Summary of Safety and Probable Benefit

I. GENERAL INFORMATION

Device Generic name(s):	Enzyme-linked Immunosorbent Assay (ELISA) for the *in vitro* quantitative measurement of Soluble Mesothelin Related Peptide (SMRP) in human serum
Device Trade name(s):	MESOMARK® Assay
Applicant's name and address	Fujirebio Diagnostics, Inc 201 Great Valley Parkway Malvern, PA 19355, USA
Humanitarian Device Exemption (HDE) number:	H060004
Humanitarian Use Device (HUD) Designation Number:	06-0169
HUD#:	06-0169
Date of Humanitarian Use Device (HUD) Designation:	September 8, 2006
Date of Panel recommendation:	None
Date of Good Manufacturing Practice Inspection	November 06-14, 2006
Date of notice of approval to the applicant	January 24, 2007

II. INDICATIONS FOR USE

The Fujirebio Diagnostics Inc. (FDI) MESOMARK™ is an enzyme linked immunosorbent assay (ELISA) for the quantitative measurement of Soluble Mesothelin Related Peptides (SMRP) in human serum.

Measurement of SMRP may aid in the monitoring of mesothelioma patients diagnosed with epithelioid or biphasic mesothelioma. MESOMARK values must be interpreted in conjunction with all other available clinical laboratory data.

Humanitarian Device. Authorized by Federal Law for use as an aid in the monitoring of patients diagnosed with mesothelioma. The effectiveness of this device for this use has not been demonstrated.

III. CONTRAINDICATIONS

There are no known contraindications for the FDI MESOMARK™ Assay.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions are stated in the product labeling.

V. DEVICE DESCRIPTION

1. Kit description

The MESOMARK kit is available in one (1) test size:

- 96 Tests (Part Number 801-900)

Materials supplied:

WASH BUFFER

1 Bottle (50 mL). Wash Buffer (20X concentrated). 0.002 M Imidazole buffered saline; containing 0.02% Tween 20. Must be diluted before use.

SUBSTRATE

1 Bottle (12 mL). Substrate <0.1% Tetramethylbenzidine (TMB) in an acidic buffer. Ready to use.

STOP SOLUTION

1 Bottle (12 mL). Stop Solution 1% Hydrocholic Acid. Ready to use.

COATED PLATE

1 Plate (96 wells). Plastic microtiter wells coated with 4H3 murine monoclonal antibody in foil pouch with desiccant. Ready to use.

CONJUGATE

1 Bottle (12 mL). Conjugate. 569 murine monoclonal antibody conjugated to horseradish peroxidase enzyme with protein (bovine) stabilizer. Contains Proclin 300/Gentamicin as preservatives. Ready to use.

CALIBRATOR A/DILUENT

1 Bottle (100 mL). Calibrator A/Assay Diluent. PBS buffer with protein (bovine) stabilizer. Contains Proclin 300/Gentamicin as preservatives. Ready to use.

CALIBRATOR

1 Bottle each (1.0 mL). Calibrator B to F contain 569 reactive antigen (recombinant) prepared in PBS buffer with (bovine) stabilizer. Contains Proclin 300/Gentamicin as preservatives. Ready to use.

Calibrator	Value (nM)
CAL A	0
CAL B	2
CAL C	4
CAL D	8
CAL E	16
CAL F	32

CONTROLS

1 Bottle each (1.0 mL). Controls contain 569 reactive antigen (recombinant) prepared in PBS buffer with (bovine) stabilizer. Contains Proclin 300/Gentamicin as preservatives. Ready to use.

Control	Target Concentration (nM)	Range (nM)
LOW CONTROL	4.5	3.6 - 5.4
HIGH CONTROL	13.5	10.8 – 16.2

2. Test Principle

MESOMARK is a two-step immunoassay used to quantitate the presence of the Soluble Mesothelin Related Peptides (SMRP) in human serum using enzyme immunoassay technology with colorimetric detection in a standard ELISA microplate sandwich assay format. Two separate monoclonal antibodies are used (4H3 and OV569); one for capturing SMRP, the other for detection of SMRP. Detection is accomplished by the addition of a standard chromogenic substrate that binds to the HRP-labeled monoclonal antibody. A direct relationship exists between the amount of SMRP in sample and the optical density (OD) detected by the spectrophotometric microtiter plate reader.

VI. ALTERNATIVE PRACTICES OR PROCEDURES

The efficacy of mesothelioma treatment regimens post diagnosis is routinely measured by radiologic imaging techniques and clinical signs and symptoms. There is currently no in vitro diagnostic test or tumor marker approved or cleared by the FDA for managing patients diagnosed with mesothelioma.

