FOR INFORMATION ONLY.
WHEN PERFORMING
THE ASSAY ALWAYS REFER
TO PACKAGE INSERT
SUPPLIED
WITH THE KIT

CanAg CEA EIA

REF 401-10

IVD

CE

Instructions for use. 2013-06

EN	**EXPLANATION OF SYMBOLS**
BG	**ОБЯСНЕНИЕ НА СИМВОЛИТЕ**
CS	**VÝZNAM SYMBOLŮ**
DA	**SYMBOLFORKLARING**
DE	**ERKLÄRUNG DER SYMBOLE**
EL	ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
ES	**SIGNIFICADO DE LOS SÍMBOLOS**
ET	**SÜMBOLITE SELGITUS**
FR	**EXPLICATION DES SYMBOLES**
HR	**OBJAŠNJENJE SIMBOLA**
HU	**JELMAGYARÁZAT**
IT	**SPIEGAZIONE DEI SIMBOLI**
LT	SIMBOLIŲ PAAIŠKINIMAI
LV	**SIMBOLU SKAIDROJUMS**
NL	**VERKLARING DER SYMBOLEN**
NO	**SYMBOLFORKLARING**
PL	OBJAŚNIENIE SYMBOLI
PT	**EXPLICAÇÃO DOS SÍMBOLOS**
RO	SEMNIFICAŢIA SIMBOLURILOR
RU	ОБОНАЧЕНИЯ
SV	**SYMBOLFÖRKLARING**
SK	**VÝZNAM SYMBOLOV**
SL	**RAZLAGA SIMBOLOV**
SR	**OBJAŠNJENJE SIMBOLA**
TR	SEMBOLLERİN AÇIKLAMALARI

Use By/Годно до/Použitelné do/ Holdbar til/Verwendbar bis/ Ημερομηνία λήξης/Fecha de caducidad/Kõlblik kuni/ Utiliser jusque/Rok valjanosti/ Felhasználható/Utilizare entro/ Sunaudoti iki/Izlietot līdz/Houdbaar tot/Brukes innen/Użyć przed/ Prazo de validade/Expiră la/ Использовать до/Använd före/ Použite´né do/ Uporabno do/ Upotrebljivo do/Son Kullanma Tarihi

LOT

Batch code/Номер на партида/ Číslo šarže/Lotnummer/ Chargenbezeichnung/Αριθμός Παρτίδας/Código de lote/Partii kood/Code du lot/Kod serije/ Sarzsszám/Codice del lotto/ Partijos kodas/Partijas kods/Lot nummer/Partikode/Kod partii/ Código do lote/Număr de lot/ Номер лота/Lotnummer/Číslo šarže/Številka serije/Kod partije/ Parti Kodu

Date of manufacture/Дата на производство/Datum výroby/ Produktionsdato/Herstellungsdatum/ Ημερομηνία παραγωγής/Fecha de fabricación/Valmistamise kuupäev/ Date de fabrication/Datum proizvodnje/ Gyártási idő/Data di produzione/ Pagaminimo data/Ražošanas datums/ Productiedatum/Fremstillingsdato/ Data produkcji/Data de fabrico/Data fabricaţiei/Дата производства/ Tillverkningsdatum/Dátum výroby/Datum izdelave/Datum proizvodnje/Üretim tarihi

Temperature limitation/ Температурни граници/ Teplotní omezení/ Temperaturbegrænsning/ Temperaturbegrenzung/ Περιορισμοί θερμοκρασίας/ Límites de temperatura/ Temperatuuri piirang/ Limite de température/ Temperaturno ograničenje/ Hőmérsékletre vonatkozó korlátozás/ Limiti di temperatura/ Temperatūriniai apribojimai/ Temperatūras ierobežojums/ Temperatuurbeperking/ Temperaturbegrensninger/ Temperatury graniczne/ Limite de temperatura/ Limite de temperatură/ Температурный режим/ Temperaturbegränsning/ Teplotné obmedzenie Omejitev temperature/ Temperaturno ograničenje/ Sıcaklık sınırlaması/

