CA 19-9™

RADIOIMMUNOASSAY

FUJIREBIO DIAGNOSTICS CA 19-9™ RIA

Radioimmunoassay for the quantitative determination of the CA 19-9 tumor associated antigen in serum or plasma of patients that have been diagnosed with pancreatic cancer.

The Fujirebio Diagnostics CA 19-9™ RIA is based on the use of the 1116NS19-9 antibody which is available exclusively through Fujirebio Diagnostics, Inc. and its licensed distributors. Performance characteristics of kits which employ the 1116NS19-9 antibody are not transferable to diagnostic kits using different antibodies.

The CA 19-9 assay level of a given specimen may vary with assays from different manufacturers due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the CA 19-9 assay used. Values obtained with the different assay methods cannot be used interchangeably. If, in the course of monitoring the patient, the assay method used for determining the CA 19-9 levels is changed, additional sequential testing should be carried out to confirm baseline values.

This radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories, blood banks or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. In the United States, its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license, of the U.S. Nuclear Regulatory Commission (NRC) or of a State with which the commission has entered into an agreement for the exercise of regulatory authority.

INTENDED USE

The Fujirebio Diagnostics CA 19-9™ RIA, an *in vitro* diagnostic test for the quantitative measurement of the CA 19-9 tumor associated antigen, in human serum or plasma, is indicated for the serial measurement of CA 19-9 to aid in the management of patients diagnosed with cancers of the exocrine pancreas. The test is useful as an aid in:

Monitoring of disease status in those patients having confirmed pancreatic cancer who have levels of serum or plasma CA 19-9 above the cutoff, at the time of diagnosis.

CA 19-9 values must be interpreted in conjunction with all other available clinical and laboratory data before a medical decision is determined.

Patients known to be genotypically negative for the Lewis blood group antigen will be unable to produce the CA 19-9 antigen even in the presence of malignant tissue. Phenotyping for the presence of the Lewis antigen may be insufficient to detect true Lewis antigen negative individuals. Even patients who are genotype positive for the Lewis antigen may produce varying levels of CA 19-9 based on gene dosage effect.

SUMMARY AND EXPLANATION

CA 19-9 assay levels are defined by using the 1116NS19-9 antibody[1,2] to measure reactive determinants on a high molecular weight glycoprotein[3] in serum or plasma. Initial clinical studies[4-7] now confirmed world-wide, indicate that CA 19-9 assay levels are frequently elevated in the serum of patients with cancers of the pancreato-biliary system (PBS) (*i.e.* pancreas, gallbladder, biliary tract). In addition, elevated levels of CA 19-9 have been observed in other malignancies such as lung cancer, other gastrointestinal cancers and in some nonmalignant disorders.

PRINCIPLES OF THE PROCEDURE

The Fujirebio Diagnostics CA 19-9™ RIA is a solid-phase radioimmunoassay based on the forward sandwich principle. Polystyrene beads coated with a mouse monoclonal antibody against CA 19-9 are incubated with a serum or plasma specimen, or the appropriate standards or controls. During the incubation, the CA 19-9 antigen present in the specimen is specifically bound to the mouse monoclonal antibody on the solid support phase. Unbound material present in the specimen is removed by aspiration and washing of the beads. The same mouse monoclonal CA 19-9 labeled with ^{125}I is then incubated with the beads and binds to the CA 19-9 antigen already bound to the beads. Unbound labeled antibody is removed by aspiration and washing. The bound radioactivity is determined by counting the beads in a gamma counter. A standard curve is obtained by plotting the CA 19-9 antigen concentration of the standards *versus* bound radioactivity. The CA 19-9 antigen concentrations of unknown patient specimens are determined from the standard curve and are directly proportional to the concentration of the bound radioactive molecules.