VII. MARKETING HISTORY

This MESOMARK assay was first sold on March 1, 2005 to European and Australian distributors. Kits have been sold through these distributors into the following markets: Australia, New Zealand, Germany, Austria, Belgium, Croatia, France, Italy, Norway, The Netherlands and Switzerland. The MESOMARK kits sold into these markets have been used by private and hospital pathology laboratories, reference laboratories, cancer hospitals and research institutions for patient testing and clinical and scientific research.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The risks associated with the use of the MESOMARK Assay arise from false positive or false negative results. The potential adverse effects of the device on health could be:

1. A falsely elevated MESOMARK result could lead to a medical decision causing unnecessary additional diagnostic workup for disease progression and subsequent treatment.

2. A falsely low MESOMARK result could lead to medical decision depriving the patients of necessary diagnostic workup and subsequent treatment.

The MESOMARK is not indicated as the sole diagnostic tool in monitoring disease progression; it must be used in conjunction with the information from a complete clinical evaluation including clinical signs and symptoms and medical imaging modalities.

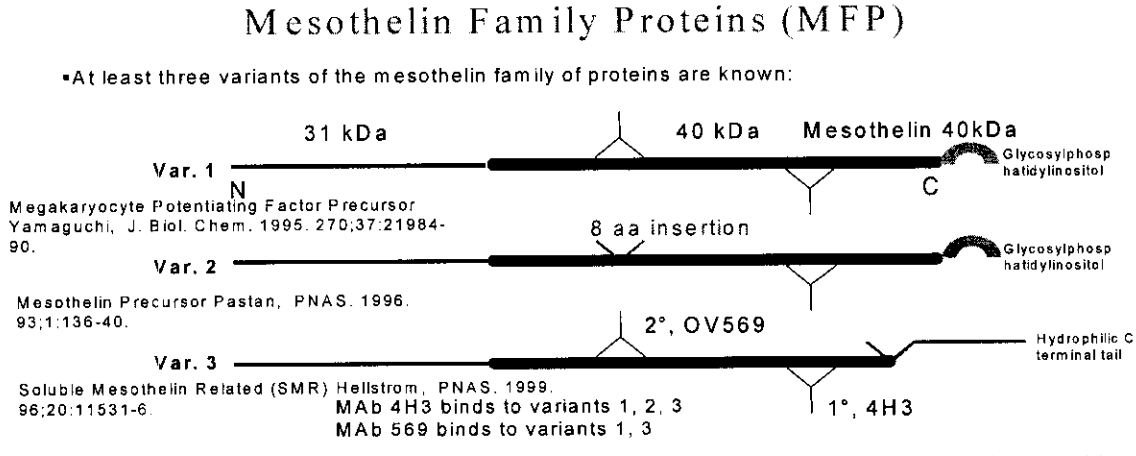
VIII. SUMMARY OF PRECLINICAL STUDIES

In-house and external studies were conducted to assess the analytical performance characteristics of the FDI MESOMARK assay.

A. Laboratory Studies

1. Antigen and antibody characterization

Mesothelin Variants



a) There are at least three (3) variants for mesothelin family protein. The 569 reactive antigen (Soluble Mesothelin Related Protein SMRP or Mesothelin Variant 3) has 591 amino acids and predicted molecular weight of a 80 kDa, and is a putative

secreted protein. However, it was not evident the 80 kDa SMRP or its processed form (44 kDa) existed in patient serum. It has been recently demonstrated that the most frequently expressed mesothelin family protein at the surface of cells from ovarian carcinomas and certain other tumors was Mesothelin Variant 1 (Megakaryocyte Potentiating Factor or MPF). SMRP was less frequently expressed in tumors (Hellstrom I et al. Cancer Epidemiol Biomarkers Prev (2005) 15(5) pp1014-1020). It was unclear, among the three known variants of mesothelin family protein, which was relevant to epithelioid and biphasic malignant mesothelioma. MESOMARK might detect other Mesothelin variants such as MPF (Variant 1) in serum but not SMRP (Variant 3). It has not been demostrated that the SMRP is a serum protein.

b) Normal subjects have low baseline expression levels of SMRP which could be up-regulated as the result of cancer treatment such as in thrombocytopenia or drug actions.

c) MESOMARK monoclonal antibody was originally made by immunizing mice with ovarian cancer cells (not with purified antigen from malignant mesothelioma). A second set of hybridoma (including mAB 4H3) was made to the antigen to which OV569 binds. The mAB 4H3 recognized a different antigenic epitope of the same mesothelin family protein purified from supernatants of ovarian and lung carcinoma cell lines. On Western Blot, mAB OV569 recognizes a 44 kDa shed SMRP fragment, as well as an additional unidentified high molecular weight protein of 212 kDa from cell lysate and eluate of pleural effusion but not the predicted 80 kDa SMRP. The mAB OV569 was not mono-specific to SMRP.

d) Data from Western Blotting experiments (utilizing samples with the same matrix as authentic patient sera) that might definitively establish the specificity of mAB OV569 and mAB 4H3 were not provided.