IVD

In Vitro Diagnostic Medical Device/ Медицински уред за диагностика ин витро/Diagnostický zdravotnický prostředek in vitro/Medicinsk udstyr til in vitro-diagnostik/In-vitro-Diagnostikum/ Ιατροτεχνολογικό προϊόν για διάγνωση In Vitro/Dispositivo médico para diagnóstico in vitro/In vitro diagnostiline meditsiiniseade/Dispositif médical de diagnostic in vitro/Diagnostički medicinski uređaj In Vitro/In vitro orvosdiagnosztikai eszköz/Dispositivo medico per test diagnostici in vitro/In Vitro Diagnostinė Medicinos Priemonė/Medicīniska ierīce in vitro diagnostikai/In vitro-diagnostisch medisch instrument/In vitro diagnostisk medisinsk utstyr/Wyrób medyczny do diagnostyki in vitro/Dispositivo Médico de Diagnóstico In Vitro/Dispozitiv medical pentru diagnostic in vitro/Только для диагностики In Vitro/Endast för in vitro-diagnostik/ Zdravotnícka pomôcka na diagnostiku in vitro/In vitro diagnostični pripomoček/Diagnostički medicinski uređaj In Vitro/<96> testleri için yeterlilik içerir

Contains sufficient for <96> tests/Съдържа достатъчно количество за тестове <96>/Lze použít pro <96> testů/Indeholder tilstrækkeligt/Inhalt ausreichend für <96> Prüfungen/Περιεχόμενο επαρκές για «96» εξετάσεις/Contenido suficiente para <96> ensayos/Kogusest piisab <96> testi läbiviimiseks/Contenu suffisant pour "96" tests/Sadrži dovoljno za <96> testova/A doboz tartalma <96> vizsgálat elvégzéséhez elegendő/Contenuto sufficiente per "96" saggi/Turinys skirtas atlikti <96> tyrimus/ Saturs pietiekams <96> testiem/Inhoud voldoende voor "96" testen/til "96" test/ Tilstrekkelig innhold for <96> prøver/ Wystarczy na wykonanie <96> testów/ Conteúdo suficiente para "96" ensaios/ Conţinut suficient pentru 96 de teste/ Содержит достаточные количества для «96» определений/Innehåller tillräckligt till "96" antal tester/Obsah postačuje na tento počet testov: <96>/Vsebina zadostuje za <96> testov/Sadržina dovoljna za <96> testova/<96> testleri için yeterlilik içerir

REF

Catalogue number/Каталожен номер/ Katalogové číslo/Katalognummer/ Bestellnummer/Αριθμός καταλόγου/ Número de catálogo/Kataloogi number/ Numéro de catalogue/Kataloški broj/ Katalógusszám/Numero di catalogo/ Katalogo numeris/Numurs katalogā/ Katalogusnummer/Katalognummer/ Numer katalogowy/Número do catálogo/ Număr de catalog/Номер по каталогу/ Produktnummer/Katalógové číslo/ Katološka številka/Kataloški broj/ Katalog numarası

Consult Instructions for Use/ Прочетете инструкцията за употреба/Konzultujte s návodem k použití/Se brugsanvisning/Siehe Gebrauchsanweisung/Συμβουλευτείτε τις Οδηγίες σχετικά με τη χρήση/ Consulte las instrucciones de uso/ Vt kasutusjuhendit/Consulter le mode d'emploi/Pročitajte upute za uporabu/ Olvassa el a használati utasítást/ Consultare le istruzioni per l'uso/Dėl naudojimo žiūrėkite instrukcijas/Izlasiet lietošanas instrukciju/Raadpleeg de instructies voor gebruik/Les instruksene før bruk/Sprawdzić w instrukcji użycia/ Consulte as Instruções de Utilização/ Consultaţi instrucţiunile de utilizare/ Обратитесь к инструкции по применению/Se bruksanvisning/ Prečítajte si návod na používanie/ Pročitajte uputstvo za upotrebu/ Kullanım Talimatlarına Bakınız