REAGENTS

1. BUFFER — 1 vial (10 mL) CA 19-9 sample buffer: 50 mM sodium citrate (pH 5.0) with pentachlorophenol (0.056%) as a preservative.
2. STANDARDS — 5 vials (0.8 mL each) standards: 15, 30, 60, 120 and 240 U/mL* in defibrinated normal human plasma** with sodium azide as a preservative.
3. CONTROLS — 2 vials (0.8 mL each) controls CA 19-9: (assay values on vial labels) in defibrinated normal human plasma** with sodium azide as a preservative.
4. COATED BEADS — 100 1116NS19-9 antibody (Mouse, Monoclonal) Coated Beads.
5. TRACER — 2 vials (10 mL each) 1116NS19-9 antibody (Mouse, Monoclonal) ^{125}I in sodium citrate buffer with a bovine derived stabilizer, containing pentachlorophenol as a preservative. Radioactivity maximum: 0.64 µCi/mL (23.7 kBq/mL).
6. STANDARD/DILUENT — 1 vial (5.8 mL) CA 19-9 0 Standard/Diluent, 0 U/mL* in defibrinated, delipidated normal plasma** with Pluronic F-68 and sodium azide as preservatives.

* CA 19-9 assay values are expressed as units per mL (U/mL). A unit is an arbitrary value related to an FDI-maintained reference standard. There is no generally available reference standard at this time..

** Tested in accordance with current FDA-required assays for blood-borne pathogens.

Materials supplied perform 100 tests. If any component remains after the performance of 100 tests (100 beads), DO NOT USE. REAGENTS FROM DIFFERENT KIT LOTS MUST NOT BE MIXED.

WARNING AND PRECAUTIONS FOR USERS

1. FOR *IN VITRO* DIAGNOSTIC USE ONLY. Not for internal or external use in humans or animals.
2. Do not use kit components beyond the expiration date.
3. Do not mix reagents from different kit lots.
4. Avoid microbial contamination of reagents in vials.
5. Do not eat, drink, smoke or apply cosmetics in any laboratory in which radioactive materials are handled.
6. Do not pipette reagents and samples by mouth.
7. A lab coat or other suitable protective clothing and disposable gloves should be worn throughout the testing procedure.
8. All spillage should be immediately wiped up and work area properly decontaminated. All contaminated radioactive material should be discarded in the radioactive waste.
9. The user should store the radioactive material until used, in the original shipping package or in a container providing equivalent radiation protection. The storage refrigerator should be properly marked with a radiation hazard sign. Pursuant to a Certificate of Registration received after filing form NRC-483, laboratories may receive products containing ^{125}I in units not exceeding 10 microcuries each, and may not possess at any one time, at any one location of storage or use, a total amount of ^{125}I in excess of 200 microcuries. A Fujirebio Diagnostics CA 19-9™ RIA 100 test kit contains two vials of tracer, each of which does not exceed 10 microcuries. Licensees in Agreement States are to refer to the appropriate regulations of their own state. Radioactive waste is to be disposed of into appropriately labeled waste containers, according to State or Federal requirements. Radioactive material should be stored in a properly designated area.
10. Test components contain sodium azide as a preservative. Because sodium azide may form explosive lead or copper azide in plumbing, it is recommended that drains be thoroughly flushed with water after disposal of solutions containing sodium azide.
11. **WARNING: Although these reagent standards, controls and diluent have been tested and found non-reactive for antibodies to HIV, HCV and for HBsAg, there are no assurances that the reagents do not contain infectious agents. Therefore, handle and dispose of reagent samples and clinical specimens as if they were potentially infectious.**

STORAGE INFORMATION

1. Store all reagents at 2°C to 8°C when received. Avoid exposure of reagents to excessive heat or light during storage or incubation. DO NOT FREEZE. The expiration date of the kit is printed on the box.
2. Bring reagents to room temperature before use. (Bead containers should be brought to room temperature before opening, and tightly capped thereafter.) Immediately after use, return reagents to 2°C - 8°C.

INSTRUMENT

Commercially available well-type gamma counters are routinely employed to determine radioactivity. Reference should be made to the instruction manual supplied with the instrument. It is recommended that counters used have efficiencies of greater than 40%. If counter efficiency is less than 40%, longer counting periods should be used.

SPECIMEN COLLECTION AND HANDLING

Serum or plasma may be tested with the Fujirebio Diagnostics CA 19-9™ RIA. Specimens should be clear, non-icteric, non-lipemic, and non-hemolyzed. Serum specimens may be collected with or without separation gel. Plasma specimens may be collected using citrate, heparin, ACD-A or EDTA as anticoagulants.

