hCEA [I-125] IRMA KIT

(REF: RK-38CT)

The ^{125}I-hCEA IRMA system provides a direct *in vitro* quantitative determination of human carcinoembryonic antigen (hCEA) in human serum in the range of 0-180 ng/mL. Each kit contains materials sufficient for 100 assay tubes permitting the construction of one standard curve and the assay of 41 unknowns in duplicate.

Introduction

Carcinoembryonic antigen (CEA) is a cell-surface glycoprotein with a molecular weight of 180-200kD, that occurs in high levels in colon epithelial cells during embryonic development. Levels of CEA are significantly lower in colon tissue of adults, but can become elevated when inflammation or tumours arise in any endodermal tissue, including in the gastrointestinal tract, respiratory tract, pancreas and breast.

An overexpression of CEA protein has been detected in a variety of adenocarcinomas, including gastric, pancreatic, small intestine, colon, rectal, ovarian, breast, cervical and non-small-cell lung cancers. CEA is also expressed by epithelial cells in several non-malignant disorders, including diverticulitis, pancreatitis, inflammatory bowel disease, cirrhosis, hepatitis, bronchitis and renal failure and also in heavy smokers.

Therefore CEA should not be regarded as a tumour-specific marker for the screening of general population for undetected cancers. However, the determination of CEA levels provides important information about patient prognosis, recurrence of tumours after surgical removal and effectiveness of therapy.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.

The ^{125}I labelled signal-antibody binds to an epitope of the CEA molecule spatially different from that recognised by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a **capture antibody - antigen - signal antibody** complex, also referred to as a "sandwich".

During a 1-hour incubation period with shaking the immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes are washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of hCEA, the unknown concentration of hCEA in patient samples can be determined.

Contents of the kit

1. 1 bottle of TRACER (21 mL), ready to use, containing about 740 kBq ^{125}I-anti-hCEA and capture anti-hCEA in buffer with red dye and 0.1 % NaN_3.

2. 7 vials of STANDARDS (7 x 1mL), ready to use, containing appr. 0, 1.6*, 3, 10, 30, 90, 180 ng/mL hCEA (WHO 1st IS 73/601 Int.Std.) in human serum with 0.1% NaN_3.

(*for exact concentration see on the label)

3. 2 vials of CONTROL SERA (2 x 1 mL), low (CI) and high (CII). Human sera with 0.1% NaN_3. Ready to use. The concentrations of the control sera are specified in the quality certificate enclosed.

4. 1 vial of SAMPLE DILUENT (5 mL), ready to use, prepared in equine serum.

5. 2 boxes of COATED TUBES, ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

6. 1 bottle of WASH BUFFER CONCENTRATE (20 mL), containing 0.2% NaN_3. See *Preparation of reagents.*

Quality certificate, Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (50, 200 and 2000 µL), distilled water, vortex mixer, shaker, plastic foil, adsorbent tissue, gamma counter.

Recommended tools and equipment

Repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-mL pipette).

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Hemolyzed and lipemic specimens may give false values and should be avoided.

Samples with a CEA concentration higher than 180 ng/mL should be diluted with the Diluent (D) and re-assayed. Recommended dilution: 10-fold (450 µL D + 50 µL sample).

Preparation of reagents, storage

Store the reagents between 2-8°C after opening. At this temperature each reagent is stable until the expiration date of the kit. The actual expiration date is given on the package label and in the quality certificate.

Add the wash buffer concentrate (20 mL) to 700 mL distilled water to obtain 720 mL wash solution. Upon dilution store at 2-8°C until the expiration date of the kit.

CAUTION!

Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide , refer to Table 1.)

1. Equilibrate reagents and samples to room temperature before use.
2. Label coated tubes in duplicate for each standard, control sera and samples. Optionally, label two test tubes for total count (T).
3. Homogenize all reagents and samples by gentle mixing to avoid foaming.
4. Pipette 50 µl of standards, controls and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
5. Pipette 200 µl of tracer into each tube.
6. Seal all tubes with a plastic foil. Fix the test tube rack firmLy onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube (min. 600 rpm).
7. Incubate tubes for 1 hour, shaking at room temperature.
8. Add 2.0 mL of diluted wash buffer to each tube. Decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
9. Return the tube-rack to an upright position and repeat step-8 one more time.
10. Count each tube for at least 60 seconds in a gamma counter.
11. Calculate the CEA concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		**50**		
Control			**50**	
Sample				**50**
Tracer	**200**	**200**	**200**	**200**
Shake for 1 hour at room temperature				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (CPM) for each pair of assay tubes.

