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Summary of Safety and Effectiveness Information  
PR-3 ELISA Test Kit

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II. Description of Device

The PR-3 ELISA test is an enzyme-linked immunosorbent assay (ELISA) for the detection and semi-quantitation of antibodies to Proteinase-3 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 Wegener's granulomatosis. FOR IN VITRO DIAGNOSTIC USE.

The PR-3 ELISA test is an enzyme linked immunosorbent assay to detect IgG, IgM, and IgA antibodies to Proteinase-3. Purified PR-3 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 PR-3 ELISA test is substantially equivalent to IFA. Equivalence is demonstrated by the following comparative results:

## Performance Characteristics

1. Sensitivity and Specificity - The PR-3 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 PR-3.

**Table 1**  
**Sensitivity and Specificity of the PR-3 ELISA Kit Relative to IFA**

		Wampole PR-3 ELISA			
		Positive Index $\geq 1.10$	Equivocal 0.91-1.09	Negative $\leq .90$	Total
IFA	Positive	53*	0	1***	54
	Negative	8**	0	173****	181
	Total	61	0	174	235

Relative Sensitivity =  $53/54 = 98.2\%$     95% confidence interval = 94.5 - 100%

Relative Specificity =  $173/181 = 95.6\%$     95% confidence interval = 92.5 - 98.6%

Relative Agreement =  $226/235 = 96.2\%$     95% confidence interval = 93.7 - 98.7%

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

\*Fifty sera were from patients diagnosed with Wegeners granulomatosis or microscopic polyangiitis with a c-ANCA pattern. Two sera were from patients diagnosed with microscopic polyangiitis with a p-ANCA pattern. One sera was from a patient diagnosed with microscopic polyangiitis with ANA thus making the c-ANCA pattern impossible to read.

\*\* Eight sera were from patients diagnosed with Wegeners granulomatosis or microscopic polyangiitis that were negative for ANCA

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

\*\*\*\*One hundred and fifty five serum were from normals that were negative for ANCA. Eighteen sera were from patients diagnosed with microscopic polyangiitis with either a p-ANCA pattern or ANA or 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 PR-3 ELISA and ELISA**

		PR-3 ELISA			Total
		Positive Index $\geq 1.10$	Equivocal 0.91-1.09	Negative $\leq 90$	
Alternate ELISA	Positive	58	0	5**	63
	Equivocal	1	0	11	12
	Negative	2*	0	158	160
	Total	61	0	174	235

Relative Sensitivity =  $58/63 = 92.1\%$  95% confidence interval = 85.3 - 98.9%

Relative Specificity =  $158/160 = 98.8\%$  95% confidence interval = 97.0 - 1.00%

Relative Agreement =  $216/223 = 98.9\%$  95% confidence interval = 94.5 - 99.2%

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

\* Both serum were from patients diagnosed with Microscopic Polyangiitis

\*\* All five sera were from normals

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

**Table 3 Clinical Sensitivity and Specificity of PR-3 ELISA**

	PR-3 ELISA			Total
	Positive Index $\geq 1.10$	Equivocal 0.91-1.09	Negative $\leq 90$	
Wegener's granulomatosis	39	0	1	40
Microscopic polyangiitis	22	0	18	40
Other autoimmune sera	0	0	21	21
Normals	0	0	155	155
Total	61	0	195	256

Clinical Sensitivity Wegener's granulomatosis =  $39/40 = 97.5\%$

95% confidence interval = 92.6 - 100%

Clinical Sensitivity Microscopic polyangiitis =  $22/40 = 55.0\%$

95% confidence interval = 39.3 - 70.7%

Clinical Specificity Other autoimmune sera =  $21/21 = 100\%$

95% confidence interval = 85.9 - 100%

Clinical Specificity Normals =  $155/155 = 100\%$

95% confidence interval = 98.1-100%

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

The 95% confidence intervals for the clinical specificities were calculated assuming one false positive.

## 2. Precision

The precision of the PR-3 kit was determined by testing nine different sera ten 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**

Serum#	Assay 1 (n=10)			Assay 2 (n=10)			Assay 3 (n=10)			Inter assay (n=30)		
	<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>S.D.</u>	<u>C.V.</u>
1	8.70	0.634	7.29%	8.92	0.479	5.37%	9.37	0.465	4.96%	9.00	0.586	6.52%
2	2.99	0.345	11.54%	3.03	0.211	6.96%	2.92	0.201	6.89%	2.98	0.256	8.59%
3	2.68	0.289	10.81	2.76	0.145	5.25%	2.70	0.144	5.32%	2.71	0.200	7.38%
4	2.52	0.177	7.04%	2.52	0.163	6.47%	2.53	0.242	9.54%	2.52	0.190	7.54%
5	10.33	0.344	3.34%	11.95	0.293	2.45%	10.29	0.200	1.95%	10.86	0.837	7.71%
6	0.17	0.074	42.79%	0.15	0.056	37.79%	0.010	0.076	80.36%	0.14	0.074	53.76%
7	0.08	0.037	47.16%	0.06	0.039	68.22%	0.04	0.037	84.50%	0.06	0.039	65.51%
8	1.28	0.079	6.13%	1.31	0.071	5.37%	1.21	0.079	6.53%	1.27	0.086	6.74%
9	1.08	0.059	5.45%	1.18	0.105	8.92%	1.06	0.083	7.91%	1.10	0.097	8.82%

## 3. Linearity

The ISR values were determined for serial twofold dilutions of five positive sera. The ISR values were compared to log2 of dilution by standard linear regression. The data in Table 5 indicates that the assay has a linear relationship with serum dilution.

**Table 5**

Serum	Neat	1:2	1:4	1:8	1:16	1:32	1:64	r
1	7.50	6.04	4.17	2.61	1.26	0.63		0.991
2	2.96	2.09	1.47	0.97	0.37			0.995
3	8.44	6.98	5.22	3.66	2.36	1.37	0.60	0.992
4	2.24	1.88	1.23	0.63				0.993
5	3.24	1.94	0.98	0.43				0.984

#### 4. Cross reactivity.

Sera containing antibodies to potentially cross reactive antigens were assayed on the PR-3 ELISA. The data in Table 6 indicate that antibodies to alternate autoimmune antigens do not cross react with the PR-3 ELISA kit.

**Table 6**

<b>Serum #</b>	<b>Antibody Specificity</b>	<b>PR-3 Index Value</b>	<b>Interpretation</b>
1.	Ro	0.09	-
2.	Ro	0.11	-
3.	Ro	0.14	-
4.	La	0.07	-
5.	La	0.05	-
6.	La	0.08	-
7.	Sm	0.24	-
8.	Sm	0.30	-
9.	Sm	0.36	-
10.	RNP	0.14	-
11.	RNP	0.09	-
12.	RNP	0.16	-
13.	Jo-1	0.05	-
14.	Jo-1	0.20	-
15.	Jo-1	0.12	-
16.	Scl-70	0.13	-
17.	Scl-70	0.07	-
18.	Scl-70	0.11	-
19.	dsDNA	0.38	-
20.	dsDNA	0.24	-
21.	dsDNA	0.36	-