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$\Sigma=96$ tests



6335-16

MICROWELL ELISA

CA-242 ENZYME IMMUNOASSAY TEST KIT

NAME AND INTENDED USE

Pancreatic & rectal CA242 Assay is a solid phase enzyme linked immunosorbent assay (ELISA). This test provides quantitative measurement of CA-242 antigen to aid in the clinical evaluation of symptomatic patients suspected of having pancreatic cancer, colo-rectal and other related diseases. . (For Professional Use Only).

SUMMARY AND EXPLANATION OF TEST

The CA-242 is a sialylated carbohydrate antigen present on mucinous type of glycoproteins in carcinomas of many organs(1,2,3). The CA-242 antigen is shedded from the tumor and the CA-242 can be detected in serum from patients with carcinomas.

In the normal healthy subjects and subjects with benign diseases, the CA-242 levels are low, while elevated levels are commonly found in patients with gastro-intestinal cancer(3). By identifying the colo-rectal cancer patients at an early stage of the disease, primary diagnosis, often relies on occult blood testing, and on radiological endoscopic examination of the large bowel. CEA is widely used for the monitoring and prognostic assessment of patients with colo-retal cancer while the clinical utility of CEA is limited due to the low sensitivity in early stages of cancer. CEA showed higher sensitivity for rectal cancer than for colonic cancer, while the opposite was true for CA-242(4). However, a combination of CA-242 with CEA will improve with higher sensitivity for both rectal and colonic cancer. CA-242 is better than CA-199 in the diagnosis of pancreatic cancer because of its higher specificity, and it may be useful in the screening of localized or respectable tumors(5).

PRINCIPLE OF THE ASSAY

Pancreatic & rectal CA-242 cancer quantitative Assay is a solid phase enzyme-linked immunosorbent system employing plastic wells coated with streptavidin. The sample, standards and controls and biotinylated anti-CA-242 antibodies are allowed to incubate in the wells. During the incubation, specific cancer antigen(CA-242) is bound to CA-242 antibodies on the wells. Unbound CA-242 antigen is removed by washing the wells with buffer. Enzyme conjugate is added to each well. After the incubation, unbound enzyme conjugate is washed off and the amount of bound peroxidase is proportional to the concentration of the CA -242 antigen present in the sample. Upon addition of the chromogen substrate, the intensity of colour developed is proportional to the concentration of CA-242

antigen in the sample and may be quantified by use of a photometric well reader at 450 nm wavelength.

WARNING AND PRECAUTION

1. The gastro-rectal CA-242 assay is a quantitative test. It is designed for in vitro diagnostic use only.
2. The components in this kit are intended for use as an integral unit. The components from different lots should not be mixed and used.
3. References contains human serum should be treated as potentially infectious. All human bases products should be using appropriate precaution.
4. Handle all reference standards and test samples as potentially infectious agents. The standards were found negative for hepatitis B and HIV & HCV antibodies. However, because no test methods offer complete assurance of the absence of the HIV I/II, HCV and hepatitis B virus, or other infectious agents, these materials should be handled at Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers of Disease Control/National Institutes of health Manual "Biosafety in Microbiological and Biomedical Laboratories", 1984.
5. Never pipet by mouth. Avoid contact with skin.

MATERIALS PROVIDED

1. Microwell Strips (96 wells): Streptavidin coated wells. 8 x 12 strips.
2. Biotinylated capture antibody Solution(11 ml)
3. Specimen Diluent (11 ml): or zero standards.
4. Washing Buffer Concentrate (20X) (50 ml): Prepare working solution by adding purified water to 1 liter.
5. Enzyme Conjugate (11 ml): Anti-CA-242 antibodies conjugated to horseradish peroxidase.
6. Reference Standards(0.75 ml each vial)calibrated to 5, 25, 50, 100, and 200 U/ml
7. Substrate Solution A (11 ml): Buffer solution containing peroxide.
8. Chromogen Solution B (11)Tetramethylbenzidine
9. Well holder ; For securing individual wells.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-well reader with wavelength at 450 nm..
2. Pipetor with tips for measuring 25 ul and 100 ul.
3. 1 N of Sulfuric acid (1N H₂SO₄) for stop solution.
4. Clean plastic washing bottle of 1000 ml capacity for use in washing micro-wells with working washing buffer during testing procedures.

REAGENT PREPARATION

Prepare the working washing buffer by adding the entire contents of the Wash Buffer Concentrate to 1000 ml distilled water in a clean plastic wash bottle. Mix gently to dissolve. Store at room temperature.

STORAGE AND STABILITY

1. Store the kits at 2-8°C and keep micro-wells in a dry bag with desiccants.
2. Reagents are stable until expiration of the kit. Solution A and Solution B should be colorless; if the solution turns blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture and allow clotting. Separate the serum by centrifugation at room temperature. If sera cannot be immediately assayed, they may be stored at -20oC for at least six months. Avoid repeated freezing and thawing of samples. Specimens obviously contaminated with

bacteria should not be used. Specimens turbid with high lipid concentrations should be clarified prior to assay.