CONT

Contents of kit/Съдържание на набора/ Obsah soupravy/Kittets indhold/Inhalt des Kits/Περιεχόμενα του κιτ/Contenido del kit/Komplekt sisaldab/Contenu du kit/Sadržaj opreme/A készlet tartalma/ Contenuto del kit/Rinkinio turinys/ Komplekta saturs/Inhoud van de set/ Settets innhold/Zawartość zestawu/ Conteúdo do kit/Conţinutul setului/ Компоненты набора/Kit innehåll/ Obsah súpravy/Vsebina kompleta/Sadržaj opreme/Kitin içindekiler

Biological risks/Биологическа опасност/Biologická rizika/Biologisk fare/Biologische Gefahren/Βιολογικοί κίνδυνοι/Riesgos biológicos/ Bioloogilised ohud/Risques biologiques/ Biološki rizici/Biológiai kockázatok/Rischi biologici/Biologinis pavojus/Bioloģiskais risks/Biologische risico's/Biologiske risikoer/Zagrożenie biologiczne/Riscos biológicos/ Biologisk risk/Pericole biologice/Биологическая опасность/ Biologicky rizikové/Biologické riziká/ Biološki rizici/Biyolojik riskler

ORIG | HUM

Human/С човешки произход/Lidské/ Humant/Human/Δείγματα αναφοράς/ Humano/Inimpäritolu/Humaine/Ljudskog porjekla/Humán/Origine Umana/ Žmogaus kilmės/Cilvēku izcelsmes/ Human/Menneske/Ludzka/Humano/ Origine umană/Человеческого происхождения/Human/Ľudské/ Humanega izvora/Ljudskog porekla/İnsan

ORIG | MOU

From mouse/С миши произход/Myší/ Fra mus/Maus/Από ποντίκι/de ratón/ Hiirtelt/De souris/Mišijeg porjekla/ Egérből/Murino/Pelės kilmės/No peles/ Van muizen/Fra mus/Mysia/Do rato/De la şoareci/Мышиного происхождения/ Från mus/Myšie/Mišjega izvora/Mišijeg porekla/Fareden

ORIG | BOV

Bovine/С говежди произход/ Hovězí/Bovin/Rind/από βοοειδή/ Bovino/Veistelt/Bovine/Rogate stoke/ Szarvasmarha/Bovino/Jaučio/No liellopa/Bovien/Bovin/Wołowy/Bovino/ Origine bovină/крупного рогатого скота/Från ko/Hovädzie/Govejega izvora/Rogate krupne stoke/Bovin

Reconstitute with/Разтваряне с/ Rozřeďte pomocí/Rekonstitueres med/ Rekonstituieren mit/Ανασύσταση με/ Reconstituir con/Lahjendamine/ Reconstituer avec/Rekonstituirajte s/ Feloldáshoz/Ricostituire con/Atkurti, ištirpdant su/Atšķaidīt ar/Reconstitutie met/Rekonstitueres med/Odtworzyć za pomocą/Reconstituir com/A se reconstitui cu/Растворить в/ Rekonstituera med/Rozrieďte pomocou/ Rekonstituirajte z/s/Ponovno formiranje sa/Yeniden oluşturulur

Manufacturer/Производител/Výrobce/ Producent/Hersteller/Κατασκευαστής/ Fabricante/Tootja/Fabricant/Proizvođač/ Gyártó/Fabbricante/Gamintojas/ Ražotājs/Fabrikant/Produsent/ Producent/Fabricante/Producător/ Производитель/Tillverkare/ Výrobca/ Izdelovalec/Proizvođač/Üretici

EN

CanAg CEA EIA

Instructions for use

Enzyme immunometric assay kit
For 96 determinations

INTENDED USE

The CanAg CEA EIA kit is intended for the quantitative determination of the cancer associated antigen CEA in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

Carcinoembryonic antigen (CEA) is a glycoprotein, which was first identified in patients with colonic carcinoma and in epithelial tumours of endodermal origin (gastrointestinal tract) by Gold and Freedman (1). The CEA molecule is quite heterogeneous due to the carbohydrate contents (50-60 %) and depending on the purification procedure employed. It is soluble in perchloric acid and has a molecular weight of about 175.000–200.000 Daltons (2). Immunological and genetic characterization of CEA has identified a family of CEA-like molecules sharing common antigenic determinants. The most relevant CEA-like molecule is NCA (non-specific cross-reacting antigen) synthesized both by normal and pathological tissues. The problem of cross-reacting CEA-like molecules when assaying CEA is possible to overcome by the use of monoclonal antibodies. The CanAg CEA EIA is based on two mouse monoclonal antibodies against the Gold epitopes IV and V (3, 4).

