

IV SUMMARY OF SAFETY AND EFFECTIVENESS

MAR 17 1998

General Information

Generic Name: Enzyme Linked Immunosorbent Assay for the Detection and Semi-Quantitation of Human Antibodies to Mouse IgG (HAMA).

Trade Name: ImmuSTRIP® HAMA IgG

Applicant's Name and Address: Immunomedics, Inc.
300 American Road
Morris Plains, NJ 07950

ImmuSTRIP® is a registered trademark of Immunomedics, Inc. All references herein to ImmuSTRIP® are to the registered trademark.

A. INDICATIONS FOR USE

The ImmuSTRIP® HAMA IgG ELISA Test System is a direct enzyme-linked immunosorbent assay for the detection and semi-quantitation of human antibodies to mouse IgG¹. Significant titers of human anti-mouse antibody (HAMA) have been associated with patients receiving injections of murine monoclonal antibody for diagnostic and/or therapeutic purposes²⁻⁶.

Background

The expanding use of murine-derived monoclonal antibodies for in-vivo diagnostic and therapeutic procedures has resulted in an increased incidence of HAMA titers in patients receiving such antibodies. Circulating levels of HAMA may bind with the injected antibody, forming complexes that may adversely affect the biodistribution and pharmacokinetics of the injected antibody⁷⁻⁸. In order to avoid injecting murine monoclonal antibodies into such patients who would receive no benefit, there is a need for an assay to predict potential complexation⁸⁻¹⁰. Additionally, HAMA has been shown to significantly interfere with many commercial assays utilizing murine-derived monoclonal antibodies, resulting in both false-positive and false-negative results¹¹⁻¹⁸.

B. DEVICE DESCRIPTION

The ImmuSTRIP® HAMA IgG ELISA Test System is a direct enzyme linked immunosorbent assay for the semi-quantitative detection of human antibodies to mouse IgG (HAMA).

The HAMA assay is a two-stage test carried out in plastic microwell strips which have been coated with mouse IgG, whole molecule.

In the first stage, mouse IgG conjugated to horseradish peroxidase (conjugate) is added to the microwell. Diluted test sample is then added and incubated for a specified length of time. If antibody to the mouse IgG is present in the test sample, bridging will occur with the solid phase mouse IgG, the test sample antibody, and the conjugate. If antibody is not present in the test sample, the unbound conjugate will be removed in the subsequent washing step.

In the second stage, enzyme substrate is added to the microwell. If bound conjugate is present, the substrate will be reduced; the reduced end product of the catalytic reaction oxidizes the colorless chromogen resulting in a colored end product. Acid is added to stop the reaction and fix the color.

The color intensity is proportional to the amount of bound conjugate and, therefore, to the amount of precipitable antibody present in the sample. The color intensity is measured with a microwell strip reader.

The ImmuSTRIP® HAMA ELISA Test System may be performed manually or with existing microtiter equipment. Results, which are determined by optical density at 488-492 nm, are available in less than one hour. The test has been standardized against primate anti-mouse IgG serum and has a sensitivity of approximately 40 ng/ml. Final values are reported as nanograms of precipitable antibody equivalents per ml.

The assay system is a rapid, efficient, and semi-quantitative method. Specific reagent formulation has eliminated the problem of background interference currently existing in this type of assay.

C. **ALTERNATIVE PRACTICES AND PROCEDURES**

Current Methods to Measure HAMA

Currently, the research methods that are available to measure HAMA are (1) Radioimmunoassay (RIA), (2) radial immunodiffusion (RID), (3) Ouchterlony, (4) rocket electrophoresis, (5) precipitation analysis and (6) passive hemagglutination. The aforementioned methodologies are currently being performed on samples from patients who have been injected with murine monoclonal antibodies^{4,7,8}. These methods may be insensitive, labor intensive, and/or require as many as 2-3 days for the results to be available.

D. **MARKETING HISTORY**

From 1988 to 1994, Immunomedics, Inc. has manufactured the ImmuSTRIP[®] HAMA IgG ELISA Test System and it has been available for research use only. Since 1994 Scimedx Corp., 400 Ford Road, Denville, New Jersey 07839, has manufactured the ImmuSTRIP[®] HAMA IgG ELISA for Immunomedics, Inc.

E. **POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH**

When this device is used according to the instructions provided, accurate assay results should be obtained. An error in the assay, however, which would produce a falsely low result could adversely affect the biodistribution and pharmacokinetics of the subsequent antibody injection. In this case, the quality of the imaging or therapy intended could be adversely affected. A falsely elevated result could lead to a medical decision resulting in the delay of a diagnostic or therapeutic procedure utilizing a murine-based monoclonal antibody.

