You are receiving this information because you have been diagnosed with malignant mesothelioma, a rare form of cancer, associated with exposure to asbestos. In this disease, cancer (malignant) cells are found in:

- **the sac lining the chest (the pleura),**
- **the lining of the abdominal cavity (the peritoneum), or**
- **the lining around the heart (the pericardium).**

Certain proteins, called biomarkers, may be released into the blood by these cancer cells. One or more samples of your blood can be tested for a specific biomarker called Soluble Mesothelin-Related Peptide or SMRP. Measuring the amount of SMRP in your blood, along with all other available clinical information, may aid in the monitoring of patients diagnosed with epithelioid or biphasic mesothelioma. You must voluntarily decide whether or not you want to have this blood test.

MESOMARK®

Patient Information

The information in this brochure may contain unfamiliar words or phrases. Please ask the doctor or a member of their staff to explain any words or phrases that you do not clearly understand.

FUJIREBIO™ Diagnostics, Inc.

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MESOMARK®

The MESOMARK assay is a "humanitarian use" device test developed by Fujirebio Diagnostics, Inc. This test device is approved by the U.S. Food and Drug Administration (FDA) as a "humanitarian use" device, which means the effectiveness of this device for this test has not been demonstrated.

What is involved in the testing?

If you choose to take part in this testing, you will be asked to provide one or more samples of blood (approximately 2 teaspoons each time). No additional visits to your physician are required. The blood will be obtained from a vein using a needle. Your blood sample will be sent to a laboratory for testing. The blood test results will be used along with all other available clinical and laboratory data to make decisions about your care.

Risks of testing

False Negative Test Result. Because the SMRP protein is not produced by every mesothelioma tumor, a false negative test result is possible. A false negative test means that a test which should have given a positive result gives a negative one in error. The risk of a false negative MESOMARK test is minimized since your doctor will NOT be using the MESOMARK result alone, but in addition to all your other clinical and laboratory information.

Benefits of testing

The effectiveness of this device has not been demonstrated, however, the measurement of SMRP may allow your doctor to have additional information on your treatment response.

What are the costs?

The cost of the blood test may not be reimbursable by your health insurance; therefore, you may be required to pay part or all of the costs associated with this testing. You may want to check with your insurance company to see if this test is covered.

Your alternatives

You may choose not to have this testing and may withdraw from participating in this testing at any time. Your decision to have or withdraw from this testing will not influence the availability of future medical care and will involve no penalty or loss of benefits to which you are otherwise entitled.

Who to call with questions

If you have any questions or problems during this testing, or if you think that you may have experienced an injury related to this testing, you should contact the doctor who ordered this blood test for you.

If you have any questions as a Humanitarian Use Device participant, please contact Kim Lerner, Chairman of the Investigational Review Board (IRB) ***toll free (877) 888-IIRB (4472)*** *during regular business hours (Eastern Standard Time). The IRB is a group of people who provide oversight review of this type of medical testing as required by Federal regulations.*

MESOMARK®

FUJIREBIO Diagnostics, Inc.

NAME: MESOMARK®

INTENDED 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 patients diagnosed with epithelioid or biphasic mesothelioma. MESOMARK values must be interpreted in conjunction with all other available clinical and 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.

SUMMARY AND EXPLANATION OF THE TEST

Malignant mesothelioma (MM) is a highly aggressive neoplasm with poor prognosis. It arises primarily from the surface serosal cells (mesothelial cells) of the pleura and, less commonly, of the pericardium or peritoneum. The estimated annual incidence in white males is approximately 7 to 13 cases per million in the United States (U.S.).[1,2,3,4] Because of occupational asbestos exposure and the long latency period of 30 to 40 years, the current annual incidence of approximately 3000 new cases (U.S. only) is not expected to decrease significantly in the short term.[5] Epidemiologic studies have established exposure to asbestos fibers as the primary cause of MM with as many as 80% of the pleural mesothelioma patients having been exposed to asbestos.[3,6] Up to 8 million people in the U.S. have been occupationally exposed to asbestos over the last five decades during mining and milling of asbestos and in diverse manufacturing processes that use the material. Today, many public and private buildings still contain asbestos, including 10% to 15% of schools in the U.S.

