

40-008-355001- GenWay Biotech/AMDL- ELISA DR-70® (FDP) Test

Intended Use

The DR-70® (FDP) ELISA is designed for RESEARCH USE ONLY for the quantitative measurement of DR-70® (FDP) in human serum. Independent laboratories must establish and validate their own cut-off values within their local sample population for the use of the GenWay Biotech DR-70® ELISA (FDP) Test as an aid in the detection of cancer in conjunction with other assays. A US-based sample study has been provided as a guide to DR-70® (FDP) Levels. Additionally, serial testing using the GenWay Biotech DR-70® ELISA (FDP) can be used as an aid in monitoring the disease progression in samples who have been previously diagnosed with cancer. Results of DR-70® (FDP) testing should be used in conjunction with other clinical modalities that are standard of care for monitoring disease progression in these samples.

Summary and Explanation of Test

The GenWay Biotech DR-70® ELISA (FDP) Test measures both Fibrin and Fibrinogen Degradation Products (FDP) in human serum samples. While the production of FDP is restricted in healthy individuals, FDP are over produced by proteolytic enzymes, such as plasmin and thrombin, released by cancer cells¹ and as a by-product of other cancer-related processes^{2,4}. Current assays for FDP are only used for measuring direct coagulation-related phenomena. In addition, other FDP based-assays are functionally restricted by measuring an individual, specific FDP component, such as D-dimer, as a representative of this group. In contrast, the DR-70® (FDP) immunoassay detects the full complement of FDP components and does not miss any of the cancer signal generated by the two independent, but related, pathways responsible for FDP production in cancer progression. In this way, the DR-70® (FDP) immunoassay may be considered a "barometer" for cancer by detecting the large increases in coagulation breakdown products (FDP) that are known to be caused by cancer.

The DR-70® (FDP) Immunoassay measures FDP generated from both of the major cancer induced FDP production pathways. The FDP measured by the DR-70® (FDP) Immunoassay in serum from colorectal cancer samples was primarily produced by plasmin cleavage of either fibrinogen or fibrin. Availability of the original substrate (fibrinogen or fibrin) is dependent on the levels of the proteolytic enzyme Thrombin, the Fibrin/ogen Degradation Products (FDP) detected by the DR-70® (FDP) immunoassay will include fragments D and E as well as D-dimer. Additional intermediate products from either cleavage reaction may be detected by the assay. Measuring multiple FDP species prevents the DR-70® (FDP) Immunoassay from underestimating the cancer-related levels of FDP.

Because FDP leaks from tumors into surrounding fluids⁵, elevated FDP levels can be measured in the urine of Subjects with bladder cancer, in the plasma of lung cancer Subjects^{6,9}, and in the serum of other cancer samples. The utility of FDP measurements in cancer has been suspected for years; however refined assays had not been developed that were able to quantitatively measure FDP with the sensitivity required. The US FDA has cleared a bladder cancer screening test that measures FDP qualitatively in urine (K970353, Organon Teknika, Ltd.)⁷.

Researchers have established a strong link between increased FDP levels and cancer^{5, 10}, which is based on multiple factors including: a cancer-caused redirection of the coagulation cascade and a cancer-related increase in proteolysis within tumors as they grow and metastasize. Clinical studies reveal that measuring FDP levels, either with the DR-70® (FDP) test or with other related tests, has significant value for a variety of cancers¹¹⁻¹⁹. Other studies demonstrate that FDP levels correlate with the cancer stage²⁰⁻²⁶ and with the cancer progression^{24, 26, 27}, as quantified by the number of lymph node metastases. Clinical research efforts have shown that pretreatment measurements of FDP levels have prognostic significance for post-treatment survival^{12, 25, 28-32}. In addition to survival prognoses, pretreatment FDP values may be used to indicate when adjuvant systemic treatments are required for surgical Subjects^{30, 32}.

In published clinical studies^{12, 13, 16, 33-37}, the the GenWay Biotech DR-70® ELISA (FDP) immunoassay was used to detect FDP levels in 7,469 samples. Among these clinical studies, DR-70® (FDP) assay results consistently correlate with either the positive detection or positive progression of a variety of cancers. The majority of the clinical data from the DR-70® (FDP) immunoassay are focused on lung, breast, ovarian and colorectal cancers. A complete list of references follows.