F. **SUMMARY OF STUDIES**

1. Pertinent Publications

- a. Kaladas PM, et al. Detection of Human Anti-Murine Antibody (HAMA) Following Infusion of OncoScint® CR103. Comparison of ImmuSTRIP® ELISA with a Double Antigen Radiometric Assay. *Antib Immunoconj Radiopharm* 1993; 4:309-317.
- b. Massuger L, et al. Specific and Nonspecific Immunoassays to Detect HAMA After Administration of Indium-111-Labeled OV-TL 3 F(ab')₂ Monoclonal Antibody to Patients with Ovarian Cancer. *J Nucl Med* 1992; 33:1958-1963.
- c. Seybold K, Trinkler M, Frey L, et al. Antigenicity of Different Antigranulocytes Antibodies Assessed by HAMA Follow-up in Patients Undergoing Immunoscintigraphic Detection of Infections. Presented at the German Nuclear Medicine Congress, Cologne, Germany, April 15-17, 1993.
- d. Behr TM, et al. Phase I/II Clinical Radioimmunotherapy with an Iodine-131-Labeled Anti-Carcinoembryonic Antigen Murine Monoclonal Antibody IgG. *J Nucl Med* 1997; 38: 858-870.

2. Summary of non-clinical studies

Preclinical laboratory studies were conducted to determine the purity and specificity of the reagents.

- a. Production of the baboon anti-mouse antibody for the ImmuSTRIP[®] HAMA IgG Reference Standard.

Immunogen Preparation

Description of Antibody

The Immunomedics anti-CEA monoclonal antibody used as the immunogen was NP-4, later referred to as IMMU-4. IMMU-4 is a Class-III anti-CEA antibody of the immunoglobulin IgG₁ subclass. It is specific for CEA, not reacting with antigens that share CEA-related epitopes, such as meconium antigen and normal cross-reactive antigens. To prepare the IgG, ascites was produced in viral antibody-free mice with the IMMU-4 hybridoma cell line. The ascites was aseptically removed, centrifuged to remove cells, and the supernatant solution was frozen and stored at -80° C. After thawing, the ascites was further clarified by passing through an ion-exchange column, using pH and ionic conditions that prevented binding of the IMMU-4 to the ion-exchange matrix. IgG was isolated from the clarified supernatant solution by Protein A affinity chromatography, and further purified by ion-exchange chromatography. Purity and identity of the purified IMMU-4 IgG were proven by immunoelectrophoresis and SDS gel electrophoresis. (Refer to Section VI, Volume III).

Lampire Biologicals, located at 217 Farmschool Road, Ottsville, PA was contracted by Immunomedics to perform the immunization of the baboons, and to maintain the animals.

Immunization Schedule

Immunogen: 0.5 ml immunogen (IMMU-4 IgG, 1 mg/ml) was emulsified in 0.5 incomplete Freund's adjuvant and divided into aliquots for four subcutaneous injection sites per animal.

<u>Schedule</u>		
<u>Week of</u>	<u>Boost</u>	<u>Bleed</u>
10/30/86	1.0 ml	50 ml
11/06/86	1.0 ml	0 ml
11/13/86	1.0 ml	0 ml
11/20/86	1.0 ml	0 ml
11/27/86	1.0 ml	50 ml
12/25/86	1.0 ml	50 ml
12/11/86	1.0 ml	50 ml
01/08/87	1.0 ml	50 ml
01/22/87	1.0 ml	50 ml
02/05/87	1.0 ml	50 ml
11/09/87	1.0 ml	20 ml
11/28/87	1.0 ml	100 ml
12/07/87	1.0 ml	100 ml
12/21/87	1.0 ml	100 ml

b. **Preparation of the ImmuSTRIP[®] HAMA reference standard**

Serum samples from the immunized baboons were collected during the immunization procedure. These samples were tested by RID until significant levels of anti-mouse antibody were achieved. Large bleeds of the animals were then performed and serum samples were pooled and frozen at -80°C. Aliquots of the pooled sera were assayed by RID and the HAMA activity was determined. A dilution of the pooled sera, to a concentration of 220 ng of precipitable antibody equivalents/ml, was prepared in phosphate buffered saline, sterile filtered and maintained as a reference standard.

c. Performance Characteristics

1. Reproducibility

Within run and between run assay reproducibilities were evaluated by performing the ImmuSTRIP[®] HAMA IgG assay utilizing low, medium and high control samples. The control samples were prepared by enriching normal human serum with purified HAMA. The assigned control values were as follows:

low control = 50 ng/ml
 medium control = 75 ng/ml
 high control = 200 ng/ml

Within run coefficients of variations (percent CVs) were calculated for replicates of three.

Between run CVs were calculated for replicates of three, from assays performed on different days. The CVs for within run values were $\leq 10\%$, and between run CVs were $< 5\%$.