Although malignant mesothelioma remains a relatively uncommon malignancy, it continues to represent an important cause of mortality in numerous areas worldwide, e.g. England, Wales, continental Europe, and Australia. Recent estimates suggest that in coming decades, as much as one percent of deaths among men in the United Kingdom currently aged 49 to 54 may be due to mesothelioma.[7] Age standardized incidence rates for mesothelioma in men range from around 8 per 100,000 in Scotland, England, and The Netherlands to 2 to 4 per 100,000 in the US, France, Italy, and Germany; and 1 per 100,000 in Spain.[5,8] The corresponding rate for Australia is estimated to be around 6 per 100,000.[9]

The clinical characteristics of mesothelioma include a male: female ratio of greater than three to one, a median age of 60 years (incidence rises steadily with age and is approximately tenfold higher in men aged 60 to 64 years as compared with those aged 30 to 34)[10] and an overall survival rate of approximately 6 to 10 months depending on the stage and presentation of disease.[11,12] On biopsy, these tumors are usually epithelial or papillary tumors. A small proportion, perhaps 7% to 10%, are sarcomatoid, which have a poorer differentiation phenotype and are associated with shorter survival. About 30% of mesotheliomas are biphasic or mixed at presentation.

The MESOMARK assay measures soluble molecules that are related to the mesothelin/Megakaryocyte Potentiating Factor (MPF) family of proteins and recognized by the monoclonal antibody OV569.[13] The reactivity of OV569 is low for normal human tissues except for the mesothelium. Soluble members of the mesothelin/MPF family of proteins have been reported in the sera of patients with tumors of mesothelial origin.[13,14]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

Principle of the Assay

The MESOMARK assay 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.

REAGENTS

96 Tests
Materials Supplied

1. WASH BUFFER — 1 Bottle (50 mL). Wash Buffer (20X concentrated). 0.002M Imidazole buffered saline, containing 0.02% Tween 20. **Must be diluted before use.**
2. SUBSTRATE — 1 Bottle (12 mL). Substrate. < 0.1% Tetramethylbenzidine (TMB) in an acidic buffer. **Ready to use.**
3. STOP SOLUTION — 1 Bottle (12 mL). Stop Solution. 1% Hydrochloric Acid. **Ready to use.**
4. COATED PLATE — 1 Plate (96 wells). Plastic microtiter wells coated with 4H3 murine monoclonal antibody in foil pouch with desiccant. **Ready to use.**
5. 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.**
6. CALIBRATOR/DILUENT — 1 Bottle (100 mL). Calibrator A/Assay Diluent. PBS buffer with protein (bovine) stabilizer. Contains Proclin 300/Gentamicin as preservatives. **Ready to use.**
7. CALIBRATORS — 1 Bottle each (1.0 mL each). Calibrators B to F contain 569 reactive antigen (recombinant) prepared in PBS buffer with protein (bovine) stabilizer. Contains Proclin 300/Gentamicin as preservatives. **Ready to use.**

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

Standardization

The MESOMARK assay values are expressed as nM (nanomolar) and are related to a Fujirebio Diagnostics, Inc maintained reference preparation. The calibrators for the MESOMARK product are manufactured gravimetrically and are referenced to this standard prepared by Fujirebio Diagnostics, Inc. There is no internationally recognized SMRP standard available at this time.

8. CONTROLS — 1 Bottle each (1.0 mL each). Controls contain 569 reactive antigen (recombinant) prepared in PBS buffer with protein (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

Materials Required But Not Provided

1. Disposable tip micropipettes to deliver volumes of 5 µL, 10 µL, 25 µL, 100 µL, and 1 mL (multichannel pipette preferred for dispensing reagents into microtiter plates).
2. Adhesive plate covers.
3. Deionized water.
4. Plate Shaker (700 rpm ± 25 rpm).
5. Clean, disposable plastic/glass test tubes, approximate capacities 5 mL and 10 mL.
6. Range of standard, clean labware consisting of, at least, 15 mL and 100 mL beakers, 1 mL, 5 mL, and 10 mL pipettes.
7. Absorbent paper towels.
8. Automatic microtiter plate washer or laboratory wash bottle.
9. Spectrophotometric microtiter plate reader with 450 nm filter.
10. Disposable gloves, safety glasses, and other appropriate protective garments.
11. Biohazard infectious waste containers.
12. Safety pipetting devices for 1 mL or larger pipettes.
13. Timer.