Principle of the Assay

The GenWay Biotech DR-70® ELISA (FDP) assay is a standard sandwich assay, utilizing removable strips in a 96-well micro titer plate format. The wells are coated with affinity purified rabbit anti-DR-70® (FDP) polyclonal antibodies. The DR-70® (FDP) in diluted sera (1:200) is captured from the sera by the antibodies immobilized on to the well of the micro titer plate. After a wash step, anti-human fibrinogen antibodies conjugated to horseradish peroxidase are added to the wells. If the DR-70® (FDP) antigen is present, the anti-human fibrinogen peroxidase complex will bind to the captured tumor marker to form an immunological sandwich with the immobilized antibodies. After a second wash step, the enzyme substrate 3,3',5,5'-tetramethylbenzidine (TMB) is added to the well. The end point is read in a micro plate reader at 450 nm once the reaction is stopped with 0.1N HCl. The intensity of the color formed is proportional to the amount of DR-70® (FDP) in the serum. The amount is quantified by interpolation from a standard curve using the calibrators provided with the kit.

Materials Provided (DR-70® (FDP) Kit Components, Cat. No. DR2101)

| | |
|-----------|--|
| DR2201 | DR-70® (FDP) Antibody-Coated Wells, (sufficient for a 96 well Plate) |
| DR2301 | Enzyme Antibody Conjugate: 1 vial (12 ml) |
| DR2401A | Low Control: 1 vial (500 µl) |
| DR2401B | High Control: 1 vial (500 µl) |
| DR2501A-E | DR-70® (FDP) calibrators: 5 vials (500 µl each) at concentrations of: 0, 0.625, 2.5, 5.0, and 10.0 µg/ml |
| DR2901A | Diluent Buffer Concentrate (5X): 1 vial (40 ml) |
| DR2091B | Wash Buffer Concentrate (20X): 1 vial (50 ml) |
| DR2601 | TMB Substrate: 1 vial (12 ml) |
| DR2701 | Stop Solution: 1 vial (12 ml) |
| N/A | Dilution/Transfer Plate (96 well uncoated Plate) |

Materials Required But Not Provided

The following materials are not provided but are required to perform DR-70® (FDP) analysis manually using the GenWay Biotech DR-70® ELISA (FDP):

- 8-channel micropipettor that delivers 100 µl
- Micropipettor that delivers 10 µl
- Adjustable micropipettor that delivers 100 - 1000 µl
- Vortex mixer
- Plate washer
- Plate reader that reads the 450 nm (kinetic) wavelengths
- Computer with software to operate the reader and printer and for data reduction
- Solution basins

Warnings and Precautions

1. The GenWay Biotech DR-70® ELISA (FDP) assay contains human blood components. The starting materials were tested and were found to be negative for hepatitis B surface antigen (HBsAg) and for HIV-1 p24 (core) antigen. No known test method can offer complete assurance that products derived from human blood will not transmit infection. Therefore, all human blood derivatives should be handled as though they contain an infectious agent. Handle these reagents and human specimens using established good laboratory working practices.
2. **Do not** pipette by mouth.
3. Wear protective clothing, disposable gloves and goggles throughout the testing procedure.
4. All spills should be wiped up promptly and any surfaces contaminated with serum must also be sterilized with appropriate disinfectant such as a 10% v/v solution of sodium hypochlorite.
5. The reagents contain 50 µg/ml gentamicin and 2.5 µg/ml amphotericin B as preservatives. Therefore, caution should be taken in handling the reagents and in disposal of the spent reagents.
6. Kits should not be used beyond the expiration date.
7. Residual reagents should be discarded. They must not be pooled and reused.
8. Avoid exposing the TMB substrate to strong light during storage or prior to dispensing.
9. GenWay Biotech DR-70® (FDP) ELISA is intended for RESEARCH USE ONLY.
10. The GenWay Biotech DR-70® ELISA (FDP) assay has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. The "hook effect" phenomenon may occur at DR-70® (FDP) results exceeding 200 µg/ml.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified 2° - 8° C.