Results for within run and between run evaluations are summarized below:

Run #	Low Control (50 ng/ml)			Medium Control (75 ng/ml)			High Control (200 ng/ml)		
	ng/ml	SD	CV (%)	ng/ml	SD	CV (%)	ng/ml	SD	CV (%)
1	53.4	± 5.2	9.7	76.3	± 2.1	2.8	186.6	± 5.9	3.2
2	56.0	± 2.5	4.5	72.5	± 0.5	0.7	192.8	± 17.7	9.2
3	51.4	± 1.8	3.5	71.5	± 3.2	4.5	193.3	± 19.4	10.0
Mean	53.6	± 2.3	4.3	73.4	± 2.5	3.4	190.9	± 3.7	1.9

Lot to lot reproducibility was evaluated by assaying six prepared serum controls and 15 normal serum samples using three lots of ImmuSTRIP[®] HAMA IgG reagents. The HAMA results were obtained from three standard curves prepared from the reference standard provided with each lot; all results are expressed as a mean of triplicate values. For the 15 normal serum samples, negative for HAMA (≤ 74 ng/ml), a maximum difference of 6.6 ng/ml was obtained across the three lots.

Six serum controls were prepared by enriching negative serum with varying concentrations of purified HAMA. The range in concentration was 50 ng/ml to 400 ng/ml, and maximum differences across the three lots were as follows:

HAMA Concentration	Maximum Difference
50 ng/ml	9.2 ng/ml
75 ng/ml	17 ng/ml
100 ng/ml	1.4 ng/ml
200 ng/ml	33.8 ng/ml
300 ng/ml	19.4 ng/ml
400 ng/ml	44 ng/ml

2. Sensitivity

The sensitivity of the ImmuSTRIP® HAMA IgG is 37 ng of precipitable antibody equivalents/ml. Recommended dilutions of the 220 ng precipitable antibody equivalent/ml reference standard results in a four point curve, prepared as follows:

Standard	Concentration ng Antibody/mL	Volume of HAMA Ref STD (μ L)	Volume of Specimen Diluent (μ L)	Test Dilution
(S1)	220	-	-	Undiluted
(S2)	110	200	200	1:2
(S3)	55	100	300	1:4
(S4)	37	100	500	1:6

The lowest concentration prepared from the reference standard is equal to 37 ng precipitable antibody equivalents/ml (S4), and is the labeled sensitivity of the assay. The recommended dilution of test sample is 1:2, therefore, a "negative" HAMA result has been defined as ≤ 74 ng, precipitable antibody per mL, (37 X 2 [dilution factor]).

3. Specimen Dilution

Multiple dilution of four positive HAMA specimens were prepared in specimen diluent and assayed with the ImmuSTRIP[®] HAMA IgG test system.

The following table illustrates the results of this study:

<u>Sample #</u>	<u>Dilution</u>	<u>Expected</u> (ng/ml)	<u>Actual</u> (ng/ml)	<u>%CV</u>
1	--	*N/A	217.9	
	1:2	109.0	102.9	2.9
	1:4	54.5	50.9	3.4
2	--	*N/A	376.3	
	1:2	188.2	186.4	0.4
	1:4	94.1	98.4	2.2
3	--	*N/A	334.6	
	1:2	167.3	153.0	4.5
	1:4	83.7	73.0	6.8
4	--	*N/A	179.3	
	1:2	89.7	86.1	2.0
	1:4	44.9	47.0	2.4

*Not Applicable.

The ImmuSTRIP[®] HAMA IgG assays requires an initial sample dilution of 1:2. The first HAMA value recorded in the "actual" column is the HAMA result of this initial sample dilution. Further dilutions of the initial sample dilution occurred; (1:2 and 1:4); and expected vs. actual results were compared. The percent CV of the mean was calculated for expected vs. actual results. The average CV for the four specimens was 3.08 with a range of 0.4% to 6.8%.

4. Recovery

A normal human serum pool was spiked with known levels of purified HAMA ranging from 50 ng/ml to 400 ng/ml. Each spiked sample was assayed in quadruplicate with the ImmuSTRIP[®] HAMA IgG ELISA assay. For each known concentration of HAMA, the following values resulted, calculated from the mean of the quadruplicate values at each concentration.

Recovery (%)
ImmuSTRIP[®] HAMA IgG

HAMA Concentration ng/ml	Lot 1628		
	ng/ml	Recovery %	CV %
50 ng/ml	53.1	106.2	6.7
75 ng/ml	76.0	101.3	2.1
100 ng/ml	98.6	98.6	4.2
200 ng/ml	186.6	93.3	2.4
300 ng/ml	290.8	96.9	2.6
400 ng/ml	373.9	93.5	0.6

5. Specificity

Discussion and Tabulation of Results from a Normal, Apparently Healthy Population

Dianon Systems, Inc. Stratford, CT, determined the presence of HAMA in serum samples from an apparently healthy population consisting of 250 males and 214 females, 104 of which were pregnant, with ImmuSTRIP® HAMA IgG.

In this patient population, 98% of the males, 100% of the non-pregnant females and 96.1% of the pregnant females were negative (< 74 ng/ml) with the ImmuSTRIP® HAMA IgG assay. Naturally occurring HAMA, in varying concentrations and frequency of occurrence, has been reported in normal, apparently healthy populations^{8, 20-23}. The degree of frequency has been suggested to be dependent on the sensitivity of the assay method used to determine the presence of HAMA²³.