WARNINGS AND PRECAUTIONS

1. IVD

   For *in vitro* Diagnostic Use.
2. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
3. Do not use the MESOMARK kit after the expiration date printed on the outer carton label.
4. Do not cross contaminate reagents. Always use fresh pipette tips when drawing from stock reagent bottles.
5. Always use clean, preferably disposable, glassware for all reagent preparation.
6. Allow foil pouches to warm to room temperature before opening. This avoids condensation on the inner surface of the pouch, which may contribute to a deterioration of Coated Plate strips intended for future use.
7. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section.
8. Always keep the upper surface of the Coated Plate strips free from excess fluid droplets.
9. Reagents and buffer over-spill should be blotted dry on completion of the manipulation.
10. Do not allow the wells to completely dry during an assay.
11. Disposal or decontamination of fluid in the waste reservoir from either the plate washer or trap for vacuum line in the manual system should be in accordance with guidelines set forth in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens.[15]
12. Automatic or semi-automatic EIA processors or liquid handling systems should be qualified specifically for use with the MESOMARK kit by demonstration of equivalence to the manual processing methods.
13. Consistent with good laboratory practice, it is recommended that all pipetting devices (manual or automatic), timers, and thermometers be regularly calibrated according to the manufacturer's instructions or internal Quality Control procedures.
14. Care must be taken to ensure that specimens are dispensed correctly to each test well. If a specimen is inadvertently not added to a well, the result for that well will be non-reactive, regardless of the actual result of the specimen.

Safety Precautions

1. This product may require the handling of human specimens. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[15] Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[16-18]
2. The Conjugate, Calibrators, Controls, and Calibrator A/Assay Diluent contain a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (3:1), which is a component of Proclin 300, and are classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

   

   R43: May cause sensitization by skin contact.
   S24: Avoid contact with skin.
   S35: This material and its container must be disposed of in a safe way.
   S37: Wear suitable gloves.
   S46: If swallowed, seek medical advice immediately and show this container or label.
3. The Stop Solution contains Diethylene Glycol and is classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

   

   R36: Irritating to eyes.
   R38: Irritating to skin.
   S24: Avoid contact with skin.
   S25: Avoid contact with eyes.
   S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
   S36/39: Wear suitable protective clothing and eye/face protection.

Handling Precautions

1. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Bio-safety Level 2.[15-18]
2. The MESOMARK kit contains reagents that are optimized and balanced for each kit lot. Do not interchange reagents from kits with different lot numbers. Do not interchange vial caps or stoppers either within or between kits.
3. Do not use the MESOMARK kit beyond the expiration date.
4. Do not mix reagents from different MESOMARK kits.

Storage Instructions

1. The MESOMARK kit should be stored at 2-8°C and should not be used beyond the expiration date.
2. Once opened, the MESOMARK kit components, including working strength Wash Buffer, may be stored at 2-8°C for 28 days or until the expiration date on the label,

whichever is earlier, provided that desiccated conditions are maintained. Unused Coated Plate strips should be resealed in their original foil pouch along with the sachet of desiccant.

Indications of Reagent Deterioration

The MESOMARK kit may be considered to have deteriorated if:

1. A control value is out of the specified range; it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting.
2. The MESOMARK kit fails to meet the required criteria for a valid test (see **Quality Control Procedures**).
3. Reagents become visibly cloudy or develop precipitate. **Note:** Wash Buffer, when cold, normally develops crystalline precipitates that re-dissolve on heating at 37°C.
4. The Substrate turns dark blue. This is likely to be caused by contamination of the Substrate.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

1. The MESOMARK kit is intended for use with serum. Specimens should be collected aseptically by venipuncture.
2. If serum specimens are to be stored, they may be stored at 2-8°C for up to 24 hours. However, the serum specimens should be frozen at –20°C or below if storage periods greater than 24 hours are anticipated. Specimens that have been frozen and thawed should be thoroughly mixed before testing.
3. If serum specimens are to be shipped, they should be packaged and labeled in compliance with federal and international regulations pertaining to the transportation of clinical specimens and etiologic agents. Serum specimens may be shipped refrigerated (2-8°C) on wet ice for up to 24 hours or frozen (–10°C or colder) on dry ice.

PROCEDURE

Rinse Cycle

Efficient rinsing to remove unbound serum sample components is a fundamental requirement of enzyme immunoassay procedures. The MESOMARK assay utilizes two standard five-rinse cycles, one at the end of each of the first two incubation steps. Automatic plate washers may be used provided they meet the following criteria:

1. All wells are completely aspirated.
2. All wells are filled to the rim (350 µL) during the rinse cycle.
3. Wash Buffer is dispensed at a good flow rate.
4. The microtiter plate washer is well maintained to prevent contamination from previous use. Manufacturer's cleaning procedures are followed diligently.