If the test is to be run within 24 hours after collection, the specimen should be stored in the refrigerator at 2°C to 8°C. If testing will be delayed more than 24 hours, specimens should be frozen at -20°C.

PROCEDURE

Materials provided:

Fujirebio Diagnostics CA 19-9™ RIA Kit, 100 TEST UNIT
(See Reagents for complete listing of kit contents.)

An appropriate number of the following accessories is provided for performance of the CA 19-9 RIA:

- Reaction trays.
- Transfer trays pre-filled with counting tubes (For transfer of beads from reaction trays).
- Adhesive cover sealers.

Materials required but not provided:

- Precision pipettes with disposable tips to deliver 0.1 mL (± 1%), 0.2 mL (± 1%).
- Distilled or deionized water for use in bead-washing operation.
- Device for delivery of wash solution, such as Cornwall syringe, Gorman-Rupp pump or equivalent.
- An aspiration device for washing coated beads, *e.g.,* cannula, aspiration tip, or commercial multiwash device with vacuum source and a liquid trap for retaining aspirated fluids.
- Water bath capable of maintaining temperature at 37°C ± 2°C.
- A well-type gamma counter.
- Bead dispenser device, *e.g.,* commercial single or multiple bead dispenser or nonmetallic forceps.
- Rectilinear graph paper.

PERFORMANCE OF TEST

CAUTION: BRING SPECIMENS AND REAGENTS TO ROOM TEMPERATURE (20°C TO 30°C) BEFORE USE. USE A CLEAN PIPETTE OR DISPOSABLE TIP FOR EACH TRANSFER TO AVOID CROSS-CONTAMINATION.

ASSAY PROCEDURE

NOTE: EACH STANDARD, CONTROL AND SPECIMEN SHOULD BE ASSAYED IN DUPLICATE EACH TIME TEST IS PERFORMED.

1. Adjust temperature of water bath to 37°C (± 2°C).
2. Identify reaction tray wells on data sheet for testing the standards, controls and specimens. Six standards and two controls should be run with each series of unknown samples. Reaction trays containing standards and controls should be subject to the same manipulations and incubation times as the sera or plasma being tested.
3. Pipette 0.1 mL (100 µL) of CA 19-9 RIA sample buffer solution into each reaction well using precision pipette and tips.
4. Pipette 0.1 mL (100 µL) of standards, controls and specimen samples into their assigned wells.
5. Dispense one bead into each reaction well. Beads may be transferred by use of clean forceps or by use of a single or multiple bead dispensing device.
6. Apply a cover sealer to each tray. Make sure that each bead is completely covered by the specimen. Gently tap the tray to assure mixing of the solutions to eliminate any air bubbles trapped in the reaction wells. Be careful not to splash liquid onto cover.
7. Incubate the trays in the 37°C (± 2°C) water bath for three hours (± 5 minutes).
8. At the end of the three hour incubation, remove trays from the water bath. Carefully remove and discard the adhesive covers.
9. Wash each bead (See Wash Procedure for details).
10. Add 0.2 mL (200 µL) of 1116NS19-9 antibody (Mouse, Monoclonal) ^{125}I to each well containing a bead.
11. Apply cover sealer to each tray. Make sure that beads are completely covered with liquid. Tap the tray gently to release any air bubbles that may be trapped in the solution.
12. Incubate the trays for three hours (± 5 minutes) at room temperature (20°C - 30°C).
13. At the end of the three hour incubation, remove and discard the adhesive sealers. Aspirate the liquid and wash each bead (See Wash Procedure for details).
14. Transfer the beads from the reaction trays to the tube rack transfer system by aligning the numbers and letters on the bottom of the transfer system with the numbers and letters on the reaction tray. Invert both tube rack and reaction tray simultaneously. Tap the tray lightly to transfer the beads to the counting tubes. Tear off the protective flap of the transfer system only after the beads have been transferred.
15. Mark or identify all tubes either prior to or after transfer of the beads.
16. Place the counting tubes into a suitable well-type gamma counter. Count the radioactivity in each tube for one minute. All standards, controls, and unknown samples must be counted together.