Specimen Collection and Handling

Serum is required for the assay. No other specimen types should be used. The sample volume required for analysis is 10 µl.

No special sample preparation is necessary. Results are not affected by fasting status or time of sample collection. A venous blood sample is collected aseptically directly into an SST tube. Store upright at room temperature until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assay.

Once the serum is separated from the clot, samples may be stored at 2° - 8° C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at -20° C, or below, for up to one year.

Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, slowly bring frozen samples to room temperature (18° - 25°C) and mix gently.

Procedure

I. Reagent Preparation

A. DILUENT BUFFER

Bring all reagents to room temperature (18-25°C) before preparing the working reagent. Add the entire contents of the DILUENT BUFFER CONCENTRATE (5X) (40 ml) to an additional 160 ml of deionized or distilled water. Mix thoroughly and label appropriately.

B. WASH BUFFER

Add the entire contents of the WASH BUFFER CONCENTRATE (20X) (50 ml) to an additional 950 ml of deionized or distilled water. Mix well and label appropriately.

C. CALIBRATORS AND CONTROLS

Kit calibrators and controls are treated in an identical fashion to the samples and must be run with each and every run. They must be diluted 1:200 with the DILUENT BUFFER prior to testing. The procedure to be followed is: Label one 12x75 mm glass test tube for each of the two controls and each of the five calibrators. Dispense 2.0 ml of DILUENT BUFFER into each one of the tubes. Vortex the controls and the calibrators to mix well, then dispense 10 µl of each into the correspondingly labeled tubes. Mix these tubes again.

II. Serum Sample Dilutions

Note: Only serum samples can be used in this assay, not whole blood or plasma.

Dilute each sample serum specimen 1:200 as follows:

- Label 12 x 75 mm glass tubes for each serum sample.
- Dispense 2.0 ml of DILUENT BUFFER into each of the tubes.
- Vortex the serum samples and then dispense 10 µl of each into the correspondingly labeled tubes.

III. Low and High Controls

Two levels of control, normal and abnormally elevated, are included in the GENWAY BIOTECH DR-70® ELISA(FDP).

Assay control specimens should be run as if they were sample samples.

Quality control material to be run with this assay is defined by individual laboratory policy and may include reference controls in addition to the quality control materials provided in the DR-70 assay kit. Those should be run with each assay even if other QC materials are used.

IV. Procedural Notes

- All specimens should be tested in duplicate.
- All kit components and serum to be tested must be allowed to come to room temperature.
- Do not use expired reagents.
- Break off the number of strips needed for the test and place in a micro-titer plate holder.
- Unused micro-titer plate strips should be kept in a sealed mylar bag with desiccants and stored at 2° - 8° C.
- Water should be tested at least once per month and should be free of particulate matter including bacteria. The pH of the water should also be routinely tested. For further information, consult the NCCLS document "Preparation and Testing of Reagent Water in the Clinical Laboratory," NCCLS Document C3-A2, Volume 11 No. 13, originally approved as a guideline by NCCLS in August 1991.