For each rinse cycle the machine should be set to five consecutive washes. On completion of the cycle, invert the microtiter plate and tap firmly on absorbent paper towels. Check for any residual Wash Buffer in the wells and blot the upper surface of the wells dry with a paper towel.

Alternatively, the following manual system may be employed:

1. Aspirate well contents using a vacuum line fitted with a trap.
2. Fill all wells to the brim with Wash Buffer dispensed from a squeeze-type laboratory wash bottle.
3. Aspirate all wells.
4. Repeat steps 2 and 3, four times.
5. Invert the plate and tap firmly on absorbent paper towels.

Preparation for the Assay

CONTROLS

Note: Controls are provided at working strength and do not require further dilution. Controls should be included on each Coated Plate. The high control and low control should always be tested in duplicate and the mean of the duplicates must have a CV ≤ 15%.

CALIBRATORS

Note: Calibrators are provided at working strength and do not require further dilution. Calibrators should be included on each Coated Plate. All Calibrators should always be tested in duplicate. The values for the Calibrators are printed on the vials.

WASH BUFFER

Prepare working strength Wash Buffer (conc. 1X) by diluting 1 part concentrate with 19 parts of deionized water.

Serum Specimens

Note: Serum specimens do require dilution. The serum specimens should always be tested in duplicate.

Assay Procedure

1. Allow all reagents to reach room temperature (20-25°C).
2. Prepare 1:101 dilutions of the patient serum samples using Calibrator A/Assay Diluent; e.g., add 10 µL patient serum to 1.0 mL Calibrator A/Assay Diluent. **Note: Calibrators and Controls do not require dilution.** Mix the dilutions carefully with a vortex mixer immediately before use.
3. Select sufficient Coated Plate strips to accommodate all diluted samples, Calibrators, and Controls. Fit the Coated Plate strips into the holding frame. Generate plate map according to specimen: identify using the letter/number cross-reference system molded into the plastic frame.
4. Dispense 100 µL of each Calibrator, Control, and diluted samples into appropriate wells. **Note: All Calibrators, Controls, and diluted samples should be tested in duplicate.**
5. Apply adhesive plate cover.
6. Incubate covered plate on a plate shaker (700 rpm ± 25 rpm) at room temperature (20-25°C) for 60 (±5) minutes.
7. Aspirate the contents of the wells and wash the plate with the prepared working strength Wash Buffer as described in the rinse cycle section.
8. Pipette 100 µL of Conjugate into each well, apply adhesive plate cover, and place the plate on a plate shaker (700 rpm ± 25 rpm) at room temperature (20-25°C) for 60 (±5) minutes. **Protect from light.**
9. Aspirate the Conjugate from the wells and wash the plate as described in the rinse cycle section.
10. Dispense 100 µL of Substrate into each well. A multichannel pipette should be used for best results. Apply adhesive plate cover. Incubate the plate on a plate shaker (700 rpm ± 25 rpm) at room temperature (20-25°C) for 15 (±2) minutes. **Protect from light.**
11. Stop the reaction by adding 100 µL of Stop Solution (1% Hydrochloric acid) to each well. The blue solution should change to a uniform yellow color. Gently tap the wells to mix. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.

12. Within 30 minutes of adding the Stop Solution, read the absorbance values at 450 nm using a spectrophotometric microtiter plate reader blanked on air unless the manufacturer specifically recommends otherwise.

13. **Immediately return all unused reagents for storage at 2-8ºC after use.**

RESULTS

Interpretation of Results

Method 1

The calibration curve can be constructed manually, or in a suitable graphing program by plotting the mean absorbance for each Calibrator on the y-axis *versus* the concentration of the Calibrator (value printed on vial) on the x-axis. Plot the curve by a 'best-fit' regression method. Determine the concentration of the Controls and specimen samples by using a four-parameter logistic equation for best results.

Method 2

For plate readers, which utilize proprietary data reduction software, the data reduction software may be used to create the calibration curve. Software providing a four-parameter logistic curve-fitting routine provides best results.

QUALITY CONTROL PROCEDURES

The MESOMARK assay results should be considered valid if:

1. The Calibrator a.u. for duplicates are ≤ 15% CV for the mean absorbance readings ≥ 0.2 a.u. For Calibrators with a mean a.u. < 0.2, the individual replicates are within ± 0.03 a.u. of the mean a.u.

2. Additionally, the ratio of the mean absorbance of Calibrator B to the mean absorbance of Calibrator A (B/A ratio) is > 2.0.