WASH PROCEDURE

Semi-Automated

Commercial rinsing/aspiration systems which are semi-automated are recommended. Each well is aspirated, the beads are washed with 5 mL of distilled or deionized water, and wash fluid is removed by aspiration. Repeat this wash procedure two additional times for a total rinse volume of 15 mL. To ensure an adequate washing, beads must be lifted off the bottom of the reaction well during the wash process.

Manual

Aspirate each well using a disposable pipette or cannula attached to a vacuum source. Rinse each bead by placing the pipette or cannula, attached to the vacuum source, adjacent to the bead in the bottom of the well and slowly add, with a Cornwall Syringe or equivalent, 5 mL of distilled or deionized water. The bead must be totally immersed throughout the wash procedure. Care should be taken not to overflow the well. Wash fluid is removed by aspiration. Repeat the wash procedure two additional times for a total wash volume of 15 mL.

RESULTS

1. Construct a standard curve by plotting the average counts per minute obtained for each standard on the vertical (Y) axis versus the corresponding "known" concentration of each standard on the horizontal (X) axis using rectilinear graph paper. Connect the points with straight-line segments.
2. Using the average counts per minute, CA 19-9 assay results can be determined for each control and unknown specimen.
3. If the specimen required additional dilution to give a concentration value which falls on the standard curve, the concentration value determined on the horizontal (X) axis from the standard curve must be multiplied by the dilution factor.

QC CRITERIA

1. Ensure that the values for the controls fall within the limits indicated on the vial labels.
2. Values for duplicates should be within 15% of the mean cpm; duplicate values that differ from the mean by greater than 15% should be considered suspect and the sample should be retested. However, for the 0 U/mL Standard/Diluent, and samples with less than 300 cpm, duplicates may differ more than 15% from the mean cpm.

Repeat test if assay is considered invalid. Consult Technical Service if assay fails repeat test.

PROCEDURE FOR ASSAY OF SPECIMEN WITH GREATER THAN 240 U/ML (OPTIONAL)

If in an initial assay, a specimen is found to contain greater than 240 U/mL, dilute the specimen with CA 19-9 diluent as necessary to obtain a result that is less than 240 U/mL. For example, a ten-fold dilution is prepared by adding 0.05 mL (50 µL) specimen to 0.45 mL (450 µL) of CA 19-9 diluent. Mix thoroughly before assaying. Perform the assay according to Assay Procedure. Multiply the value in U/mL by a factor of 10 (See example in Tables 1 and 2 and Figure 1).

FUJIREBIO DIAGNOSTICS CA 19-9™ RIA PROCEDURE SUMMARY

(BRING ALL REAGENTS AND SPECIMENS TO ROOM TEMPERATURE)

ASSAY PROCEDURE

First Incubation:

1. Adjust Water Bath to 37°C ± 2°C.
2. Pipette 0.1 mL of CA 19-9 RIA sample buffer to each well.
3. Pipette 0.1 mL of standards, controls and specimen into each appropriate well of reaction tray.
4. Dispense one 1116NS19-9 antibody-coated bead into each well.
5. Apply adhesive cover sealer and gently tap tray to ensure that beads are covered and that air bubbles are released.
6. Incubate for 3 hours ± 5 minutes at 37°C ± 2°C.
7. Remove cover sealer, aspirate the liquid and wash each bead three times with 5 mL distilled or deionized water.

Second Incubation:

1. Pipette 0.2 mL of ^{125}I 1116NS19-9 antibody onto all beads.
2. Apply adhesive cover sealer and gently tap tray to cover beads and release bubbles.
3. Incubate for 3 hours ± 5 minutes at room temperature (20°C to 30°C)
4. Remove cover, aspirate the liquid from wells and wash beads as in Step 7.
5. Remove all excessive liquid from tray by aspiration.
6. Transfer beads to the counting tubes.
7. Count tubes for 1 minute.

RESULTS

1. Construct standard curve by plotting average counts per minute for the standards (Y axis) *versus* "known" standard concentration (X axis). Connect points with straight line segments.
2. Determine CA 19-9 assay results for unknown specimens from the standard curve.
3. A change in CA 19-9 values of greater than 20%, in U/mL, between serial draws may indicate a change in disease status.