Rheumatoid Factor Samples

An evaluation of the specificity of ImmuSTRIP® HAMA IgG was performed by assaying 57 serum samples having various concentrations of rheumatoid factors (RF). RF are autoantibodies directed to the Fc fragment of human and other mammalian IgG molecules¹⁹. In this study, serum RF concentrations, expressed in International Units (IU), varied in the range from negative (normal) to 3000 IU (high). All positive samples were from patients diagnosed with rheumatoid arthritis. Dianon Systems, Inc. sequestered these samples from Universal Reagents, Inc., and Scantibodies Laboratories, Inc. and assayed these RF samples following the ImmuSTRIP® HAMA IgG manufacturer's directions. All samples were run in duplicate, the mean of the duplicate values was reported. The patient population from which these samples had been collected either had no previous exposure to mouse protein or such exposure was unknown. Twenty-one (36.8%) of the RF samples resulted in falsely elevated HAMA test results (> 74 ng/ml). There was no apparent correlation between the level of RF in the sample and the severity of interference with the ImmuSTRIP® HAMA IgG assay.

6. Stability

Real time stability data were determined on 12 lots of ImmuSTRIP[®] HAMA IgG kits stored at 2° to 8°C. These data are reported as valid or invalid based on the criteria for a valid assay.* These stability data support a shelf life of 12 months.

NOTE: The studies are on-going for lot 1636.

ImmuSTRIP [®] HAMA IgG Lot #	Day 0	3 Months	6 Months	9 Months	12 Months
1604	Valid	Valid	Valid	Valid	Valid
1606	Valid	Valid	Valid	Valid	Valid
1607	Valid	Valid	Valid	Valid	Valid
1608	Valid	Valid	Valid	Valid	Valid
1611	Valid	Valid	ND	Valid	Valid
1613	Valid	Valid	Valid	Invalid	Valid
1620	Valid	Valid	ND	ND	Valid
1623	Valid	Valid	Valid	Valid	Valid
1631	Valid	Valid	Valid	Valid	ND
1633	Valid	Valid	Valid	Valid	Valid
1634	Valid	Valid	Valid	Valid	Valid
1636	Valid	Valid	Valid	TBD	TBD

ND = Not Done

TBD = To be determined

*Criteria for a Valid Assay:

Slope: 0.005 to 0.015 Correlation coefficient (R^2): ≥ 0.950

Y-intercept: ≤ 0.200

3. Clinical Studies

Clinical studies were conducted at two medical institutions and at one reference laboratory. The investigators and their institutions were: Robert M. Sharkey, Ph.D., Center for Molecular Medicine and Immunology (CMMI), H. Abdel-Nabi, M.D., State University of New York at Buffalo, Dept. of Nuclear Medicine, and Dianon Systems, Inc., Stratford, CT (a commercial reference laboratory).

The objectives of the clinical studies were to:

- a. retrospectively determine the incidence of HAMA in serum samples from a normal, apparently healthy population.
- b. retrospectively evaluate the ImmuSTRIP[®] HAMA IgG assay to detect and quantitate HAMA in patients receiving murine-derived monoclonal antibodies for diagnostic and/or therapeutic purposes.
- c. prospectively evaluate the ImmuSTRIP[®] HAMA IgG assay to detect and quantitate HAMA in patients receiving a murine-derived monoclonal antibody fragment for imaging colorectal cancer, (CEA-Scan[™], *Arcitumomab*).
- d. compare the ImmuSTRIP[®] HAMA IgG assay values to those obtained with a reference method.
- e. determine the clinical impact of elevated HAMA levels on pharmacokinetics and dosimetry in patients receiving ¹³¹I-labeled murine anti-carcinoembryonic antigen (CEA) monoclonal antibody.

The results of each of the above studies are summarized on the following pages.

Summary of Clinical Studies:

a. Distribution of HAMA values in a normal population

Blood samples obtained from 464 healthy individuals (250 males and 214 females, 104 of which were pregnant) were assayed with ImmuSTRIP® HAMA IgG by Dianon Systems, Inc., Stratford, CT.

Results were obtained following the manufacturer's directions for use; all samples were assayed in duplicate and final values are expressed as the mean of the duplicate results.

The following table summarizes the distribution of HAMA values in an apparently healthy population.