2. Imprecision Analysis
The MESOMARK assay precision is <15% total CV. A study was performed as described per the Clinical Laboratory Standard Institute (CLSI) Protocol EP5-A2 "Evaluation of Precision Performance of Clinical Chemistry Devices, Approved Guideline - Second Edition". Three panels were assayed, using two kit lots, in replicates of two at two separate times per day for 20 days at two sites. Data from this study are summarized below.*

Panel Member	Reagent Lot	Site	N	Mean Conc. (nM)	Within Run		Total	
					SD	%CV	SD	%CV
1	1	1	80	3.85	0.07	1.9	0.21	5.5
		2	80	4.26	0.23	5.3	0.43	10.1
	2	1	80	4.17	0.08	1.8	0.18	4.4
		2	80	4.47	0.21	4.8	0.45	10.0
2	1	1	80	7.44	0.08	1.1	0.30	4.0
		2	80	8.16	0.28	3.4	0.46	5.6
	2	1	80	8.00	0.15	1.9	0.38	4.7

Panel Member	Reagent Lot	Site	N	Mean Conc. (nM)	Within Run		Total	
					SD	%CV	SD	%CV
		2	80	8.59	0.20	2.3	0.48	5.6
3	1	1	80	16.54	0.31	1.9	1.31	7.9
		2	80	18.01	0.39	2.2	0.91	5.0
	2	1	80	17.97	0.31	1.7	0.86	4.8
		2	80	19.02	0.43	2.3	2.09	11.0

* Representative data

Additional Imprecision Study using spiked specimens

A study was performed at the manufacturer site to assess the precision of MESOMARK at low concentrations of antigen (< 2nM). One defibrinated plasma panel member (Low Panel) and one MESOMARK Control (Low Control) containing 569 reactive antigen (recombinant) prepared in PBS buffer with protein (bovine) stabilizer were tested in duplicate using one lot of reagents at two separate times per day for a total of 20 days. The average total imprecision (%CV) of MESOMARK for the Low Panel and Control members in this study was calculated to be ≤ 7.6%. The observed imprecision is comparable to that observed for the higher ranges of the assay and supports the precision claim of ≤15%.

Total Precision	Low Control	Low Panel
# of Observations	80	80
Mean	1.3 nM	1.5 nM
Standard Deviation	0.1 nM	0.1 nM
Within-Run %CV	6.4%	4.4%
Total %CV	7.6%	5.7%

3. Recovery

A study was performed to determine recovery for the MESOMARK test. Known concentrations of 569-reactive antigen were added to five (5) independent normal human serum samples throughout the assay range. The concentration of 569-reactive antigen was determined using the MESOMARK assay, and the resulting percent recovery was calculated. The MESOMARK assay mean recovery is 100% ± 15% with average recovery at each level of added analyte = 107% (Range = 103%-113%). Representative data from this study are summarized below.*

Sample	Endogenous Assay Value (nM)	569-Reactive Antigen added (nM)	Observed Assay Value (nM)	Percent Recovery**
1	3.96	0	3.96	N/A
		3.2	7.49	105
		6.4	10.39	100
		16.0	21.25	106
		24.0	31.11	111
2	4.25	0	4.25	N/A
		3.2	8.65	116
		6.4	10.03	94

Sample	Endogenous Assay Value (nM)	569-Reactive Antigen added (nM)	Observed Assay Value (nM)	Percent Recovery**
		16.0	20.95	103
		24.0	27.62	98
3	3.35	0	3.35	N/A
		3.2	7.39	113
		6.4	10.02	103
		16.0	21.20	110
		24.0	31.50	115

* Representative data; results in individual laboratories may vary from these data.

** Percent Recovery Calculation:

% Recovery = 100 x (measured concentration)/(endogenous concentration + added concentration)

4. Dilution Linearity

   A study was performed for the MESOMARK assay modeled after the CLSI Protocol EP6-P2[21]. Known concentrations of 569-reactive antigen were added to five (5) independent normal human serum samples followed by dilution with assay diluent. The MESOMARK value was determined for each dilution and the percent recovery was calculated. The MESOMARK assay mean dilution linearity is 100% ± 15% with average recovery across the five diluted samples = 109% (Range = 99%-113%). Representative data from this study are presented below.

