granulomatosis with a c-ANCA pattern. Four sera were patients diagnosed with microscopic polyangiitis, that were positive for ANA thus making the p-ANCA pattern impossible to read.

\*\*One serum was from a patient diagnosed with microscopic polyangiitis with a p-ANCA pattern.

\*\*\*Sixty serum were from patients diagnosed with microscopic polyangiitis or Wegeners granulomatosis that had c-ANCA patterns or negative for ANCA. One hundred fifty three serum were from normals negative for ANCA.

The same group of clinical sera were tested on an legally marketed ELISA device to determine the relative sensitivity and specificity to an alternate ELISA. The data in Table 2 summarizes the data.

**Table 2 Comparison of MPO ELISA and ELISA**

		<b>MPO ELISA</b>			<b>Total</b>
		<b>Positive Index <math>\geq 1.10</math></b>	<b>Equivocal 0.91-1.09</b>	<b>Negative <math>\leq 90</math></b>	
<b>Alternate ELISA</b>	<b>Positive</b>	6	0	1**	7
	<b>Equivocal</b>	4	0	7	11
	<b>Negative</b>	11*	0	204	215
	<b>Total</b>	21	0	212	233

Relative Sensitivity =  $6/7 = 85.7\%$  95% Confidence interval = 59.3% - 100%

Relative Specificity =  $204/215 = 94.9\%$  95% Confidence interval = 91.9% - 97.9%

Relative Agreement =  $210/222 = 94.6\%$  95% Confidence interval = 91.6% - 97.6%

The 95% confidence intervals were calculated using the normal method.

\* Eight serum were from patients diagnosed with Microscopic polyangiitis. Two serum were from patients diagnosed with Wegeners Granulomatosis. One serum was a normal.

\*\* The serum was a normal.

The clinical sera and the potentially cross-reactive sera were grouped and the clinical sensitivity and specificity of the MPO ELISA assay was calculated. The data in Table 3 summarizes the data.

**Table 3 Clinical Sensitivity and Specificity of MPO ELISA**

	MPO ELISA			Total
	Positive Index $\geq 1.10$	Equivocal 0.91-1.09	Negative $\leq 90$	
Microscopic polyangiitis	18	0	22	40
Wegener's granulomatosis	2	0	38	40
Other autoimmune sera	0	0	21	21
Normals	1	0	152	153
Total	20	0	233	254

Clinical Sensitivity Microscopic polyangiitis =  $18/40 = 45.0\%$   
 95% confidence interval = 29.3 - 60.7%

Clinical Specificity Wegener's granulomatosis =  $38/40 = 95.0\%$   
 95% confidence interval = 88.1 - 100%

Clinical Specificity Other autoimmune sera =  $21/21 = 100\%$   
 95% confidence interval = 85.9 - 100%

Clinical Specificity Normals =  $152/153 = 99.4\%$   
 95% confidence interval = 98.0 - 100%

The 95% confidence intervals were calculated using the normal method.

The 95% confidence intervals for the clinical specificity for other autoimmune sera were calculated assuming one false positive.

## 2. Precision

The precision of the MPO kit was determined by testing nine different sera eleven times each on three different assays. The data is summarized in Table 4. With proper technique the user should obtain C.V.'s of less than 15%.

**Table 4**

	Assay 1 (n=11)			Assay 2 (n=11)			Assay 3 (n=11)			Inter Assay(n=33)		
	<u>X</u>	<u>S.D.</u>	<u>C.V.</u>	<u>X</u>	<u>S.D.</u>	<u>C.V.</u>	<u>X</u>	<u>S.D.</u>	<u>C.V.</u>	<u>X</u>	<u>SD</u>	<u>CV</u>
1	4.60	0.137	2.99%	4.39	0.222	5.06%	4.53	0.347	7.67%	4.5	0.258	5.73%
2	5.13	0.136	2.66%	4.97	0.236	4.74%	5.12	0.432	8.44%	5.08	0.295	5.81%
3	3.53	0.227	6.43%	3.21	0.217	6.75%	3.32	0.192	5.79%	3.36	0.248	7.38%
4	2.76	0.218	7.90%	2.51	0.338	13.45%	2.74	0.282	10.29%	2.67	0.297	11.13%
5	0.41	0.079	19.19%	0.37	0.067	17.92%	0.44	0.070	15.93%	0.41	0.076	18.58%
6	0.04	0.021	46.53%	0.05	0.017	35.78%	0.04	0.024	59.49%	0.04	0.020	46.08%
7	0.05	0.17	36.14%	0.05	0.018	38.94%	0.05	0.028	60.79%	0.05	0.021	45.24%
8	0.92	0.049	5.32%	0.84	0.054	6.42%	0.88	0.060	6.85%	0.88	0.063	7.14%
9	1.08	0.064	5.95%	0.94	0.055	5.91%	0.97	0.04	4.10%	0.97	0.094	9.67%

X = Mean MPO Value  
 S.D. = Standard Deviation  
 C.V. = Coefficient of Variation

## 3. Linearity

The MPO index values were determined for serial twofold dilutions of five positive sera. The index values were compared to  $\log_2$  of dilution by standard linear regression. The data in table #5 indicates that the assay is semi-quantitative.

**Table 5**  
**Linearity**

Serum	Neat	1:2	1:4	1:8	1:16	1:32	1:64	r
1	3.03	2.43	1.77	1.12	0.51			0.999
2	3.57	2.67	1.83	1.05	0.52			0.997
3	2.93	2.21	1.57	0.99	0.65			0.994
4	3.72	3.07	2.51	1.74	1.06	0.61		0.998
5	4.32	3.97	3.52	2.82	2.06	1.31	0.77	0.992

Linear regression compared MPO ISR to  $\log_2$  of dilution.

#### 4. Cross Reactive Data

Sera containing high level of antibodies to potentially cross reactive antigens were assayed on the MPO ELISA kit. The data in table 6 indicate that antibodies to alternate autoimmune antigens do not cross react with the MPO ELISA kit.

**Table 6**  
**Cross Reactive Data**

<b>Serum #</b>	<b>Antibody Specificity</b>	<b>MPO Index Value</b>	<b>Interpretation</b>
1.	SM	0 15	-
2.	SM	0 36	-
3.	SM	0 23	-
4.	RNP	0 10	-
5.	RNP	0 17	-
6.	RNP	0 09	-
7.	Ro	0 12	-
8.	Ro	0 07	-
9.	Ro	0 09	-
10.	La	0 07	-
11.	La	0 07	-
12.	La	0 06	-
13.	Scl-70	0 10	-
14.	Scl-70	0 12	-
15.	Scl-70	0 08	-
16.	Jo-1	0 09	-
17.	Jo-1	0 10	-
18.	Jo-1	0 10	-
19.	dsDNA	0 22	-
20.	dsDNA	0 08	-
21.	dsDNA	0 27	-