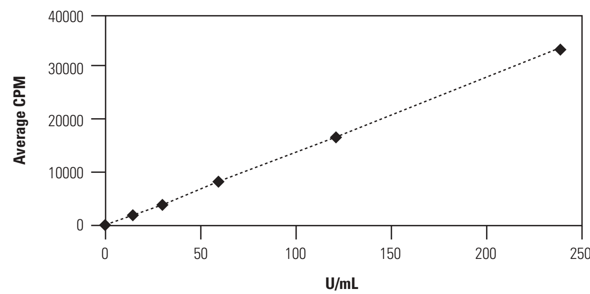
Table 1: EXAMPLE OF STANDARDS

U/mL-on vial	0	15	30	60	120	240
cpm	320	2344	3909	8588	17999	32662
	365	2074	4076	8173	15551	34734
Average cpm	342.5	2209	3992.5	8380.5	16775	33698

Table 2: EXAMPLE OF ASSAY VALUES OF UNKNOWN SPECIMENS

Specimen	cpm	Average cpm	U/mL From Curve	Multiply Dilution Factors	U/mL
A	5207 5716	5461.5	40.0	NA	40.0
B	47639 45822	46730.5	>240	—	—
C	27243 28427	27835	198.4	NA	198.4
Diluted 1:10 Specimen B	11139 9846	10492.5	75.1	X 10	751

Figure 1: FUJIREBIO DIAGNOSTICS CA 19-9™ RIA EXAMPLE OF STANDARD CURVE



LIMITATIONS OF THE PROCEDURE

1. Patients known to be genotypically negative for the Lewis blood group antigen will be unable to produce the CA 19-9 antigen even in the presence of malignant tissue. Phenotyping for the presence of the Lewis antigen may be insufficient to detect true Lewis antigen negative individuals. Even patients who are genotype positive for the Lewis antigen may produce varying levels of CA 19-9 based on gene dosage effect.
2. The assay should not be performed on clotted, icteric, hemolyzed, or lipemic samples.
3. Rare specimens have been observed in which very high CA 19-9 levels may cause diminished binding. While this prozone or "hook" effect is not typical, an erroneous unit value below 240 U/mL may be found. Results up to 1,250,000 U/mL did not produce prozone effect.
4. Human anti-mouse antibodies (HAMA) may be present in samples from patients who have received mouse monoclonal antibodies for immunotherapy. Such sample may show false elevated or depressed values when tested with this method. Results for patients suspected of having such antibodies should be carefully evaluated and interpreted in the context of the clinical status of the patient.
5. The Fujirebio Diagnostics, Inc. CA 19-9™ RIA can be used with serum and plasma prepared using different anti-coagulants (ACD-A, Citrate, EDTA, and Heparin) (see Table 3). However, it is recommended that if the specimen type is changed during patient monitoring, the patient should be re-baselined to negate any potential biases due to specimen type.

Table 3: PLASMA *VERSUS* SERUM RESULTS

CA 19-9 Level (%SST)

Sample #	Serum SST	Serum no SST	Plasma EDTA	Plasma Heparin	Plasma ACD-A	Plasma Citrate	Mean Recovery
1	100.0	91.8	100.9	90.8	108.3	111.0	102.8
2	100.0	81.7	82.1	91.0	104.0	94.7	93.0
3	100.0	94.0	84.6	123.6	118.1	101.9	107.1
4	100.0	86.6	90.9	77.0	91.2	95.3	88.6
5	100.0	100.0	108.4	92.1	106.5	108.6	103.9
Mean (%)		**90.8**	**93.4**	**94.9**	**105.6**	**102.3**	**99.1**

ANALYTICAL RANGE

The analytical range of the Fujirebio Diagnostics CA 19-9™ RIA assay is 0 – 240 U/mL.