Healthy Subjects	PERCENT (%)				
	< 10 ng/ml	10-74 ng/ml	75-99 ng/ml	100-199 ng/ml	200-299 ng/ml
250 Males	41.2	56.8	0.8	0.4	0.8
110 Females	49.1	50.9	0	0	0
104 Pregnant Females	35.6	60.5	1	1	2

The ImmuSTRIP® HAMA IgG assays defines a "negative" as ≤ 74 ng/ml. In this healthy population, 98% of the males, 100% of the non-pregnant females and 96.1% of the pregnant females were negative for HAMA. The presence of antibodies to mouse immunoglobulin in the serum of healthy individuals has been documented in several studies^{8, 20-23}. Thompson et al. found interfering heterophilic antibodies in 92 samples (9.1%)²⁰. Boscato and Stuart, using an assay specifically designed to detect the heterophilic antibodies, demonstrated their presence in 40% of 66 normal serum samples²¹. Courtenay-Luck et al. reported anti-murine antibody immune reactivity in sera from all the healthy controls (n=24) included in their study²². The high variability of the reported incidences of heterophilic antibodies may be a result of the different sensitivities of the assays used²³.

- b. A second clinical study was performed to determine the clinical utility of the ImmuSTRIP® HAMA IgG ELISA assay to detect and semi-quantitate HAMA in patients receiving injections of murine-derived monoclonal antibodies.

Twenty-six patients were provided by Dr. Robert Sharkey of the Center for Molecular Medicine and Immunology (CMMI), Newark, NJ and 51 patients were provided by Dr. Hani Abdel-Nabi of the VA Medical Center, Buffalo, NY.

All patients evaluated in the study had received at least one injection of a murine-derived, intact IgG antibody for imaging and/or therapeutic purposes. The total number of patients required for clinical utility study was determined based on the assumption that 30-50% of elevated HAMA levels can be detected by any assay method as documented in the literature.^{4, 7, 8, 18, 21} To ensure that there is a 95% chance of estimating the detection rate with ImmuSTRIP® HAMA IgG to be no less than 10% of the minimum rate, for any method, a sample size of at least 65 HAMA determinations would be required.

A total of 305 pre- and post-injection specimens from 77 patients were assayed with ImmuSTRIP® HAMA IgG ELISA test system. Seventy-seven of the 305 samples were pre-injection samples collected from patients with no known exposure to murine-derived protein prior to injection of murine-derived monoclonal antibody. One pre-injection sample, per patient, was collected and assayed with ImmuSTRIP® HAMA IgG ELISA test system.

A total of 228 post-injection samples were collected from these 77 patients at various intervals after the injection of a murine-derived monoclonal antibody. Although an effort was made to collect post-injection samples within the specified intervals, it was not always possible due to death, loss to follow-up, or unavailability of the patients. Alternatively, a substantial number of patients had samples collected in excess of the required intervals for post-injection testing. In either event, all assay results have been reported.

The following table represents the number of pre- and post-injection samples collected at various intervals:

# of Patients (Pre-injection Samples)	# of Post- 1 st injection Intervals	# of Post- 1 st injection Samples	# of Pre- 2 nd injection Samples	# of Post- 2 nd injection Samples	# of Pre- 3 rd injection Samples	# of Post- 3 rd injection Samples
11	1	11	3	5	1	2
25	2	50	3	6	1	1
32	3	96	2	6		
5	4	20				
3	5	15				
1	6	6				
77 Total Patients		Total Samples 198	Total Samples 8	Total Samples 17	Total Samples 2	Total Samples 3
GRAND TOTAL PRE-AND POST-INJECTION SAMPLES = 305						

Of the 11 patients with one post-1st-injection sample, three patients went on to receive a second injection of murine-derived antibody. There were three pre-2nd-injection samples; two of the three patients had two post-2nd-injection samples, and one patient had one post-2nd-injection sample, for a total of five post-2nd-injection samples. One of the three patients went on to receive a third injection of murine-derived antibody. There was one pre-3rd-injection sample; that patient had two post-3rd-injection samples, for a total of two post-3rd-injection samples.

Of the 25 patients with two post-1st-injection samples, three patients went on to receive a second injection of murine-derived antibody. There were three pre-2nd-injection samples and all patients had two post-2nd-injection samples, for a total of six post-2nd-injection samples. One of the three patients received a third injection of murine-derived antibody; one pre-3rd-injection sample and one post-3rd-injection sample were assayed.

Of the 32 patients with three post-1st-injection samples, two patients went on to receive a second injection of murine-derived antibody. There were two pre-2nd-injection samples; one patient had two post-2nd-injection samples, and one patient had four post-2nd-injection samples, for a total of six post-2nd-injection samples.

In summary, there were eight patients of the 77 who received a second injection of murine-derived antibody; two of the eight patients went on to receive a third injection of murine-derived antibody.

The results of both retrospective clinical studies to determine the utility of ImmuSTRIP® HAMA IgG in patients receiving murine-derived monoclonal antibodies are summarized as follows:

Of the 26 patients provided by Dr. Robert Sharkey of the Center for Molecular Medicine and Immunology (CMMI), Newark, NJ, 21 patients (80.8%) were positive for HAMA post-injection with murine-derived antibodies. The ImmuSTRIP® HAMA IgG assay result was positive (> 74 ng/ml) at least once during the post-injection test interval(s).