Sample	Dilution Factor	Expected Value (nM)	Observed Value (nM)	Percent Recovery[2]
1	Undiluted	26.69	26.69	N/A
	1:1.1	24.02	24.21	101
	1:1.4	18.68	20.05	107
	1:2	13.35	15.01	112
	1:2.5	10.68	12.39	116
	1:3.3	8.01	9.11	114
	1:5	5.34	6.16	115
	1:10	**2.67**	**3.29**	**123[3]**
	1:20	**1.33**	**1.57**	**118[3]**
2	Undiluted	26.53	26.53	N/A
	1:1.1	23.88	24.15	101
	1:1.4	18.57	19.39	104
	1:2	13.26	15.14	114
	1:2.5	10.61	12.24	115
	1:3.3	7.96	9.19	115
	1:5	**5.31**	**6.26**	**118[3]**
	1:10	2.65	2.78	105
	1:20	1.33	1.51	114
3	Undiluted	25.91	25.91	N/A
	1:1.1	23.31	21.58	93
	1:1.4	18.13	17.93	99

Sample	Dilution Factor	Expected Value (nM)	Observed Value (nM)	Percent Recovery[2]
	1:2	12.95	11.28	87
	1:2.5	10.36	10.60	102
	1:3.3	7.77	7.19	93
	1:5	5.18	5.33	103
	1:10	**2.59**	**3.10**	**120[3]**
	1:20	1.30	1.28	99

[1] Representative data; results in individual laboratories may vary from these data.
[2] Percent Recovery Calculation:
% Recovery = 100 x (measured concentration) x (dilution factor)/undiluted concentration
[3] Level outside the acceptance limit of 15%

Dilution Linearity for Functional Sensitivity

Sample #	Dilution Factor	Observed Value	% CV	Theoretical Value	% Recovery	Mean Recovery*	R
1	1.0	2.596	6.3	2.596	100.0	92.8	0.989
	1.1	2.503	2.8	2.360	106.1		
	1.4	1.832	6.2	1.854	98.8		
	2.0	0.929	4.8	1.298	71.6		
	2.5	0.993	0.8	1.038	95.6		
	3.3	0.782	2.3	0.787	99.4		
	5.0	0.443	4.3	0.519	85.3		
2	1.0	4.604	2.7	4.604	100.0	93.6	0.998
	1.1	4.192	4.0	4.185	100.2		
	1.4	2.998	5.8	3.289	91.2		
	2.0	2.120	1.7	2.302	92.1		
	2.5	1.654	2.9	1.842	89.8		
	3.3	1.285	4.1	1.395	92.1		
	5.0	0.885	4.4	0.921	96.1		
3	1.0	1.770	5.9	1.770	100.0	94.1	0.987
	1.1	1.710	6.3	1.609	106.3		
	1.4	1.222	4.9	1.264	96.7		
	2.0	0.645	5.0	0.885	72.9		
	2.5	0.687	5.8	0.708	97.0		
	3.3	0.523	3.5	0.536	97.5		
4	1.0	1.808	3.5	1.808	100.0	96.2	0.983
	1.1	1.726	3.4	1.644	105.0		
	1.4	1.249	6.0	1.291	96.7		
	2.0	0.640	9.7	0.904	70.8		
	2.5	0.714	0.5	0.723	98.7		
	3.3	0.547	11.5	0.548	99.8		
	5.0	0.383	0.7	0.362	105.9		
5	1.0	8.317	6.2	8.300	100.0	84.9	0.998
	1.1	7.369	7.2	7.561	97.5		

Sample #	Dilution Factor	Observed Value	% CV	Theoretical Value	% Recovery	Mean Recovery*	R
	1.4	5.552	0.1	5.941	93.5		
	2.0	3.852	4.1	4.159	92.6		
	2.5	2.737	3.8	3.327	82.3		
	3.3	1.971	9.2	2.520	78.2		
	5.0	1.250	3.5	1.663	75.1		
	10.0	0.622	4.9	0.832	74.8		

Assay linearity was evaluated by measuring dilution linearity created from five samples (concentration range from 0.4 to 26.69 nM). Dilutions were evaluated using percent recovery determination.

Percent Recovery Calculation:
% Recovery = 100 x (measured concentration) x (dilution factor)/undiluted concentration.

These results indicate that assay results are linear with dilution within the range of 5.0 to 27.0 nM within 15% recovery.

5. Interfering Substances
Mean assay recoveries in the presence of interfering substances are 100% ± 15%. Recovery studies were performed to compare sera containing the following compounds at the indicated concentrations with control sera.*

INTERFERING SUBSTANCE

Test Compound	**Test Concentration**
Bilirubin	20mg/dL
Hemoglobin	500mg/dL
Total Protein	12g/dL
Triglycerides	3g/dL

CHEMOTHERAPEUTIC AGENTS

Test Compound	**Test Concentration**
Cisplatin	1.53 mg/mL
Carboplatin	0.34 mg/mL
Alimta	1.3 mg/mL
Gemcitabine	300 µM

*Representative data; results in individual laboratories may vary from these data.