ANALYTICAL PERFORMANCE CHARACTERISTICS

Analytical Sensitivity (Minimal Detectable Dose)

Analytical sensitivity or minimal detectable dose (MDD) is defined as the lowest antigen concentration which can be differentiated from a sample with no antigen. It is determined as the concentration of antigen for which the mean cpm value is two standard deviations (2SD) above the mean zero standard as read from the standard curve. The analytical sensitivity was determined by testing twenty replicates of the zero standard/diluent and determining the mean plus 2SD of the zero standard. The analytical sensitivity was determined to be 0.9 U/mL. This level of analytical sensitivity is well below the established "cutoff" of 37 U/mL[10].

Interfering Substances

The appropriate NCCLS guideline[8] was followed to determine possible sources of interference with the Fujirebio Diagnostics CA 19-9™ RIA kit. The substances and the testing concentration are listed in Table 4.

Table 4: SUBSTANCE AND TESTING CONCENTRATION

Substance	Concentration in mg/mL (unless noted)
Hemoglobin	0.50
Bilirubin	0.60
HAMA	Titer: 16636
HAMA	Titer: 1664
Lipemia/Lipids/Triglycerides	10.00
PAR® TDM 1*	Level 1
PAR® TDM 2*	Level 2
PAR® TDM 3*	Level 3
Heparin	50 U/mL
5'fluorouracil/Adrucil	1.00
Acetaminophen/Tylenol	0.20
Adriamycin/Doxorubicin-HCL	0.10
Cyclophosphamide/Cytoxan	0.25
Paclitaxel	3.50E-06
Tamoxifen – CITRATE SALT	0.13
Amethopterin – HYDRATE, Methotrexate	4.50
Acetylsalicylic acid/Aspirin	0.50
Cisplatin – DICHLORIDE	0.10
Lidocaine – HCL/Xylocaine	0.06
Aminoglutethimide	0.40
Cyclosporin A	2.97E-06
Folinic Acid/Leucovorin	1.10
Cisplatin – DICHLORIDE	1.00
Tobramycin – SULFATE SALT	1.45E-02
Propanolol – HCL/Inderal	5.00E-03
Quinidine gluconate/Duraguin, Quinaglute	0.05
Digoxin	5.00E-06
Caffeine	0.10
Phenytoin SODIUM SALT/Dilantin	0.10
Salicylate/Salicylic acid – SODIUM SALT	0.50
Cortisol/Hydrocortisone DISODIUM SALT	1.00
Valproic acid – SODIUM SALT/Depakene	0.50
Novatrone/Mitoxanntrone	0.50
Theophylline/Aminophylline	0.25
Gentomycin – SULFATE	0.12
Lithium carbonate/Eskalith	3.50E-02
Mitomycin C	6.00E-03

* PAR® TDM Levels 1-3 are therapeutic drug cocktails manufactured by Medical Analysis Systems, Inc. PAR is a registered product of Medical Analysis Systems, Inc.

Only human anti-mouse antibodies (HAMA), at a titer of 16,636, showed potential interference with the assay. This concentration of human anti-mouse antibody is extremely high. HAMA was also tested at a lower titer of 1664 and showed no effect on the assay. All other substances that were tested with the Fujirebio Diagnostics CA 19-9™ RIA kit showed no interference at the levels tested.

RECOVERY STUDIES

Ten (10) patient samples were spiked with purified CA 19-9 antigen at three (3) different levels. These spiked samples were compared to samples in which only CA 19-9 diluent was added. Measured values minus expected values were calculated and compared to the amount of antigen added to the samples. Results were reported as percent recoveries (% Recovery) and compared to the acceptable percent Recovery Limits calculated using the precision of the assay diluent buffer (which was run as samples, both spiked and unspiked).

For the Low spike samples, where the values of added antigen were approximately 8 U/mL, acceptable limits of % Recovery were calculated as being 87.9 – 112.1%. For the Mid spike samples, where the values of added antigen were approximately 29 U/mL, acceptable limits of % Recovery were calculated to be 81.9 – 118%. For the High spike samples, where the values of added antigen were approximately 90 U/mL, acceptable limits of % Recovery were calculated to be 93.0 – 107.0%. These data are listed in Table 5.