Of the 51 patients provided by Dr. Hani Abdel-Nabi of the VA Medical Center, Buffalo, NY, 21 patients (41.2%) were positive for HAMA post-injection with murine-derived antibodies. Dr. Nabi's laboratory prepared a 1:10 dilution of patient sample with specimen diluent prior to assay with ImmuSTRIP® HAMA IgG. Immunomedics, Inc. initially recommended a 1:10 dilution, but subsequently changed the recommended sample dilution to 1:2. Specimen dilution studies resulted in acceptable expected vs. actual values and percent CV's, therefore no difference was expected in 1:2 vs. 1:10 sample dilution HAMA values. The ImmuSTRIP® HAMA IgG assay was positive (> 400 ng/ml: sensitivity 40 ng/ml X 10 [dilution factor]), at least once during the post-injection test interval(s).

c. ImmuSTRIP® HAMA and ImmuSTRIP® HAMA Fragment assay results for patients receiving CEA-Scan®, (Arcitumomab).

Two HAMA enzyme immunoassays, ImmuSTRIP® HAMA IgG and ImmuSTRIP® HAMA Fragment, were used to quantify HAMA in patient sera obtained in the CEA-Scan® Phase III study. CEA-Scan® is an FDA-approved (BLA #1205) imaging agent for colorectal cancer. (Please note that the ImmuSTRIP® HAMA Fragment ELISA assay kit is the subject of a separate 510(k) submission, therefore, clinical results are provided here for informational purposes only.) Sera subsets were also assayed for drug-reactive HAMA with an HPLC assay that is used, and has been validated, to determine purity immunoreactivity, and stability of CEA-Scan®. HAMA determined by this assay will be referred to as "drug-reactive HAMA." To perform the drug-reactive HAMA assay, a vial of CEA-Scan® is labeled with ^{99m}Tc-pertechnetate according to the package insert. Twenty ng of the labeled drug contained in one μ l of saline-HSA (1% human serum albumin in saline) is added to 100 μ l of patient serum. The specimen is incubated for one hour at 37°C, and then analyzed for drug-reactive HAMA by application of 50 μ l of the serum onto a HPLC size exclusion column, and continuously monitoring the radioactivity eluting from the column. If drug-reactive HAMA is not present in the serum, the primary peak of radioactivity elutes at a time consistent with its molecular weight of

50,000 daltons. If drug-reactive HAMA is present in the sera, the radiolabeled drug is bound by the antibody, and the resultant complex that has a molecular weight of 200,000 daltons or greater, elutes from the column prior to the elution of the 50,000 Dalton Fab'-SH. To determine if drug-reactive HAMA is directed against the constant region of the Fab', or the variable region of the Fab', 10 µg of an irrelevant murine Mab is added to the 100 µl of serum prior to addition of the labeled drug, and the assay repeated. Thus, HAMA reactive with the constant region of Fab' is neutralized and an antibody-drug complex is not formed. HAMA reactive with the variable region is not neutralized and the elution time of the radioactivity is not changed by addition of the irrelevant MAb.

Results:

Of 382 patients entered into the Phase III trials, 240 of the patients had a baseline determination and at least one follow-up measurement with ImmuSTRIP® HAMA-IgG and ImmuSTRIP® HAMA Fragment Assays (data on file at Immunomedics).

Of the 240 patients, 213 (89%) had negative baseline HAMA-IgG and HAMA Fragment test result, and remained negative after infusion of CEA-Scan®; these patients are listed in Table VII. Sera of 24 of these patients were assayed for drug-reactive HAMA (Vol. 2, Section VI, pages 140-143, Table VII, underlined patient numbers) 2-3 months post-infusion, and all of the sera were negative in this assay. Seventeen of these 213 patients received a second infusion of CEA-Scan® and all of these patients remained negative with the HAMA-IgG and HAMA Fragment Assays (Vol. 2, Section VI, Table VIII, pages 144-145). Twenty sera from 12 of these patients that received a second infusion of CEA-Scan® were assayed for drug-reactive HAMA after the second administration of the drug, and all remained negative for drug-reactive HAMA (Vol.2, Section VI, Table VIII, pages 144-145).

Of the 240 patients, 24 (10%) patients had at least one serum sample that tested positive with the ImmuSTRIP® HAMA IgG Assay and negative with the ImmuSTRIP® HAMA Fragment Assay (Vol. 2, Section VI, Table IX, page 146). Twenty-one sera from ten of these patients were tested for drug-reactive HAMA. With the exception of patient 040950, all tested negative. The baseline serum and the two post-infusion sera of patient 040950 bound approximately 50% of the labeled drug, and this HAMA was neutralized by irrelevant IgG. Therefore, this preexistent drug-reactive HAMA is directed against the constant region of the Fab'. Administration of CEA-Scan® did not significantly boost the HAMA titer in this patient. One of the ImmuSTRIP® HAMA IgG positive, ImmuSTRIP® HAMA Fragment negative patients (181801) received a second injection of CEA-Scan®

(Vol. 2, Section VI, Table VIII, page 145). The baseline HAMA-IgG was 209 ng/mL the day the second dose of CEA-Scan[®] was given, and the HAMA-IgG in the two post-infusion sera were essentially unchanged (183 ng/mL and 170 ng/mL). Both of these post-infusion sera were negative with the drug-reactive HAMA method.