V. Assay Preparation

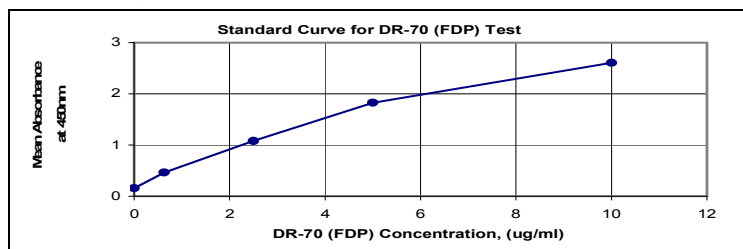
- Vortex each of the tubes of diluted controls, calibrators and Sample Serum and dispense 200 µL from each tube into two wells on the DILUTION PLATE, so that each sample will then be tested in duplicate (two adjacent wells). For example, dispense 200 µL of diluted calibrator A (diluted 1:200) into well A1 and also into well B1 of the dilution plate; 200 µL of diluted calibrator B into wells C1 and D1; 200 µL of diluted calibrator C into wells E1 and F1; 200 µL of diluted calibrator D into wells G1 and H1; 200 µL of diluted calibrator E into wells A2 and B2.
- Continue in this manner with the two diluted controls and diluted serum samples.
- Using an 8-channel pipettor, transfer from the dilution plate 100 µl diluted serum per well into the DR-70® (FDP) Antibody coated plate.
- Incubate at room temperature (22° - 28° C) for 30 minutes.
- Wash all the wells 6 times each with 300 µl per well of wash buffer. Invert and blot plate onto clean absorbent paper.
- Dispense 100 µl of the antibody - enzyme conjugate per well using an 8-channel pipettor. Incubate 30 minutes at room temperature (22° - 28° C).
- Wash as in step 3 above.
- Dispense 100 µl of the TMB substrate per well using an 8-channel pipettor.
- Cover the plate with foil and incubate in a dark area at room temperature (22° - 28° C) for 15 minutes. Stop the reaction by dispensing 100 µl of stop solution per well, using an 8-channel pipettor. Read immediately at 450 nm.

| Calibrator | Value (µg/ml) | Well | O.D. | Mean | S.D. | %CV |
|------------|---------------|------|-------|-------|-------|-----|
| A | 0.000 | A1 | 0.166 | 0.158 | 0.011 | 7.2 |
| | | B1 | 0.150 | | | |
| B | 0.625 | C1 | 0.472 | 0.465 | 0.010 | 2.1 |
| | | D1 | 0.458 | | | |
| C | 2.500 | E1 | 1.080 | 1.079 | 0.002 | 0.2 |
| | | F1 | 1.077 | | | |
| D | 5.000 | G1 | 1.823 | 1.830 | 0.010 | 0.5 |
| | | H1 | 1.837 | | | |
| E | 10.000 | A2 | 2.631 | 2.607 | 0.033 | 1.3 |
| | | B2 | 2.584 | | | |

VI. Calculation of Results

- Plot the standard curve on standard graph paper with the optical density units on the Y axis versus the µg/ml calibrator values on the X axis. Interpolate the average value for each test specimen from the standard curve. See example below.
- If data reduction software is used, use a quadratic or Four (4) parameter algorithm to define the standard curve or any of the software programs available to interpolate the sample values from the standard curve.
- If a serum value is found to be greater than the 10 µg/ml calibrator, the sample should be diluted with diluent buffer and then retested. The resultant value must then be corrected for the dilution factor.

Example of a Typical DR-70® (FDP) Standard Curve:



Evaluation of Results

I. Quality Control

Low and High Control materials in the DR-70® (FDP) kit should fall within the following concentration ranges: Low: 0.14 – 0.51 µg/ml, High: 1.4 – 5.2 µg/ml.

If one or more control sample value(s) is out of the acceptable range, it will be necessary to investigate the validity of the calibration curve before reporting sample results. Standard laboratory procedures should be followed in accordance with the regulatory agency under which the laboratory operates.

In order to monitor and evaluate the precision of the analytical performance, it is recommended that control samples with known values be assayed daily.

II. Interpretation of DR-70® (FDP) Sample Values

Part A. Cancer Screen

Prior to validating a cut-off DR-70® (FDP) values, each laboratory must establish its own normal and abnormal ranges, which are based on local population studies.

Part B. Cancer Monitoring

For each sample, a baseline reading should precede the evaluation of their DR-70® (FDP) levels. The DR-70® (FDP) value of the initial serum draw serves as the baseline reading. The value of successive serum draws are evaluated by constructing the following ratio (R); where a = the initial DR-70® (FDP) value and b = the current DR-70® (FDP) value.

$$R = \frac{b}{a}$$

When the ratio of the current DR-70® (FDP) value relative to the baseline DR-70® (FDP) value is greater than 1.15, the sample is likely to have a progression of the disease, however, results of DR-70® (FDP) testing should be used in conjunction with other clinical modalities that are standard of care for monitoring disease progression in these samples.

Limitations of the Procedure

The results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of other tests, clinical impressions, therapy, etc.).