PREPARATION FOR ASSAY

1. Bring all reagents and samples to room temperature (20-25°C) and mix gently before beginning the test.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruptions to get the most reliable and consistent results.
3. Use new disposable tips for each specimen.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.
2. Dispense 25 µl of Sample diluent into well #1 as a blank, 25 µl of standards, samples or controls into appropriated well. Add to each well 100 µl of biotinylated solution (blue color) except blank well.
3. Incubate for 120 minutes at room temperature.
4. Remove incubation mixture and rinse the wells five times with Washing Buffer.
5. Dispense 100 µl Enzyme Conjugate into each well except blank well.
6. Incubate for 60 minutes at room temperature.
7. Wash Five times with the Washing buffer.
8. Dispense 100 µl of Solution A and 100 µl of Solution B.
9. Incubate for 30 minutes at room temperature.
10. Stop reaction by adding 50 µl of 1 N sulfuric acid (stop solution) to each well.
11. Zero a micro reader on the blank and measure the absorbance of each well at 450 nm.

PROCEDURAL NOTE

1. Wash the microwells and remove water thoroughly to get the best results.
2. Pipet all reagents and samples into bottom of the well. Vortex mixing or shaking of wells after sample and reagent pipeting is not required.
3. The appropriate number of wells should be secured in a holder and all reagent and sample caps should be removed prior to the start of testing. This will permit pipetting at equally intervals without interruption. A maximum of 30 patients' samples should be assayed at one time in order to minimize error due to timing differences between specimens
4. Absorbance is a function of the time and temperature of incubation > it is recommended to have reagents, samples and needed wells ready .to ensure the equal time for each pipetting without interruption.

EXPECTED VALUES AND INTERPRETATION OF RESULTS

1. It is recommended that each laboratory should determine its own normal and abnormal ranges as to account for its local environmental factors such as diet, climate, etc.
2. A clinical study of the CA-242 quantitative was conducted in the house and results were obtained as follows: Serum samples from 236 normal subjects were assayed and showed that 96 % of the individual have CA-242 values below 15 U/ml and 4% range from 15 to 25 U/ml

APPLICATIONS & LIMITATIONS OF THE PROCEDURE

1. The CA-242 Assay should not be used in cancer screening and should not replace any established clinical examination.
2. For diagnostic purposes, CA-242 antigen value should be used as adjunct to other data available to the physician,
3. sample with CA-242 antigens levels above 200 U/ml should be diluted to obtain an accurate value.

QUALITY CONTROL

Each laboratory should utilize internal controls several levels to monitor assay performance. The controls should be treated as unknown. Results obtained should be in agreement with the assigned values of the control. Controls can be obtained from commercially available source.

PERFORMANCE CHARACTERISTICS

Precision

Intra Assay: Three pooled sera were assayed of 8 in a single run

| Serum Samples | Mean (U/mL) | Intra-assay | |
|---------------|-------------|-------------|------|
| | | S.D. | CV% |
| A | 54.4 | 3.05 | 5.61 |
| B | 112.7 | 6.13 | 5.43 |
| C | 213.5 | 10.40 | 4.87 |

Inter Assay: Three pooled area sera were assayed in duplicate in four days.

| Serum Samples | Mean (U/mL) | Intra-assay | |
|---------------|-------------|-------------|-------|
| | | S.D. | CV% |
| A | 53.8 | 6.29 | 11.65 |
| B | 116.8 | 8.23 | 7.06 |
| C | 226.4 | 23.38 | 10.33 |

Accuracy

A serum containing 333 U/mL was diluted with a series of CA242 free serum. The dilutions were tested and the CA242 recoveries were compared with the expected concentrations.

| Sample Dilutions | CA242 level Expected (U/mL) | CA242 level Measured | (U/mL) Recovery |
|------------------|-----------------------------|----------------------|-----------------|
| Undiluted | 333.0 | | |
| 1:1/5 | 266.5 | 287.0 | 107.7 |
| 1:1/4 | 249.8 | 244.4 | 97.8 |
| 1:1/3 | 222.0 | 203.0 | 91.4 |
| 1:1 | 166.5 | 165.5 | 99.3 |
| 1:2 | 111.0 | 112.2 | 101.1 |
| 1:4 | 83.0 | 86.3 | 104.0 |

Known CA242 samples were spiked with different concentrations of CA242 with same volume. Samples were then tested and the CA242 recoveries compared with the expected concentrations as illustrated:

| CA242 U/mL | CA242 Spiked (U/mL) | Expected Value (U/mL) | Measured Value (U/mL) | Recovery % |
|------------|---------------------|-----------------------|-----------------------|------------|
| 5.0 | 25.0 | 15.0 | 14.3 | 95.3 |
| 5.0 | 85.0 | 45.0 | 45.8 | 101.8 |
| 5.0 | 158.0 | 81.5 | 84.7 | 103.9 |
| 25.0 | 85.0 | 55.0 | 56.5 | 102.7 |
| 25.0 | 158.0 | 91.5 | 97.3 | 106.3 |
| 100.0 | 158.0 | 129.0 | 117.3 | 90.9 |

Specificity

The CA242 ELISA assay only recognizes the CA 242 antigens. The following compounds were tested for cross reactivity of the assay. The cross reactivity to other compounds which might be present in patient samples is not detected at the concentrations given below. Cross reactivity were not found at the concentrations stated with PSA (120 ng/mL), PAP (60 ng/mL), CEA (18248 ng/mL), AFP (10,000 ng/mL), CA125 (1000/ U/mL).

However Crude Antigen CA153 and CA 199 will react with CA 242 in this test.

MINIMAL DETECTABLE CONCENTRATION

The detectable limit of CA242 ELISA assay is 1 U/mL. The minimal detectable concentration of CA242 is defined as that of CA 242 which corresponds to the absorbance that is two standard deviation from the mean absorbance of 10 replicate determination of the sample diluent (0/mL)/

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