CEA is secreted from tumour cells and is a widely used serological marker of gastrointestinal carcinomas, lung cancer and breast cancer. In colorectal cancer, the clinical use of CEA testing for monitoring response to therapy and for documenting progressive disease is well established (5, 6). CEA may also be present in benign gastrointestinal inflammatory diseases or in hepatobiliary diseases. These observations make it necessary to emphasize that the CEA assay should not be used as a cancer-screening test.

PRINCIPLE OF THE TEST

The CanAg CEA EIA is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique. Calibrators, controls and patient samples are incubated together with biotinylated Anti-CEA monoclonal antibody and horseradish peroxidase (HRP) labelled Anti-CEA monoclonal antibody in Streptavidin coated microstrips. After washing, buffered Substrate/ Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetra-methylbenzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue colour will develop if antigen is present.

The intensity of the colour is proportional to the amount of CEA present in the samples.

The colour intensity is determined in a microplate spectrophotometer at 620 nm (or optionally at 405 nm after addition of Stop Solution). Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The CEA concentrations of patient samples are then read from the calibration curve.

REAGENTS

- Each CanAg CEA EIA kit contains reagents for 96 tests.
- The expiry date of the kit is stated on the label on the outside of the kit box.
- Do not use the kit beyond the expiry date.
- Do not mix reagents from different kit lots.
- Store the kit at 2–8° C. Do not freeze.
- Opened reagents are stable according to the table below provided they are not contaminated, stored in resealed original containers and handled as prescribed. Return to 2-8° C immediately after use.

Component	Quantity	Storage and stability after first opening
MICROPLA		
Microplate	1 Plate	2–8° C until expiry date stated on the plate

12 x 8 wells coated with Streptavidin. After opening, immediately return unused strips to the aluminium pouch, containing desiccant. Reseal carefully to keep dry.

CEA Calibrators			6 vials	2–8° C until expiry date stated on the vials
CAL	CEA	0	0 µg/L 1 x 8 mL	
CAL	CEA	2	2 µg/L 1 x 0.75 mL	
CAL	CEA	5	5 µg/L 1 x 0.75 mL	
CAL	CEA	15	15 µg/L 1 x 0.75 mL	
CAL	CEA	50	50 µg/L 1 x 0.75 mL	
CAL	CEA	75	75 µg/L 1 x 0.75 mL	

Human CEA in a Tris-HCl buffered salt solution containing bovine serum albumin, an inert yellow dye and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use. CAL | CEA | 0 should also be used for dilution of samples.

Component	Quantity	Storage and stability after first opening
CEA Controls	2 vials	2–8° C until expiry date stated on the vials
CONTROL | CEA | 1	1 x 0.75 mL	
CONTROL | CEA | 2	1 x 0.75 mL	

Human CEA in a Tris-HCl buffered salt solution containing bovine serum albumin, and 0.01 % methyl-isothiazolone (MIT) as preservative. Ready for use.

BIOTIN | Anti-CEA		
Biotin Anti-CEA	1 x 15 mL	2–8° C until expiry date stated on the vial

Biotin Anti-CEA monoclonal antibody from mouse, approximately 3 µg/mL. Contains phosphate buffered saline (pH 7.2), bovine serum albumin, bovine immunoglobulin, blocking agents, Tween 20, an inert blue dye and 0.01 % methyl-isothiazolone (MIT) as preservative. To be mixed with Tracer, HRP Anti-CEA before use.