POTENTIALLY INTERFERING CLINICAL CONDITIONS
The MESOMARK assay was evaluated using specimens with Human Anti-mouse Antibodies (HAMA) and Rheumatoid Factor (RF) to further assess the assay specificity. Ten specimens positive for HAMA and five specimens positive for RF were evaluated for percent (%) recovery with an additional 5 and 12.5 nM 569-

reactive antigen spiked into each specimen; mean percent (%) recovery results are summarized in the following table.*

Clinical Condition	Number of Specimens	Mean Percent Recovery (Range)
HAMA	10	99% (86% - 112%)
RF	5	105% (100% - 112%)

*Representative data; results in individual laboratories may vary from these data.

6. Sample Stability Testing
   Fifty-one (51) samples were analyzed within 36 hours, Day 3 and Day 7 from draw and at subsequent times and kept at + 2-8°C for temperatures of storage, and alternating cycles of freeze-thaw. Results of testing at various storage conditions and durations are listed in the table below. Samples are stable at the conditions and times indicated in the table.

Storage Condition	Duration
+ 20 - 25 °C	No data
+ 2-8°C	420 days
- 10 °C	No data
Freeze-thaw	10 cycles (24 Hours)

7. Serum vs. Plasma Comparison
   Not applicable

8. Limit of Detection and Limit of Quantification
   The Lowest Limit of Detection (LOD) of MESOMARK was determined by running calibrator curves, controls and testing the MESOMARK Calibrator A/Assay Diluent (0 nM) in replicates of 25. This testing was performed using two kit lots (n = 50 replicates of Calibrator A/Assay Diluent). Analytical sensitivity corresponds to the upper limit of the 95% confidence interval of the zero calibrator and represents the lowest concentration of antigen that can be distinguished from zero. The analytical sensitivity of MESOMARK is established as 0.3 nM.

   The functional sensitivity and imprecision at the minimal detectable limit was assessed by serial dilution of 5 patient samples. Linear regression of observed concentration versus theoretical concentration yielded a slope of 1.00 to 1.06 for the 5 samples and $R > 0.98$. The intra-assay CV was $\leq 15\%$ down to 0.2nM. These data demonstrate linearity of the MESOMARK assay from 0.2nM to 26.69 nM.

9. High Dose Hook Effect
   High dose hook effect is a phenomenon whereby very high-level specimens may read within the dynamic range of the assay. For the MESOMARK assay, no high dose hook effect was observed when samples containing up to approximately 10,291 nM of 569-reactive antigen were assayed.

10. Reagent stability

Reagent open use stability for a single reagent pack was determined with reagents stored at 2-8 °C for up to 60 weeks. The acceptance criterion was ±15% of the initial value and the B/A ratio must be > 2.0 according to the specification report. Results support the recommendation of 60 weeks storage at 2-8°C.

Additional transport stability study was performed in conjunction with this evaluation. Available data indicated the MESOMARK Assay kit was stable for 392 days (56 weeks) when shipped and stored at 2-8°C.

IX. SUMMARY OF CLINICAL INFORMATION

A. Clinical Study Objective

The major objective of this clinical study was to determine the safety of the device in histologically proven malignant mesothelioma for monitoring purpose.

The specific objectives of the study included:

- To determine the precision of the assay using serum panel set
- To verify the performance of the quality control procedures as stated in the package insert
- To determine the normal range of the assay using serum samples from an apparently healthy population
- To determine the reference ranges of the assay using serum samples from patients having various related conditions
- To evaluate concordance between MESOMARK values and disease state over time as described by the attending physician

B. Study Design

1. Apparently Healthy Subjects for Determination of Reference Range

   Values for MESOMARK were obtained on 163 women and 246 men (409 total) with no evidence of disease. This number of patients was sufficient to estimate the Normal Range with 99% confidence. All of the samples that were used in this study were obtained from either commercial vendors or specimen banks. No samples were specifically drawn for this study. The sample inclusion and exclusion criteria are as follows:

   The Inclusion criteria for normal samples are as follows:

   - Apparently healthy (self-declared)
   - Minimum 0.5 mL volume available
   - Normal appearance
   - Informed consent

   The Exclusion criteria for normal samples are as follows:

   - Concurrent illness
   - Insufficient volume
   - Stored or shipped at 4°C
   - Icteric, lipemic, hemolytic, substantial particulates
   - No consent

Values for the test assay were determined in duplicate, a cumulative distribution was established. This study was performed at one (1) site, Fujirebio Diagnostics, Inc.