Using GenWay Biotech DR-70® (FDP) ELISA, the highest concentration of DR-70® (FDP) measurable without dilution is 10 µg/ml, and the lowest measurable concentration is 0.06 µg/ml (assay sensitivity).

Hemolysis has been shown to cause higher test results in some cases and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur causing a falsely suppressed result. It is preferred that grossly lipemic samples be ultracentrifuged before any analysis.

An elevated DR-70® (FDP) may result from testing serum from samples who have pancreatic disease, heart disease, coagulation disorders, other acute infections or trauma.

DR-70® (FDP) results below the upper reference limit of normal do not indicate the absence of malignancy because samples with histopathologic evidence of cancer may have DR-70® (FDP) assay values within the range of normal individuals.

Conversely, a DR-70® (FDP) assay value exceeding the upper limit reference limit of normal does not necessarily indicate the presence of colorectal malignancy since a small percentage of healthy individuals and individuals with non-malignant conditions may have elevations in DR-70® (FDP) assay results. A DR-70® (FDP) assay value should not be interpreted as absolute evidence for the presence or absence of malignant disease of the pancreas. It should be remembered that this assay is to be used as a monitoring aid, not as an aid in diagnosis.

For a more complete understanding of the limitations of this procedure, please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert.

Expected Values

Each laboratory should determine a reference interval corresponding to the characteristics of their population.

For the serial monitoring of samples previously diagnosed with cancer, a determination was made that a meaningful increase to determine evidence of progression was 15% increase or more. Thus, if the ratio of the current DR-70® (FDP) value relative to the baseline DR-70® (FDP) value is 1.15 or higher, the DR-70® (FDP) test is deemed to be positive, otherwise it is deemed to be negative. A 15% increase from the previous visit was chosen as the threshold for significant % change for the determination of disease progression in the DR-70® (FDP) immunoassay based on the imprecision study where the total CV over all runs, days, and intra-assay was computed for each specimen analyzed.

Note: Results of DR-70® (FDP) testing should be used in conjunction with other clinical modalities that are standard of care.

I. Normal, Benign, Malignant Disease Studies

Values for the GENWAY BIOTECH-ELISA DR-70® (FDP) assay were analyzed for the different DR-70® (FDP) concentration levels within each normal, benign and malignant disease cohort and are presented in Table 1. The table is provided as a reference for informational purposes only.