Table 5: SPIKE PERCENT RECOVERY RESULTS

Patient Number	Low-Spike Percent Recovery	Mid-Spike Percent Recovery	High-Spike Percent Recovery
1	110.23%	103.42%	111.58%
2	112.12%	101.87%	96.49%
3	114.08%	102.35%	99.07%
4	121.37%	102.30%	107.50%
5	112.41%	102.89%	100.45%
6	92.31%	102.18%	99.99%
7	117.43%	100.93%	98.81%
8	101.98%	97.75%	99.10%
9	99.64%	102.43%	98.49%
10	101.78%	102.96%	99.78%
Mean	**108.34%**	**101.91%**	**101.13%**

PRECISION, REPEATABILITY, AND REPRODUCIBILITY

The appropriate NCCLS guideline[9] was followed to determine the precision of the Fujirebio Diagnostics CA 19-9™ RIA kit.

Each of three (3) sites performed one (1) run per day for thirteen (13) acceptable days with three (3) different lots of product. Test materials were assayed in random order for each run, but were tested identically across each lot of reagent under evaluation.

The total average variability ranged from 6.7% (44 U/mL) to 15.4% (8 U/mL). Day-to-day variation across sample-site-lot combinations peaked at 14.9% CV with a nadir of 0%. The maximum intra-assay variation was 18% at CA 19-9 concentration of 8 U/mL, a concentration below the first non-zero calibrator and well under the clinical cutoff of 37 U/mL. The average intra-assay variation across all sites and lots for that 8 U/mL sample was 12.5% CV. For all other concentrations tested, the average intra-assay variation did not exceed 6.5% CV.

LINEARITY

The linearity of the Fujirebio Diagnostics CA 19-9™ RIA was tested with serial dilutions of 12 individuals with elevated CA 19-9 assay values. Dilutions were prepared in CA 19-9 0 Standard/Diluent. Regression analysis comparing observed and expected CA 19-9 assay values yielded a mean slope for the twelve samples of 0.96 ± 0.031 and a mean y-intercept of 5.97 ± 7.65.

CLINICAL PERFORMANCE CHARACTERISTICS

Monitoring of Disease Status in Patients Diagnosed with Pancreatic Cancer:

The effectiveness of CA 19-9 as an aid in monitoring of disease status in patients diagnosed with pancreatic cancer was determined by assessing changes in CA 19-9 levels in serial serum sets with changes in disease status. Samples from 61 patients with a total of 234 observations were analyzed. The average number of observations per patient was 3.84. Fifty-seven percent (57%) of the positive serum sets correlated with disease progression while seventy-one (71%) of serum sets showing no significant change in the marker correlated with no progression. Table 6 presents the data in a 3x3 classification scheme.

The disease states are:

- Progression from one collection to the next collection (Progressing).
- No change in disease status (Stable).
- Reduction in the signs and symptoms of the disease from one collection to the next (Responding).

Marker changes are classified as:

- A 20% or greater increase in the marker from one collection to the next (INC).
- No significant change in the marker (|Delta CA 19-9|<20%) (NC).
- A 20% or greater decrease in the marker value from one collection to the next (DEC).

Table 6: EXPANDED DISTRIBUTION

Marker Change	Disease Status: Progressing	Stable	Responding	Total
INC	31 56.4% (1.29)	30 32.3% (.477)	4 16.0% (.190)	65 37.6%
NC	12 21.8% (.279)	41 44.1% (.789)	7 28.0% (.389)	60 34.7%
DEC	12 21.8% (.279)	22 23.7% (.310)	14 56.0% (1.27)	48 27.7%
Total	55	93	25	173

Table 7: SERIAL MONITORING DATA ANALYZED ON A PER PATIENT BASIS

Marker Change	Disease Status: Progression	No Progression	Total
20% Increase	21 (65.6%)	15	36
No Increase	11	14 (48.3%)	25
Total	32	29	61

Figures 2 and 3 are examples of serial patient monitoring graphs showing changes in CA 19-9 values with clinical course of the disease.