2. All of the samples that were used in this study were obtained from either commercial vendors or specimen banks using only retrospective samples. The sample inclusion and exclusion criteria are as follows:

   The Inclusion criteria for benign samples are as follows:
   - No concurrent malignancy
   - Diagnosis
   - Minimum 0.5 mL volume available
   - Normal appearance
   - Informed consent

   The Exclusion criteria for benign samples are as follows:
   - Concurrent malignancy
   - No diagnosis
   - Insufficient volume
   - Stored or shipped at 4°C
   - Icteric, lipemic, hemolytic, substantial particulates
   - No consent

   The Inclusion criteria for malignant samples are as follows:
   - Specific malignant condition
   - Diagnosis
   - Minimum 0.5 mL volume available
   - Normal appearance
   - Informed consent

   The Exclusion criteria for malignant samples are as follows:
   - Concurrent unrelated malignancy
   - No diagnosis
   - No information
   - Insufficient volume
   - Stored or shipped at 4°C
   - Icteric, lipemic, hemolytic, substantial particulates
   - No consent

3. Population Demographics
   The following patient cohorts were tested in the MESOMARK assay to establish the Reference Range, and to compare MESOMARK concentrations in the target population with concentrations in non-target populations of patients.

Cohort	Number
Apparently Healthy	
Normal healthy females	163

Cohort	Number
Normal healthy males	246
Non Malignant Conditions	
Hypertension/Chronic Heart Disease	100
Asbestos Exposed Individuals	61
Endometriosis	16
Malignant Conditions	
Mesothelioma – Pre Op	88
Ovarian Cancer	111
Lung Cancer	174
Colon Cancer	50
Pancreatic Cancer	52
Endometrial Cancer	25
Total	**1086**

C. Reference Range

1. Reference range of apparently healthy subjects (n = 409)
   The mean age of the cohort for females was 45.0 years and for males the average age was 46.4 years. A t-test for two independent samples (non-equal variances) indicated that these means are not significantly different (t=1.28, DF=390.4, p=0.202).However, when SMRP values were plotted versus age, a trend with increasing age was seen. The SMRP concentration in healthy subjects, the 99th order statistic was 1.5 nM.

2. Reference ranges using all cross-sectional serum samples
   A total of 677 sera from patients with various diseases were evaluated in the MESOMARK assay and results are shown in the table below.

Sample Statistics

Disease or Condition	N	Mean (nM)	Median (nM)	%>ULN	Std. Deviation (nM)	95% CI for Mean (nM)		Minimum (nM)	Maximum (nM)
						Lower Bound	Upper Bound		
Normal	409	0.393	0.315	1	0.366	0.357	0.429	0	2.144
Mesothelioma	88*	7.48	1.6	56	21.93	2.83	12.13	0	170.5
Ovarian Cancer	111	0.8	0.703	7.2	0.569	0.693	0.908	0	3.61
Endometriosis	16	0.474	0.4	0	0.328	0.3	0.649	0	1.05
Colon Cancer	50	0.609	0.512	4	0.41	0.493	0.726	0.063	1.663
Lung Cancer	174	1.944	0.611	17.2	5.817	1.08	2.808	0	287.2
Hypertension	100	0.759	0.584	12	0.591	0.641	0.876	0	2.702
Endometrial Cancer	25	0.666	0.577	0	0.303	0.541	0.791	0.212	1.464
Pancreatic Cancer	52	0.651	0.478	9.6	0.717	0.452	0.851	0	4.24
Asbestos Exposed	61	0.594	0.482	3.3	0.394	0.493	0.695	0	1.708
Total	1086								

Pre-Op Mesothelioma Patient Samples (n=88)*

The values from the pre-operative samples of 88 mesothelioma patients were stratified by stage and histology at diagnosis. The results demonstrate the different stages compared to healthy subjects are significantly different ($p \leq 0.0001$). However, the median MESOMARK values are not significantly different among the different stages ($p>0.1600$) or different histologies ($p>0.1100$).

		Mesomark Value (nMol)		% with Mesomark Values (nMol):			
Patient Type	**N**	**Mean ± StDev**	**Median**	**< 1.5**	**1.5 - 3.0**	**3.1 - 10.0**	**>10.0**
Normals	409	0.4 ± 0.4	0.3	98.8%	1.2%	0.0%	0.0%
Mesotheliomas	88	7.5 ± 21.9	1.6	47.7%	22.7%	15.9%	13.6%
	Mesotheliomas By Histology:						
Epithelioid	59	7.0 ± 15.6	1.9	40.7%	25.4%	18.6%	15.3%
Biphasic	21	2.1 ± 2.7	1.0	66.7%	14.3%	14.3%	4.8%
Sarcomatoid	8	25.1 ± 59.2	1.6	50.0%	25.0%	0.0%	25.0%
	Mesotheliomas By Stage:						
Stage I	14	5.7 ± 17.0	1.5	64.3%	28.6%	0.0%	7.1%
Stage II	22	11.9 ± 35.9	1.8	45.5%	22.7%	18.2%	13.6%
Stage III / IV	52	6.1 ± 14.3	1.9	44.2%	21.2%	19.2%	15.4%

* Note: The Preoperative malignant mesothelioma was a separate study cohort from that for monitoring study (n=31). In addition, not all 88 preoperative malignant mesothelioma cases were diagnosed with epithelioid or biphasic mesothelioma.