Table 1. Distribution in percent of serum DR-70® (FDP) values **

| Disease | # of subjects | Percent (%), 95% CI (lower-upper %)* | | | |
|---------------|---------------|--------------------------------------|-------------------|-------------------|-------------------|
| | | 0-1.4 µg/ml | 1.5-2.4 µg/ml | 2.5-4.9 µg/ml | ≥ 5.0 µg/ml |
| Normal | 420 | 94.5 (91.9, 96.5) | 5.0 (3.1, 7.5) | 0.5 (0.1, 1.7) | 0.0 (0.0, 0.9) |
| < 65 years | 337 | 96.4 (93.9, 98.2) | 3.3 (1.6, 5.8) | 0.3 (0.0, 1.6) | 0.0 (0.0, 1.1) |
| ≥ 65 years | 83 | 86.8 (77.5, 93.2) | 12.1 (5.9, 21.0) | 1.2 (0.0, 6.5) | 0.0 (0.0, 4.4) |
| Benign | 326 | 75.5 (70.4, 80.0) | 6.8 (4.3, 10.0) | 0.6 (0.1, 2.2) | 17.2 (13.2, 21.7) |
| GU Disease | 94 | 94.7 (88.0, 98.3) | 4.3 (1.2, 10.5) | 0.0 (0.0, 3.9) | 1.1 (0.0, 5.8) |
| GI Disease | 61 | 90.2 (79.8, 96.3) | 3.3 (0.4, 11.4) | 0.0 (0.0, 5.9) | 6.6 (1.8, 16.0) |
| Pancreas | 84 | 60.7 (49.5, 71.2) | 15.5 (8.5, 25.0) | 2.4 (0.3, 8.3) | 21.4 (13.2, 31.7) |
| Heart Disease | 87 | 58.6 (47.6, 69.1) | 3.5 (0.7, 9.8) | 0.0 (0.0, 4.2) | 37.9 (27.7, 49.0) |
| Malignant | 439 | 44.0 (39.3, 48.8) | 24.2 (20.2, 28.4) | 19.6 (16.0, 23.6) | 12.3 (9.4, 15.7) |
| Colon | 187 | 55.6 (48.2, 62.9) | 21.4 (15.7, 28.0) | 15.0 (10.2, 20.9) | 8.0 (4.6, 12.9) |
| Lung | 44 | 34.1 (20.5, 49.9) | 38.6 (24.4, 54.5) | 18.2 (8.2, 32.7) | 9.1 (2.5, 21.7) |
| Liver | 44 | 31.8 (18.6, 47.6) | 27.3 (15.0, 42.8) | 22.7 (11.5, 37.8) | 18.2 (8.2, 32.7) |
| Breast | 31 | 54.8 (36.0, 72.7) | 25.8 (11.9, 44.6) | 12.9 (3.6, 29.8) | 6.5 (0.8, 21.4) |
| Ovarian | 31 | 25.8 (11.9, 44.6) | 6.5 (0.8, 21.4) | 32.3 (16.7, 51.4) | 35.5 (19.2, 54.6) |
| Cervical | 28 | 50.0 (30.7, 69.4) | 28.6 (13.2, 48.7) | 7.1 (0.9, 23.5) | 14.3 (4.0, 32.7) |
| Gall Bladder | 19 | 42.1 (20.3, 66.5) | 26.3 (9.2, 51.2) | 31.6 (12.6, 56.6) | 0.0 (0.0, 17.7) |
| Pancreas | 28 | 25.0 (10.7, 44.9) | 17.9 (6.1, 36.9) | 35.7 (18.6, 55.9) | 21.4 (8.3, 41.0) |
| Gastric/Other | 27 | 22.2 (8.6, 42.3) | 33.3 (16.5, 54.0) | 29.6 (13.8, 50.2) | 14.8 (4.2, 33.7) |

*Exact binomial confidence limits.

**It is recommended that each laboratory establish its own expected reference range for the population of interest.

II. Summary of Published Clinical Studies

In summary, the GenWay Biotech DR-70® (FDP) Immunoassay has been tested in eight international clinical trials^{12, 13, 16, 33-37}. The GenWay Biotech DR-70® (FDP) ELISA test detected fourteen kinds of cancer in published clinical trials; however, there is a sufficient quantity of sample data to be statistically significant for the detection of eight out of fourteen types of cancers. Additional clinical trial data will soon be available to validate the other cancers that were detected but had few sample samples in the chart below.

Detection of Multiple Cancers by the GENWAY BIOTECH DR-70® ELISA(FDP) Test

| Type of Cancer | Effectiveness | |
|----------------|---------------|--------------|
| | Sensitivity* | Specificity* |
| Lung | 70.5% | 92% |
| Colorectal | 75.6% | 93.4% |
| Ovarian | 84% | 100% |
| Liver | 96.5% | 96.7% |
| Tongue | 73% | 92.5% |
| Breast | 65.2% | 95% |
| Stomach | 92% | 95% |
| Pancreatic | 80% | 95% |

*A normalized average was used when reported by multiple papers

Performance Testing

To determine the analytic validity of the DR-70® (FDP) immunoassay, the following performance tests were conducted.

I. Precision

Imprecision was tested on the GenWay Biotech DR-70® (FDP) ELISA using three serum pools and two quality control materials with concentrations of DR-70® (FDP) across the linear range of the assay run in quadruplicate in a randomized manner in two runs per day for twenty days at three sites using three manufactured lots. The results are presented in the table below:

Total and Components of Assay Variance and Percentage by Source

| Specimen | Site Mean (µg/ml) | Site Variance (%) | Day Variance (%) | Lot Variance (%) | Run Variance (%) | Residual Variance (%) | Total Variance |
|----------|-------------------|-------------------|------------------|------------------|------------------|-----------------------|----------------|
| Pool 1 | 0.315 | 0.001336 (23.53) | 0.000417 (8.28) | 0.000437 (8.68) | 0.000303 (6.02) | 0.002543 (50.50) | 0.005036 |
| Pool 2 | 1.389 | 0.002385 (11.00) | 0.003585 (16.53) | 0.000132 (0.61) | 0.001810 (8.34) | 0.01378 (63.53) | 0.021692 |
| Pool 3 | 2.739 | 0.01483 (20.11) | 0.008803 (11.94) | 0.000431 (0.58) | 0.005631 (7.64) | 0.04405 (59.73) | 0.073745 |
| QC1 | 0.240 | 0.001088 (23.74) | 0.000424 (9.25) | 0.000701 (15.29) | 0.000135 (2.95) | 0.002236 (48.78) | 0.004584 |
| QC2 | 2.994 | 0.02246 (20.03) | 0.01255 (11.19) | 0.008363 (7.46) | 0.001438 (1.28) | 0.06731 (60.03) | 0.112121 |

II. Spike Recovery

Serums from three normal subjects having DR-70® (FDP) values ranging from 0.3 µg/ml to 0.6 µg/ml and a control diluent buffer were spiked with a DR-70® (FDP) antibody solution to obtain expected levels ranging from 1.5 µg/ml to 10 µg/ml to represent the range of the DR-70® (FDP) calibrators. The values of DR-70® (FDP) in the spiked serum were measured and compared to the theoretical values and to values obtained for the control diluent buffer. The experiment was designed to compare responses of the analyte in a biological sample versus the standard diluent to assess for any difference in assay response. Based on the overall analysis of results, the DR-70® (FDP) immunoassay kit is a quantitative test without concerns of sample matrix affects.

| Sample | DR-70 concentration value (µg/ml) | | | | | |
|--------------------|-----------------------------------|----------------------|----------------------|----------------------|----------------------|---------------------|
| | No spike | Spike 1 1.5 µg/ml | Spike 2 2.5 µg/ml | Spike 3 5.0 µg/ml | Spike 4 7.0 µg/ml | Spike 5 10 µg/ml |
| Diluent buffer(5x) | 0 | 1.517 | 2.649 | 4.586 | 6.983 | 10.94 |
| Sample 1 | 0.428 | 1.743 | 2.908 | 4.839 | 7.057 | 13.11 |
| Sample 2 | 0.576 | 1.520 | 2.680 | 4.848 | 7.050 | 11.95 |
| Sample 3 | 0.464 | 1.598 | 2.967 | 5.193 | 6.701 | 10.88 |
| Sample mean value | 0.489 | 1.620 | 2.852 | 4.960 | 6.936 | 11.98 |
| % Mean Recovery | ----- | 107% | 108% | 108% | 99% | 110% |

III. Linearity

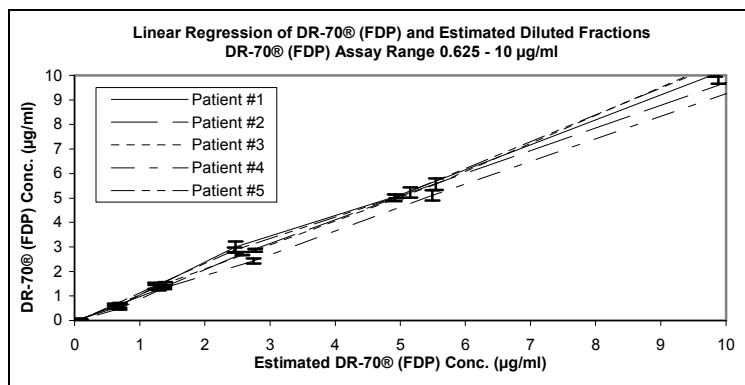
Serums from 5 colorectal cancer samples with DR-70® assay values in the range of 19.7 to 22.2 µg/ml were diluted with assay diluent buffer in a two-fold serial dilution series. For each CRC sample serum sample, a total of 9 DR-70® (FDP) dilution samples were tested. The following table lists the % difference between the actual DR-70® (FDP) concentrations and the estimated DR-70® (FDP) concentrations (1st column in table below) for each sample at each dilution. For each dilution, the average % difference is listed as well as the average % recovery. Grey boxes contain % differences per CRC sample at dilutions whose values were statistically non-linear. Values below the lowest calibrator included in the DR-70® (FDP) assay kit are in the non-linear portion of the DR-70® (FDP) assay curve.