CONJ | Anti-CEA		
Tracer, HRP Anti-CEA	1 x 0.75 mL	2–8° C until expiry date stated on the vial

Stock solution of HRP Anti-CEA monoclonal antibody from mouse, approximately 60 µg/mL. Contains preservatives. To be mixed with Biotin Anti-CEA before use.

SUBS | TMB		
TMB HRP-Substrate	1 x 12 mL	2–8° C until expiry date stated on the vial

Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetramethyl-benzidine (TMB). Ready for use.

Component	Quantity	Storage and stability after first opening
STOP		
STOP Solution	1 x 15 mL	2–8° C until expiry date stated on the vial
Contains 0.12 M hydrochloric acid. Ready for use.		
WASHBUF | 25X		
Wash Concentrate	1 x 50 mL	2–8° C until expiry date stated on the bottle
A Tris-HCl buffered salt solution with Tween 20. Contains Germall II as preservative. To be diluted with water 25 times before use.		

Indications of instability

The TMB HRP-Substrate should be colourless or slightly bluish. A blue colour indicates that the reagent has been contaminated and should be discarded.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use.

- For professional use only.
- Please refer to the US Department of Health and Human Services (Bethesda, Md., US) publication No. (CDC) 88-8395 on laboratory safety or any other local or national regulation.
- Handle all patient specimens as potentially infectious.
- Follow local guidelines for disposal of all waste material.

Caution

Material used in the preparation of human source reagent has been tested and found to be Non Reactive for HIV-1/2 Antibody, HCV Antibody and Hepatitis B Surface Antigen (HBsAg). Since no method can completely rule out the presence of blood borne diseases, the handling and disposal of human source reagents from this product should be made as if they were potentially infectious.

SPECIMEN COLLECTION AND HANDLING

The CanAg CEA EIA is intended for use with serum. Collect blood by venipuncture and separate the serum according to common procedures. Samples can be stored at 2–8° C for 2 days. For longer periods it is recommended to store the samples at –20° C or below. Avoid repeated freezing and thawing of the samples. Allow frozen samples to thaw slowly, preferably at 2–8° C over night and then bring the samples to room temperature before analysis.

PROCEDURE

Materials required but not supplied with the kit

1. **Microplate shaker**
   Shaking should be medium to vigorous, approximately 700-1100 oscillations/min.
2. **Microplate wash device**
   Automatic plate washer capable of performing 1 and 6 washing cycles with a minimal fill volume of 350 µL/well/washcycle.
   An 8-channel pipette with disposable plastic tips for delivery of 350 µL is recommended if an automatic microplate washer is not used.
3. **Microplate spectrophotometer**
   With a wavelength of 620 nm and/or 405 nm and an absorbance range of 0 to 3.0.
4. **Precision pipettes**
   With disposable plastic tips to deliver microlitre and millilitre volumes. An 8-channel pipette or respenser pipette with disposable plastic tips for delivery of 100 µL is useful but not essential.
5. **Distilled or deionized water**
   For preparation of Wash Solution.

Procedural notes

1. A thorough understanding of this package insert is necessary to ensure proper use of the CanAg CEA EIA kit. The reagents supplied with the kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use the kit reagents after the expiry date printed on the outside of the kit box.
2. Reagents should be allowed to reach room temperature (20–25° C) prior to use. The assay should only be performed at temperatures between 20–25° C to obtain accurate results. Frozen specimens should be brought to room temperature slowly and must be gently but thoroughly mixed after thawing.

3. Before starting to pipette calibrators, controls and patient specimens it is advisable to mark the strips to be able to clearly identify the samples during and after the assay.
4. The requirement for efficient and thorough washing for separation of bound and unbound antigen and reagents from the solid-phase bound antibody-antigen complexes is one of the most important steps in an EIA. In order to ensure efficient washing make sure that all wells are completely filled to the top edge with wash solution during each wash cycle, that wash solution is dispensed at a good flow rate, that the aspiration of the wells between and after the wash cycles is complete and that the wells are empty. If there is liquid left, invert the plate and tap it carefully against absorbent paper.
   - Automatic strip washer: Follow the manufacturer's instructions for cleaning and maintenance diligently and wash the required number of wash cycles prior to and after each incubation step. It's highly recommended to use *strip* process mode and *overflow* wash mode with a dispensing volume of 800 µL. The aspiration/wash device should not be left standing with the Wash Solution for long periods, as the needles may get clogged resulting in poor liquid delivery and aspiration.
5. The TMB HRP-Substrate is very sensitive for contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial to a carefully cleaned reservoir or preferably a disposable plastic tray to avoid contamination of the reagent. Be sure to use clean disposable plastic pipette tips (or respenser pipette tip).
6. Be sure to use clean disposable plastic pipette tips and a proper pipetting technique when handling samples and reagents. Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or surface of the liquid. A proper pipetting technique is of particular importance when handling the TMB HRP-Substrate Solution.