Distribution of MESOMARK Assay values (n= 1086)

	Number of Subjects	Percent (%)			
		< 1.5 nM	**1.5 - 3.0 nM**	**3.0 - 10.0 nM**	**>10.0 nM**
Apparently Healthy Females	163	98.8%	1.2%	0.0%	0.0%
Apparently Healthy Males	246	98.8%	1.2%	0.0%	0.0%
Total	409	98.8%	1.2%	0.0%	0.0%
MALIGNANT CONDITIONS					
Mesothelioma - Pre Op*	88	47.7%	22.7%	15.9%	13.6%
Ovarian Cancer	111	92.8%	5.4%	1.8%	0.0%
Lung Cancer	174	82.8%	10.9%	2.3%	4.6%
Colon Cancer	50	94.0%	6.0%	0.0%	0.0%
Pancreatic Cancer	52	90.4%	7.7%	1.9%	0.0%
Endometrial Cancer	25	96.0%	4.0%	0.0%	0.0%
NONMALIGNANT CONDITIONS					
Hypertension/Chronic Heart Disease	100	88.0%	12.0%	0.0%	0.0%
Asbestos Exposed Individuals	61	95.1%	4.9%	0.0%	0.0%
Endometriosis	16	100.0%	0.0%	0.0%	0.0%

* Note: The Preoperative malignant mesothelioma was a separate study cohort from that for monitoring study (n=31). In addition, not all 88 preoperative malignant mesothelioma cases were diagnosed with epithelioid or biphasic mesothelioma.

D. Monitoring of Disease Status in Patients Diagnosed with Mesothelioma

1. Subject and Study Design

Sera were collected retrospectively from a total of 31 patients diagnosed with mesothelioma. All patients were enrolled into an Investigator sponsored clinical research study (Protocol D1420 entitled "Collection of serum and tissue samples from patients with biopsy proven or suspected malignant disease") at Karmanos Cancer Institute at the Harper University Hospital of Wayne State University in Detroit Michigan. The Principle Investigator was Dr. Harvey Pass, Professor of Surgery and Oncology, and Chief, Thoracic Oncology. The protocol was approved by the Institutional Review Board at Wayne State University. A Protocol Amendment was approved by the same IRB for the use of the subset of serum samples tested in this retrospective trial, along with the use of a limited amount of patient information to interpret results from this study. All surviving patients provided consent for participation in this trial.

Eligibility criteria for Protocol D1420 were:
Patients with histologically documented malignant mesothelioma that is confined to the hemithorax. Patients may have been previously treated with thoracotomy with incomplete resection in the past or may have recurrent disease after distant thoracotomy.

- Patients should be considered to have disease, which is amenable to subtotal extirpation of malignant disease ("debulking"). The maximum thickness of remaining surface malignancy at any intrathoracic site must be no more than 0.5 cm. There can be multiple discontinuous sites of disease with these satisfactory dimensions ("diffuse studding" or one large area ("plaque-like").
- Patients must have an expected survival of greater than 3 months.
- Patients must be greater than or equal to 18 years.
- No history of other malignancies except treated basal cell carcinoma or carcinoma in situ of the cervix.
- Pregnant women and nursing mothers are not eligible.
- No absolute requirement for steroids.
- No history of myocardial infarction within 6 months of entry onto the protocol. No current angina or congestive heart failure requiring treatment.
- Patients must have a performance status of equal to ECOG 0, 1 or 2.
- The patient must be aware of the neoplastic nature of his/her illness, the experimental nature of the therapy, alternatives, potential benefits and risks. The patient must be willing to sign the informed consent.

All patients were referred to Dr. Harvey Pass at Wayne State University with a histologic or cytologic diagnosis of mesothelioma. The diagnosis was based on a tissue biopsy by open thoracotomy, thoracoscopy, needle biopsy, or thoracentesis. The majority of tissue samples were immunostained at outside hospitals. Staining included detection of cytokeratins, CEA, calretinin, and B72.3

2. Histologic Confirmation of Mesothelioma
The pulmonary pathologist at Wayne State University reviewed all of the slides, and further stains were performed or the diagnosis confirmed on the outside specimens. Additional antibodies for immunostaining included WT-1 and TTF.