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{1\text{-}6} / C_{I\text{-}II} / M_x \text{ (cpm)} - S_0 \text{ (cpm)}}{T\text{(cpm)}} \times 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of CEA.

Determine the CEA concentration of the unknown samples by interpolation from the

standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Automated data processing systems are also available.

Table 2. Typical assay data

Tubes	hCEA ng/mL	Count cpm	Mean cpm	B/T %
T		307879 310574	309211	
S0	0	81 88	85	0.027
S1	1. 6	1900 1898	1899	0.59
S2	3	3461 3387	3424	1.08
S3	10	10679 10443	10712	3.44
S4	30	33306 33520	33413	10.78
S5	90	87390 89529	88460	28.58
S6	180	143200 143211	143205	46.28

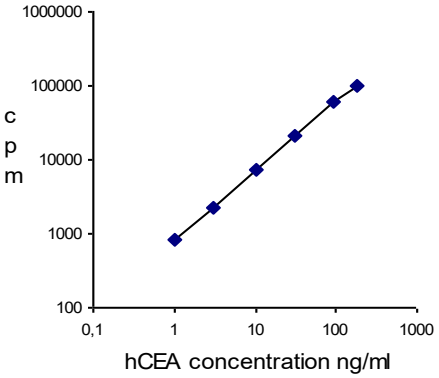

Figure 1: A typical standard curve
(Do not use to calculate unknown samples)

Characterization of assay

Specificity

No cross reactivity with NCA can be detected in normal physiological levels.

Sensitivity

The analytical sensitivity or minimum detectable limit is calculated by the interpolation of the mean counts of zero standard plus 2 standard deviation from the standard curve. Determination was carried out using 15 replicates of zero standard response.
The value of analytical sensitivity is 0.01 ng/mL measured using new tracer and 0.05 ng/mL measured using 4-week old tracer.
The functional sensitivity is a measure of the hCEA concentration that is significantly different from zero as determined by the inter-assay precision profile (22 % CV).
The value of functional sensitivity is: < 0.4 ng/mL.

Based on 120 determinations, with 60 blank and 60 low-level samples and with 95% probability, measurement limits are:
Limit of Blank (LoB): 0.035 ng/mL
Limit of Detection (LoD): 0.09 ng/mL

Precision

5 control pool samples were assayed in 10 replicates to determine intra-assay precision. Values obtained are shown below:

Sample	Replicates	Mean ng/mL	SD ng/mL	CV %
1	10	3.12	0.12	4.0
2	10	5.01	0.05	3.1
3	10	34.16	0.41	1.2
4	10	52.20	1.36	2.6
5	10	80.06	1.12	1.4

Reproducibility

To determine inter-assay precision 5 control sample pools were measured in duplicates in 15 independent assays by 3 operators using different kit batches. Values obtained are shown below:

Sample	Replicates	Mean ng/mL	SD ng/mL	CV %
1	15	0.40	0.04	9.52
2	15	3.01	0.18	6.00
3	15	11.13	0.59	5.29
4	15	22.30	0.97	4.37
5	15	47.66	1.89	3.98

Linearity – dilution test

Individual human serum samples were diluted with the sample diluent of the KIT. The diluted samples were measured according to KIT protocol.
The recovery results were in the 97.6 – 108% range.

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of hCEA. The average per cent recover for 5 serum pooles spiked with hCEA at 3 levels was: 99.4 ± 5.2%, with a range of 90% to 108%.

Expected Values

In 95% of healthy subjects, CEA levels are usually < 3.0 ng/mL.
For individuals who smoke normal CEA levels are usually < 5.0 ng/mL.
It is recommended that each laboratory determine a reference range for its own patient population.
The results obtained should only be interpreted in the context of the overall clinical picture. None of the in vitro diagnostic kits can be used as the one and only proof of any disease or disorder.

Hook effect

There is no high dose hook effect up to 15000 ng/mL.

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

Limitations

The reagents supplied in this kit are optimized to measure hCEA levels in serum.

Avoid freezing and thawing of reagents and specimens.

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precautions

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials.*
All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials.*
Bovine components originate from countries where bovine spongiform encephalopathy has not been reported. Nevertheless, components containing animal substances should be treated as *potentially infectious materials.*

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 74 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 60 days from availability.

	Use by	CONTROL	Control
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	WASHB	Wash buffer
IVD	In vitro diagnostic medical device	DIL	Serum diluent
	Manufacturer	8°C 2	Store between 2-8ºC
REF	Catalogue number		Radioactive material

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Updated: October/2021