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Summary of Safety and Effectiveness Information
EBNA-1 IgG EIA Test Kit

K951549

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II. Description of Device

The Epstein-Barr Virus Nuclear Antigen 1 (EBNA-1) IgG kit is an Enzyme-Linked Immunosorbent Assays (ELISA) for the semi-quantitative determination of IgG antibodies in human serum to EBNA-1 antigen. The ImmunoProbe anti-EBNA-1 IgG assay may be used in conjunction with other Epstein-Barr serologies (VCA IgG, VCA IgM, EA (R&D), and heterophile) as an aid in the diagnosis of infectious mononucleosis.
For In Vitro Diagnostic Use Only.

The EBNA-1 IgG EIA test is an enzyme linked immunosorbent assay to detect IgG antibodies to EBNA-1. Purified recombinant EBNA-1 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 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 EBNA-1 IgG EIA test is substantially equivalent to BioWhittaker's EBNA STAT test. Equivalence is demonstrated by the following comparative results:

Performance Characteristics

1. **Relative sensitivity and specificity.** Three different sites compared the EBNA-1 IgG EIA test relative to a commercially available EIA assay. Two of the sites were R&D laboratories at commercial companies located in Maryland and New York. The other site was a large commercial lab located in Pennsylvania. The results of the studies are compiled and summarized in Table 1. None of the performance characteristics were established with specimens from patients having nasopharyngeal carcinoma or Burkitt's lymphoma.

Table 1
EBNA-1 IgG Relative Sensitivity and Specificity

		ImmunoProbe EBNA-1 G EIA			Total
		+	eq	-	
Alternate EBNA-1 G ELISA	+	270	5	6	281
	eq	0	0	1	1
	-	0	1	74	75
	Total	270	6	81	357

Relative Sensitivity = $270/276 = 97.8$ 95% Confidence interval = 96.1% - 99.6%
 Relative Specificity = $74/74 = 100\%$ 95% Confidence interval = 96.0% - 100%
 Relative Agreement = $344/350 = 98.3\%$ 95% Confidence interval = 96.9% - 99.7%

Equivocals were not included in the above calculations.

The 95% confidence interval for relative specificity was calculated assuming one false positive

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

2. Precision. Four different sera (High Positive, Mid Positive, Low Positive, and Negative) were assayed at three different sites to determine the precision of the assay. Each sera was tested three times each, twice a day for twenty days at each of the three study sites. The inter-site coefficient of variation (CV) for each serum is presented in table 2.

Table 2
Inter Site Precision
n= 360

	Mean	Std Dev	CV%
High Positive	4.95	0.381	7.70%
Mid Positive	2.89	0.229	7.92%
Low Positive	1.41	0.152	10.78%
Negative	0.01	0.022	not applicable
Calibrator (n=240)	4.17	0.113	2.71%
High Positive Control (n=120)	6.90	0.419	6.07%

3. Linearity. The data in table 3 illustrates EBNA-1 index values for serially two fold diluted sera. The index values are compared to log₂ of dilution by standard linear regression. The data indicates that the antibody can be semi-quantitated by using a single serum dilution.

Table 3
EBNA-1 G Linearity

Serum #	Neat	1:2	1:4	1:8	1:16	1:32	1:64	SLOPE	Y INTERCEPT	r
1	8.6	5.96	3.50	1.96	0.87			1.95	-1.66	0.985
2	11.4	9.30	6.90	4.10	2.50	1.30	0.42	1.91	-2.49	0.985
3	6.90	4.90	2.7	1.5	0.69			1.58	-1.41	0.983
4	8.7	6.5	4.07	2.32	1.20	0.55		1.67	-1.95	0.979
5	9.4	7.17	4.8	3.2	1.5	0.70		1.77	-1.75	0.985

Linear regression compared Index Values to log₂ of dilution.

4. Cross-Reactivity. Serum containing IgG antibody detectable by ELISA to Herpes Simplex Virus I & II, Cytomegalovirus, and Varicella Zoster Virus were assayed. The data summarized in Table 4 indicates that antibodies to these Herpes Viruses do not cross-react with the EBNA-1 IgG EIA kit.

TABLE 4
Cross Reactivity

SERUM	EBNA-1 G	HSV1 G	HSV2 G	CMV	VZV
1	0.67 -	2.43 +	0.60 -	1.69 +	2.28 +
2	0.36 -	0.32 +	0.08 -	0.18 -	3.17 +
3	0.73 -	3.67 +	0.86 -	1.11 +	2.45 +
4	0.29 -	0.30 -	0.39 -	0.18 -	3.21 +
5	0.34 -	6.18 +	5.98 +	0.18 -	2.14 +

Sera ≥ 1.10 were considered positive.
Sera ≤ 0.90 were considered negative.

5. Evaluation of paired sera. To validate the sensitivity of the paired sera procedure 20 high positive sera were serially two fold diluted and run on the assay. From these dilutions, there were 68 pairs that had a four fold dilution where the acute sera had a value of less than 3.70. All 68 pairs demonstrated a >46% rise in ISR value, showing a significant rise in antibody. Therefore the paired sera procedure demonstrated 100% sensitivity in being able to detect a four fold increase in antibody level when the acute sera has a value of <3.70.

6. Sensitivity and Specificity Based on Serum Characterization

The serum from the first study site were characterized as seronegative (no serological evidence of past or present EBV infection), acute (VCA IgM present), or seropositive (presence of VCA IgG antibodies, no evidence of VCA IgM, indicative of past infection). The sensitivity, specificity and accuracy of the assay was determined based on this characterization. It was assumed that the EBNA-1 IgG response should be negative for seronegative and acute serum, and positive for seropositive serum. The results are summarized in Table 5.

Table 5

		Seropositive VCA IgG + VCA IgM -	Acute VCA IgM+	Seronegative VCA IgG - VCA IgM -	Total
IPI EBNA-1 IgG	Positive	89	1	1	91
	Negative	6	23	30	59
	Total	95	24	31	150

Relative Sensitivity = $89/95 = 93.7\%$

95% Confidence Interval = 88.7%-98.7%

Relative Sensitivity = $53/55 = 96.4\%$

95% Confidence Interval = 91.3%-100%

Relative Agreement = $142/150 = 94.7\%$

95% Confidence Interval = 91.0%-98.3%

Eight equivocal results were not included in the calculations.

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