% Difference Between Actual and Estimated DR-70® (FDP) Values in Linearity Study

| Estimated DR-70® (FDP) Conc. | Dilution Ratio | % Difference per CRC Sample | | | | | Average % Difference | Average % Recovery |
|------------------------------|----------------|-----------------------------|-------|-------|------|--------|----------------------|--------------------|
| | | 1 | 2 | 3 | 4 | 5 | | |
| 20 | 1 | (14.5) | (0.1) | (6.9) | 2.6 | (3.2) | (4.4) | 96 |
| 10 | ½ | (2.3) | 3.1 | (7.4) | 7.5 | (5.7) | (1.0) | 99 |
| 5 | ¼ | (2.6) | (1.4) | (0.3) | 7.0 | (2.1) | 0.1 | 100 |
| 2.5 | 1/8 | (21.2) | (5.8) | (3.2) | 11.8 | (16.8) | (7.0) | 93 |
| 1.25 | 1/16 | (11.0) | 2.0 | (8.6) | 3.6 | (14.5) | (5.7) | 94 |
| 1.125 | 1/32 | 18.1 | 5.6 | (0.1) | 20.1 | (5.8) | 7.6 | 108 |
| 0.625 | 1/64 | 13.9 | 11.8 | 12.6 | 12.9 | 16.7 | 13.6 | 114 |

█ = indicates that these dilutions were statistically non-linear

The results of the linearity study are presented in graphic form on the following page. For each of the 5 CRC sample serums, the estimated DR-70® (FDP) Conc. (µg/ml) is graphed against the actual DR-70® (FDP) Conc. (µg/ml) with the standard deviation among the 5 replicates at each point represented by the Y-axis error bars. For all of these samples, the DR-70® (FDP) concentrations were statistically found to be linearly related; except for those dilutions below a DR-70® (FDP) concentration of 0.625 µg/ml.



IV. Analytical Sensitivity

The minimal detectable concentration (MDC) of DR-70[®] (FDP) is estimated to be 0.06 µg/ml. The MDC is defined as that concentration of DR-70[®] (FDP) corresponding to the absorbance that is two standard deviations from the mean rate of absorbance of 20 replicate determinations of a zero calibrator.

V. Functional Sensitivity

The functional sensitivity was determined by diluting the lowest non-zero calibrator serially, measuring the DR-70[®] (FDP) concentration and extrapolating to the point where the CV% = 20%. Functional sensitivity for the GenWay Biotech DR-70[®] (FDP) is calculated as being 0.063 µg/ml. This compares well to the Analytical Sensitivity.

VI. Interference

Interference is defined, for purposes of this study, to be recovery outside of 10% of the known specimen mean concentration.

- Added hemoglobin (up to 500 mg/dl) does not interfere with the assay.
- Added bilirubin (up to 30 mg/dl) do not interfere with the assay.
- Lipemia, as indicated by added triglyceride (up to 1000 mg/dl), does not interfere with the assay.
- Heparin (at concentrations of 500 U/ml) do not interfere with the assay.
- The following pharmaceutical agents were tested at levels indicated and found not to cause analyte recovery outside 10%: 5'-fluorouracil (Adrucil), 1.0 mg/ml; acetaminophen, 0.2 mg/ml; adriamycin (Doxorubicin HCl), 0.10 mg/dl; coumarin, 1.4 mg/ml; cyclophosphamide (Cytosan), 0.25 mg/ml; Paclitaxel, 3.5 x 10⁻⁶ g/m²; amethopterin hydrate (Methotrexate), 4.5 mg/ml; mitoxantrone (Novatrone), 0.5 mg/ml; folic acid (Leucovorin), 1.10 mg/ml, Mitomycin C, 0.06 mg/ml; cisplatin, 0.10 mg/ml.

VII. Hook Effect

Studies were performed testing for hook effect in the GENWAY BIOTECH-ELISA DR-70[®] (FDP). No evidence of a hook effect was found up to a concentration of 250 µg/ml.

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