Protocol Sheet

CanAg CEA EIA REF 401-10

Mix the components directly before use. Use shaking conditions according to the Instructions.

Step		Vial/Plate	Procedure
1.	Prepare Wash Solution	WASHBUF 25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled water or deionized water.
	Prepare Antibody Solution	CONJ Anti-CEA BIOTIN Anti-CEA	Mix 50 µL of Tracer, HRP Anti-CEA, with 1 mL of Biotin Anti-CEA per strip:

No. of Strips	Tracer, HRP Anti-CEA (µL)	Biotin Anti-CEA (mL)
1	50	1
2	100	2
3	150	3
4	200	4
5	250	5
6	300	6
7	350	7
8	400	8
9	450	9
10	500	10
11	550	11

		10	500	10
		11	550	11
		12	600	12
2.	Wash	MICROPLA	Wash each well once with wash solution
3.	Add calibrators, controls and samples	CAL CEA 0, 2, 5, 15, 50, 75 CONTROL CEA 1, 2	25 µL in each well
4.	Add Antibody Solution	ANTIBODY SOLUTION	100 µL in each well
5.	Incubate	MICROPLA	1 hour shaking at room temperature
6.	Wash	MICROPLA	Wash each well six times with wash solution
7.	Add TMB HRP-Substrate	SUBS TMB	100 µL in each well
8.	Incubate	MICROPLA	30 min shaking at room temperature
9.	Read absorbance	MICROPLA	620 nm
Alt. 9	Add Stop Solution	STOP	100 µL in each well
Alt. 10	Incubate	MICROPLA	1 min shaking at room temperature
Alt. 11	Read absorbance	MICROPLA	Read at 405 nm within 15 min

Preparation of reagents	Stability of prepared reagent
Wash Solution	2 weeks at 2–25° C in a sealed container

Pour the 50 mL Wash Concentrate into a clean container and dilute 25- fold by adding 1200 mL of distilled or deionized water to give a buffered Wash Solution.

Antibody Solution	3 weeks at 2–8° C

Prepare the required quantity of Antibody Solution by mixing 50 µL of Tracer, HRP Anti-CEA with 1 mL of Biotin Anti-CEA per strip (see table below and the Protocol Sheet).

No. of Strips	Tracer, HRP Anti-CEA (µL)	Biotin Anti-CEA (mL)
1	50	1
2	100	2
3	150	3
4	200	4
5	250	5
6	300	6
7	350	7
8	400	8
9	450	9
10	500	10
11	550	11
12	600	12

Be sure to use a clean plastic or glass bottle for preparation of the Antibody Solution.

Alternative: Pour the content of the Tracer, HRP Anti-CEA into the vial of Biotin Anti-CEA and mix gently. Make sure that all of the Tracer, HRP Anti-CEA is transferred to the vial of Biotin Anti-CEA.

NOTE: The Antibody Solution is stable for 3 weeks at 2–8° C. Do not prepare more Antibody Solution than will be used within this period and make sure that it is stored properly.

Assay procedure

Perform each determination in duplicate for calibrators, controls and patient samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20–25° C) before use.

1. Start to prepare Wash Solution and Antibody Solution. It is important to use clean containers. Follow the instructions carefully.

2. Transfer the required number of microplate strips to a strip frame. (Immediately return the remaining strips to the aluminium pouch containing a desiccant and reseal carefully). Wash each strip once with the Wash Solution. Do not wash more strips than can be handled within 30 min.