3. The mean concentration of the Controls is within the specific control range listed in the Materials Supplied section of this package insert. Additionally, the Control concentrations for duplicates have a CV ≤ 15%.

4. The mean concentration of a sample has a CV ≤ 15% for samples with a mean absorbance reading ≥ 0.2 a.u. For samples with a mean absorbance < 0.2 a.u., the individual replicates must be within ± 0.03 a.u. of the mean.

Note:

1. An assay that results in an invalid calibrator curve or control value is invalid, and the complete set of samples should be retested.

2. Invalid sample(s) should be retested for an accurate measurement of SMRP levels. Calibrators and Controls should be run again each time samples are retested.

Procedure for Samples with SMRP Levels Greater Than the Highest Calibrator in the Assay

To obtain accurate results for patient samples with SMRP levels greater than Calibrator F it is necessary to dilute and re-test the sample. Diluting the serum specimen 10-fold is recommended. For example: Make a 10-fold dilution by adding 0.05 mL of the serum specimen originally diluted 1:101 to 0.45 mL of the MESOMARK Calibrator A/Assay Diluent. Mix thoroughly and repeat the assay according to the assay procedure. Multiply the results by 10 to determine the correct SMRP levels in the original serum specimens.

LIMITATIONS OF THE PROCEDURE

1. MESOMARK results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, and imaging studies.

2. Heterophilic antibodies in human serum may react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[19] Patients routinely exposed to animals or to animal serum products may be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

3. Individuals receiving mouse immunoglobin by parenteral routes may produce anti-mouse antibodies. Serum from such individuals may produce erroneous results.[20]

4. Representative performance data are given in the Expected Values and Specific Performance Characteristics Sections. Results obtained in individual laboratories may vary.

5. The MESOMARK assay results < 1.0 nM should be interpreted with caution as variability in low concentrations over time may result in significant changes on a percent basis.

Expected Values

The distribution of SMRP levels determined in 1086 specimens is shown in the table below:

Distribution of SMRP Levels

		Percent (%)			
	Number of Subjects	**≤ 1.5 nM**	**> 1.5-3.0 nM**	**> 3.0-10.0 nM**	**> 10.0 nM**
APPARENTLY HEALTHY					
Females	163	99	1	0	0
Males	246	99	1	0	0
MALIGNANT CONDITIONS					
Mesothelioma	88	48	23	16	14
Ovarian Cancer	111	93	5	2	0
Lung Cancer	174	83	11	2	4
Colon Cancer	50	94	6	0	0
Pancreatic Cancer	52	90	8	2	0
Endometrial Cancer	25	96	4	0	0
NONMALIGNANT CONDITIONS					
Hypertension/Chronic Heart Disease	100	88	12	0	0
Asbestos Exposed Individuals	61	95	5	0	0
Endometriosis	16	100	0	0	0

In this study, 99% of the healthy subjects had SMRP levels at or below 1.5 nM. It is recommended that each laboratory establish its own reference value for the population of interest.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The MESOMARK assay precision is ≤ 15% total CV. A study was performed as described per the National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP5-A.[21] 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 SD	Within Run %CV	Total SD	Total %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
		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; results in individual laboratories may vary from these data.

Recovery

The MESOMARK assay mean recovery is 100% ± 15%. A study was performed to determine recovery for 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. 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
		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

Average recovery at each level of added analyte = 107% (Range = 103%-113%)

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

** Percent Recovery Calculation:
% Recovery = 100 x (measured concentration)/(endogenous concentration + added concentration)

Dilution Linearity

The MESOMARK assay mean dilution linearity is 100% + 15%. A study was performed for the MESOMARK assay modeled after the National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP6-P2.[22] Known concentrations of 569-reactive antigen were added to five (5) independent normal human serum samples followed by dilution with assay diluent. Representative data from this study are presented below.*

Sample	Dilution Factor	Expected Value (nM)	Observed Value (nM)	Percent Recovery**
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
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
	1:10	2.65	2.78	105
3	Undiluted	25.91	25.91	N/A
	1:1.1	23.31	21.58	93
	1:1.4	18.13	17.93	99
	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

Average recovery across the five diluted samples = 109% (Range = 99%-113%)

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

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

Analytical Sensitivity

The sensitivity of the MESOMARK assay is 0.3 nM. 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.

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	20 mg/dL
Hemoglobin	500 mg/mL
Total Protein	12 g/dL
Triglycerides	3 g/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.

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.

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Printed in U.S.A.
250-386 USA 1/07