Figure 2: SERIAL PATIENT MONITORING GRAPH

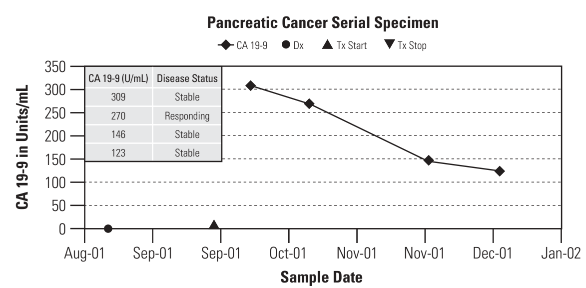
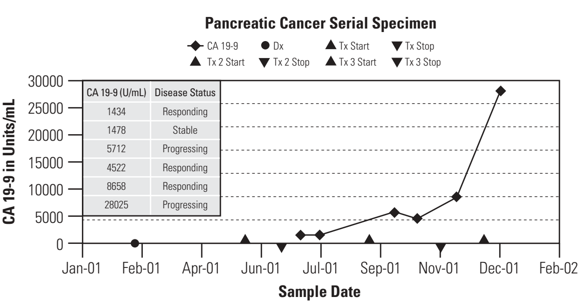


Figure 3 : SERIAL PATIENT MONITORING GRAPH



Where CA 19-9 = the measured value in U/mL, Dx = Diagnosis Date, and Tx = Treatment

CUTOFF AND DISTRIBUTION OF VALUES

The cutoff for the Fujirebio Diagnostics CA 19-9™ RIA kit was determined in 1983 by Del Villano, *et.al.*[4], using 1020 blood donor samples, 314 patients with cancer, and 323 patients with benign diseases. The cutoff of 37 U/mL was chosen to discriminate between the patients with cancer and the normal individuals or the patients with benign diseases.

Del Villano and Zurawski published another study in 1983 on the cutoff of the Fujirebio Diagnostics CA 19-9™ RIA kit[10]. In this study, 2700 healthy blood donors, 750 cancer patients and 1450 patients with benign diseases were used to verify the cutoff of 37 U/mL. The data presented in this publication supports the cutoff of 37 U/mL.

Each laboratory should establish its own reference ranges to assure proper representation of specific populations.

Apparently Healthy Subjects:

To determine the distribution of CA 19-9 values in apparently normal healthy individuals, and to confirm the cutoff of 37 U/mL, a sample of 200 women and 200 men who were apparently disease free were assessed. The distribution of values of apparently healthy subjects is presented in Table 8.

Table 8: FUJIREBIO DIAGNOSTICS CA 19-9™ RIA DISTRIBUTION OF VALUES OF APPARENTLY HEALTHY SUBJECTS

Group	Total	<37 U/mL	37-49.9 U/mL	50-69.9 U/mL	70-99.9 U/mL	≥100 U/mL
Males	200	189 (94.5%)	4 (2.0%)	5 (2.5%)	0 (0.0%)	2 (1.0%)
Females	200	186 (93.0%)	7 (3.5%)	4 (2.0%)	2 (1.0%)	1 (0.5%)

Benign Disease Cohorts:

Three hundred and ninety-nine (399) benign disease patient cohorts were assembled to determine the distribution of serum CA 19-9 values in benign diseases that may be co-existent in patients with confirmed pancreatic cancer. The distribution of values of benign diseases is presented in Table 9.

Table 9: FUJIREBIO DIAGNOSTICS CA 19-9™ RIA DISTRIBUTION OF VALUES OF BENIGN DISEASES

Diagnostic Group	Total	<37 U/mL	37-49.9 U/mL	50-69.9 U/mL	70-99.9 U/mL	≥100 U/mL
Benign Diseases of the Genitourinary Tract	99	90 (90.9%)	6 (6.1%)	2 (2.0%)	1 (1.0%)	0 (0.0%)
Benign Diseases of the Gastrointestinal Tract	100	88 (88.0%)	7 (7.0%)	3 (3.0%)	2 (2.0%)	0 (0.0%)
Benign Diseases of the Pancreas/Pancreatitis	100	94 (94.0%)	1 (1.0%)	4 (4.0%)	1 (1.0%)	0 (0.0%)
Chronic Heart Disease/ Hypertension	100	80 (80.0%)	10 (10.0%)	5 (5.0%)	5 (5.0%)	0 (0.0%)

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