TTF staining, as well as CEA and B72.3, were used to rule out adenocarcinoma, as these stains are negative in mesothelioma. The appropriate positive controls were always done to control for the staining quality. Moreover, every patient in the series was operated upon and large volumes of tissues underwent the classic immunohistochemistry panel in the Harper/Wayne State University laboratories, and in all cases the diagnosis of mesothelioma was confirmed.

3. Evaluation of Safety and Probable Benefit
Thirty of the 31 cases in the longitudinal monitoring study were histologically diagnosed as either epithelioid or biphasic malignant mesothelioma. Safe application of the MESOMARK assay is in conjunction with definitive histologic diagnosis of epithelioid or biphasic malignant mesothelioma as well as with other available clinical and laboratory data that must be incorporated into clinical decision making.

In this small study, about half of the patients experienced a post-surgical decrease of at least 50% in SMRP measured by the MESOMARK assay. Ten of 18 patients with radiologic or clinical evidence of progression had an increase of at least 30% in the MESOMARK assay result during the follow-up period. Ten of 12 patients without radiologic or clinical evidence of progression did not experience a rise of at least 30% in the MESOMARK assay result. These limited monitoring data are not adequate to demonstrate the effectiveness of the MESOMARK device as a monitoring tool.

XI. RISK / PROBABLE BENEFIT ANALYSIS

The MESOMARK Assay is intended to be used for monitoring in patients diagnosed with epithelioid or biphasic (mixed) histological subtypes of mesothelioma. The test may indicate recurrence of mesothelioma in patients following surgery, or indicate response or lack of response to systemic therapy.

The pre-clinical testing demonstrated that the test was reasonably accurate and reproducible except at the lower assay range (below 3-4 nM). The imprecision of the assay was computed as average < 15% across the range of concentrations of mesothelin, the test dilutes linearly, recovers spiked analyte within ± 15% of expected concentrations if the levels were above 3-4 nM, and shows no significant interferences from commonly used medications or cancer drugs. These data indicate that the MESOMARK Assay provides a reasonable assurance that accurate analytical measurement of soluble mesothelin related peptides in human serum when the analyte was above a threshold approximately of 3 to 4 nM. Since the majority (65-70%) of the patients diagnosed with malignant mesothelioma had SMRP

below this threshold, the safety and clinical utility of the MESOMARK Assay remained undetermined.

The limited clinical data are not sufficient to establish the effectiveness of this assay in the indicated population. Analysis of a limited population of 31 patients (30 with either the epithelioid or biphasic type of malignant mesothelioma) shows that the serum concentration of SMRP, as measured by the MESOMARK Assay, decreases following surgery, and increases in a small subgroup of patients at the time of, or prior to recurrence as detected by CT scans.

There were a few publications suggesting that SMRP concentrations may correlate with disease recurrence or progression. Thus, changes in the concentration of serum SMRP may correlate with changes in clinical status of patients with epithelioid and biphasic mesothelioma.

The current standard of care for monitoring patients with mesothelioma for recurrence or response to therapy is imaging modalities including CT scans which might not be able to determine treatment response if some specific view is not taken. Safety and cost considerations would curtail frequent use of imaging for disease monitoring. A blood test that correlates with treatment response would provide a needed tool for monitoring patients with malignant mesothelioma.

The risks associated with the use of the MESOMARK Assay arise from false positive results that would subject the patient to unnecessary work-up procedure and initiation or changing ongoing therapy; false negative results would deprive the patient of needed medical treatment. Patients whose MESOMARK concentrations do not change in parallel with disease status or false negative results could be mistakenly interpreted as well. Additional risks associated with this device include the risk associated with venipuncture.

In balancing the performance demonstrated in the pre-clinical studies and clinical studies, and the potential clinical risks and benefits associated with the device, the MESOMARK Assay demonstrates adequate safety and probable benefit to justify HDE approval. Analytical performance appears strongest when the test is used to measure SMRP in the range above 3-4 nM. The clinical study design and available data did not allow judgment on whether the device would be effective for monitoring disease progression or recurrence. Using the device is likely to provide a relative non-invasive and non-radioactive in vitro test for monitoring disease status, but any benefit to health remains undetermined.

XII. PANEL RECOMMENDATION

There is no panel meeting for this HDE.

XIII. CDRH DECISION

The applicant's manufacturing facility was inspected on November 06 to 14, 2006 and was found to be in compliance with the Good Manufacturing Practice (GMP) regulation.

CDRH has determined that, based on the data submitted in the HDE, that use of the FDI MESOMARK Assay will not expose patients to an unreasonable or significant risk of illness

or injury, and the probable benefit to health from using the device outweighs the risks of illness or injury, and issued an approval order on 24 January 2007.

XIV. APPROVAL SPECIFICATIONS

Directions for use: See the Physician's Labeling.
Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions and Adverse Events in the labeling.
Postapproval Requirements and Restrictions: See Approval Order.

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