3. Pipette 25 µL of the CEA Calibrators (CAL 0, 2, 5, 15, 50, 75), controls (c) and patient samples (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal 0	Cal 50	Unk 1				
B	Cal 0	Cal 50	Unk 1				
C	Cal 2	Cal 75	Unk 2				
D	Cal 2	Cal 75	Unk 2				
E	Cal 5	C1	etc.				
F	Cal 5	C1					
G	Cal 15	C2					
H	Cal 15	C2					

4. Add 100 µL of Antibody Solution to each well using a 100 µL precision pipette (or an 8-channel 100 µL precision pipette). Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or the surface of the liquid.

5. Incubate the frame containing the strips for 1 hour (± 5 min) at room temperature (20–25° C) with constant shaking of the plate using a microplate shaker.

6. Wash each strip 6 times, using the wash procedure described in Procedural notes item 4.

7. Add 100 µL of TMB HRP-Substrate to each well using the same pipetting procedure as in item 4. The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between the addition to the first and last well should not exceed 5 min.

8. Incubate for 30 min (± 5 min) at room temperature with constant shaking. Avoid direct sunlight.

9. Immediately read the absorbance at 620 nm in a microplate spectrophotometer.

Option

If the laboratory does not have access to a microplate spectrophotometer capable of reading at 620 nm, the absorbance can be determined as follows:

Alt. 9. Add 100 µL of Stop Solution. Mix and read the absorbance at 405 nm in a microplate spectrophotometer within 15 minutes after addition of Stop Solution.

Measurement range

The CanAg CEA EIA measures concentrations between 0.25 and 75 µg/L. If CEA concentrations above the measuring range are to be expected, it is recommended to dilute samples with CEA Calibrator 0 prior to analysis.

Quality control

CEA Control 1 and 2 may be used for validation of the assay series. Ranges of expected results are indicated on the vial labels. If values outside of the specified range are obtained, a complete check of reagents and reader performance should be made and the analysis repeated. It is recommended that each laboratory in addition prepare its own serum pools at different levels, which can be used as internal controls in order to assure the accuracy of the assay.

Reference material

The 1st International Reference Preparation IRP 73/601 may be used as a reference standard. Values for CEA Calibrators and Controls were assigned against a set of in-house reference standards whose values are traceable to IRP 73/601 using the conversion factor 13.5, i.e. 1µg/L corresponds to 13.5 IU/L.

CALCULATION OF RESULTS

If a microplate spectrophotometer reader with built-in data calculation program is used, refer to the manual for the plate reader and create a program using the concentration stated on the labels of each of the CEA Calibrators.

For automatic calculation of CEA results it is recommended to use either of the following methods:

- Cubic spline curve fit method. Calibrator 0 should be included in the curve with the value 0 µg/L.
- Spline smoothed curve fit method. Calibrator 0 should be used as plate blank.

- Interpolation with point-to-point evaluation. Calibrator 0 should be included in the curve with the value 0 µg/L.
- Quadratic curve fit method. Calibrator 0 should be included in the curve with the value 0 µg/L.

NOTE: 4-parametric or linear regression should not be used.

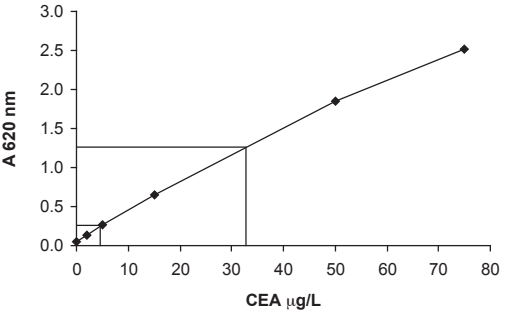
For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each CEA calibrator against the corresponding CEA concentration (in µg/L), see figure below. The unknown CEA concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen.