Only three (1.3%) of the 240 patients tested positive with the ImmuSTRIP[®] HAMA Fragment Assay at any time (patients 040405, 050602, and 051803); one other patient tested positive for HAMA-Fragment (050991), but was determined to have been injected with OncoScint CR 103 shortly before being given CEA-Scan[®] (Vol 2, Section VI, Table X, page 147). OncoScint CR 103 contains an intact IgG-MAb, and has been established to induce HAMA-IgG levels of >400 ng/mL in over 30% of patients after a single drug-infusion (Kaladas PM, et al., Antibody Immunoconjugates and Radiopharmaceuticals 4:309-317:1991).

One of the three Phase III patients (051803) that was positive with both the ImmuSTRIP[®] HAMA IgG and the ImmuSTRIP[®] HAMA Fragment also received a second injection of CEA-Scan[®], a year after the first administration (Vol. 2, Section VI, Table X, page 147). The baseline sera at the time of the second injection was negative for both HAMA-IgG and HAMA-Fragment. Both post-infusion sera remained negative for HAMA-Fragment, and only the one-month post-infusion sera demonstrated a low amount of HAMA-IgG (91.7 ng/mL). However, both post-infusion sera were strongly positive with the drug-reactive HAMA test method, and HAMA in neither of the sera was neutralized with irrelevant MAb-IgG, putative evidence that this HAMA is directed against the variable region of the Fab'.

In summary, with the exception of the patient that had received OncoScint CR 103, none of the 240 patients that were monitored in this study for induction of HAMA appear to have developed HAMA.

All clinical samples were run in duplicate, the mean of the duplicate values was reported.

- d. Comparison of the ImmuSTRIP® HAMA IgG ELISA test system values with values obtained with a double-antigen, radiometric assay method referred to as "The Reference Method."⁷

Samples, provided by Dr. Robert Sharkey of CMMI, Newark, NJ, from the 28 patients evaluated in the pre- and post- injection HAMA study were assayed by Dr. M. Khazaeli, of HAMA-KINE, Inc, 1075 Thirteenth Street South, Birmingham, Alabama. There were a total of 61 samples (19 pre-injection samples and 42 post-injection samples) from the CMMI study with sufficient quantity to perform comparative testing with the Reference Method.

The results of the ImmuSTRIP® HAMA IgG ELISA and Reference Method comparative study are summarized as follows:

Of the 19 pre-injection of murine-derived antibody samples, one sample was positive (> 74 ng/ml) with the ImmuSTRIP[®] HAMA IgG ELISA assay and three were positive (> 10 ng/ml) with the Reference Method. Each test gave a positive result on sample #36; the Reference Method resulted in two additional positive results, samples #42 and #47.

In the post-injection of murine-derived antibody samples ($n=42$), there were 30 positive (> 74 ng/ml) results (71.4%) with the ImmuSTRIP[®] HAMA IgG assay method and 27 positive (> 10 ng/ml) results (64.3%) with the Reference Method.

Table 1. Comparison of ImmuSTRIP[®] HAMA IgG (ELISA) assay results vs. double-antigen, Radiometric, (RIA) assay results in pre- and post-injection samples.

Pre-injection (n=19)

% Positive*	
ELISA	5.3 (1/19)
RIA	15.8 (3/19)

*ELISA positive > 74 ng/ml, RIA positive > 10 ng/ml.

Post-injection (n=42)

% Positive*	
ELISA	71.4 (30/42)
RIA	64.3 (27/42)

*ELISA positive > 74 ng/ml, RIA positive > 10 ng/ml.

Summary of and post- injection with murine-derived antibody (n=42)
 ImmuSTRIP® HAMA IgG (ELISA) double antigen radiometric assay (RIA)

ELISA

RIA

		NEGATIVE	POSITIVE
RIA	NEGATIVE	23.8% (10/42)	11.9% (5/42)
	POSITIVE	9.5% (4/42)	54.8% (23/42)

True Positive (TP)	=	23
False Positive (FP)	=	5
True Negative (TN)	=	10
False Negative (FN)	=	4

$$\begin{aligned} \text{Sensitivity} &= \frac{\text{TP}}{\text{TP} + \text{FN}} = 85.2\% \\ \text{Accuracy} &= \frac{\text{TP} + \text{TN}}{\text{TP} + \text{TN} + \text{FP} + \text{FN}} = 78.6\% \\ \text{Specificity} &= \frac{\text{TN}}{\text{TN} + \text{FP}} = 66.7\% \\ \text{Positive Predictive Value (PPV)} &= \frac{\text{TP}}{\text{TP} + \text{FP}} = 82.1\% \end{aligned}$$

e. Clinical impact of elevated HAMA levels on pharmacokinetics and dosimetry in patients receiving ¹³¹I-labeled murine anti-carcinoembryonic antigen (CEA) monoclonal antibody.

A total of 57 patients with CEA-expressing tumors received therapeutic doses (4-23 mg of protein and 44-268 mCi of ¹³¹I) of anti-CEA (NP-4) IgG antibody. HAMA levels were determined pre-injection and post-injection on a weekly basis until 6 weeks post-therapy and then monthly thereafter. The ImmuSTRIP[®] HAMA IgG assay was used to determine the HAMA values.

The above study was published in The Journal of Nuclear Medicine, June 1997. The publication is provided in this Section for the convenience of the reviewer (page 27). The impact of elevated HAMA levels on pharmacokinetics and dosimetry is discussed in detail under the section "Pharmacokinetics." The study demonstrated that at HAMA titers below 300 ng/ml, no effect on the clearance rates of the (injected) antibody from the blood and whole-body was apparent, whereas with titers above this threshold a rapidly increasing plasma and whole-body clearance rate was observed, which was reflected by decreasing red marrow and whole-body doses ($r = -0.6$; significantly different from zero at $p < 0.001$). Further discussion on elevated HAMA levels may be found in the "Dosimetry" section of the publication.

G. **CONCLUSIONS DRAWN FROM THE STUDIES**

The safety and effectiveness evaluations of ImmuSTRIP® HAMA IgG demonstrated the ability of the device to detect and semi-quantitate Human Anti-Mouse Antibody (HAMA) in human serum. The preclinical studies resulted in performance specifications for sensitivity, specificity, reproducibility, dilution linearity and stability that are within acceptable limits for devices of this type.

Clinical studies with regard to the distribution of HAMA values in the normal, apparently healthy population and in the pre- and post- injection with murine-derived antibody populations demonstrate agreement with the distribution of HAMA values from similar populations cited in numerous publications¹⁻²³. The clinical impact of elevated HAMA levels on pharmacokinetics and dosimetry has been demonstrated by Behr TM, et al.²⁴

Lastly, the comparative study with ImmuSTRIP® HAMA IgG vs. a double antigen radiometric assay method (Reference Method) demonstrated agreement with values obtained on the same samples in the two methods, and with values previously reported.⁷

H. REFERENCES FOR SAFETY AND EFFECTIVENESS SECTION

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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Rockville MD 20850

MAR 17 1998

Joseph E. Presslitz, Ph.D.
Vice President, Regulatory Affairs
Immunomedics®, Inc.
300 American Road
Morris Plains, New Jersey 07950

Re: K972873/S2
Trade Name: ImmuSTRIP® HAMA IgG - ELISA Test System for Human
Antibodies to Mouse IgG
Regulatory Class: II
Product Code: DAK
Dated: February 9, 1998
Received: February 13, 1998

Dear Dr. Presslitz:

We have reviewed your Section 510(k) notification of intent to market the device referenced above and we have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (Premarket Approval), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 895. A substantially equivalent determination assumes compliance with the current Good Manufacturing Practice requirement, as set forth in the Quality System Regulation (QS) for Medical Devices: General regulation (21 CFR Part 820) and that, through periodic (QS) inspections, the Food and Drug Administration (FDA) will verify such assumptions. Failure to comply with the GMP regulation may result in regulatory action. In addition, FDA may publish further announcements concerning your device in the Federal Register. Please note: this response to your premarket notification submission does not affect any obligation you might have under sections 531 through 542 of the Act for devices under the Electronic Product Radiation Control provisions, or other Federal Laws or Regulations.

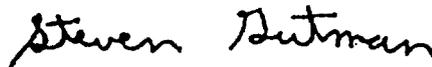
Page 2

Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88), this device may require a CLIA complexity categorization. To determine if it does, you should contact the Centers for Disease Control and Prevention (CDC) at (770)488-7655.

This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4588. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers Assistance at its toll free number (800) 638-2041 or at (301) 443-6597 or at its internet address "<http://www.fda.gov/cdrh/dsmamain.html>"

Sincerely yours,



Steven I. Gutman, M.D., M.B.A.
Director
Division of Clinical
Laboratory Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

III.

INDICATION FOR USE STATEMENT

510(k) Number (if known): K972873

Device Name: _____

Indications For Use:

The ImmuSTRIP® HAMA IgG ELISA Test System is a direct enzyme-linked immunosorbent assay for the detection and quantitation of human antibodies to mouse IgG (HAMA).

Peter Mawm
(Division Sign-Off)
Division of Clinical Laboratory Devices
510(k) Number K972873

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Prescription Use _____
(Per 21 CFR 801.109)

OR

Over-The-Counter Use _____
(Optional Format 1-2-96)