If samples in an initial analysis give CEA levels higher than 75 µg/L the samples should be diluted 1/10 and 1/100 with CEA calibrator 0 and reanalyzed to obtain the accurate CEA concentration.
1 : 10 dilution = 50 µL of specimen + 450 µL of CEA 0 µg/L
1 : 100 dilution = 50 µL of 1:10 dilution + 450 µL of CEA 0 µg/L

The CEA concentration of the undiluted sample is then obtained as follows:
Dilution 1/10: 10 x Measured value
Dilution 1/100: 100 x Measured value

Example of results

Specimen	Calibrator values	Mean abs value (A)	CEA (µg/L)
CAL CEA 0	0 µg/L	0.050	
CAL CEA 2	2 µg/L	0.131	
CAL CEA 5	5 µg/L	0.259	
CAL CEA 15	15 µg/L	0.657	
CAL CEA 50	50 µg/L	1.857	
CAL CEA 75	75 µg/L	2.519	
Specimen A		0.220	4.1
Specimen B		1.290	32.3



Example (do not use this curve or table above to determine actual assay results).

LIMITATIONS OF THE PROCEDURE

The level of CEA cannot be used as absolute evidence for the presence or absence of malignant disease and the CEA test should not be used in cancer screening. The results of the test should be interpreted only in conjunction with other investigations and procedures in the diagnosis of disease and the management of patients and the CEA test should not replace any established clinical examination.

Anti-reagent antibodies (human anti-mouse antibody (HAMA) or heterophilic antibodies) in the patient sample may occasionally interfere with the assay, even though specific blocking agents are included in the buffer.

EXPECTED VALUES

CanAg CEA was measured in 95 healthy blood donors and in 117 healthy individuals between 60 and 64 years. The lower and upper extremes of the normal range were examined using IFCC recommended non-parametric statistical treatment. The reference interval contains the central 95 % fraction of the reference distribution. Reference limits may accordingly be estimated as the 2.5 % (lower) and 97.5 % (upper) fractiles. These limits cut off a fraction of 2.5 % of the values in each tail of the reference distribution. Non-parametric estimates:

	Mean (µg/L)	SD (µg/L)	Median (µg/L)	Range (µg/L)	Upper reference limit (Central 95 % fraction)
Healthy blood donors n=95	1.3	1.0	1.0	0.5–9.1	3.2 µg/L
Healthy individuals age 60-64, n=117	2.4	1.7	1.9	0.5–8.8	7.4 µg/L

96 % of the healthy subjects had assay values below 5 µg/L.

It is recommended that each laboratory establish their own normal range to account for such local environmental factors as diet, climate, living conditions, patient selection, etc. It should also be borne in mind that the individual patient's own baseline results provides the most important reference point for interpretation of marker results. Smoking may increase CEA levels in healthy individuals.

PERFORMANCE CHARACTERISTICS

Precision

Intermediate precision was calculated according to NCCLS guideline EP5-A (7) using four levels of frozen pooled human serum containing added CEA and two different CanAg CEA EIA reagent combinations. Each sample was randomly pipetted (n=2/analysis) and analysed twice each day over 20 days.

Sample	Replicates	Mean (µg/L)	Within-run SD (µg/L)	Within-run CV %	Between-day SD (µg/L)	Between-day CV %
CEA 1	80	2.78	0.07	2.5	0.08	2.7
CEA 2	80	5.97	0.15	2.6	0.11	1.8
CEA 3	80	20.8	0.44	2.1	0.36	1.7
CEA 4	80	57.3	1.57	2.7	0.87	1.5

Detection limit

The detection limit of the CanAg CEA EIA is $\leq$ 0.25 µg/L defined as the concentration corresponding to the mean of the absorbance values of the CEA calibrator 0 plus 2 standard deviations according to formula:

$$\frac{2 \times SD\ CAL\ 0}{OD\ CAL\ 2 - OD\ CAL\ 0} \times 2\ \mu g/L$$

Recovery

Spiked serum samples were prepared by adding human CEA antigen to normal serum samples. The recovery of the added antigen was in the range 90–115 %.

Hook effect

When reading absorbance at 405 nm, i.e. using the Optional assay procedure with addition of STOP solution, no hook effect has been noticed for samples containing up to 250 000 µg/L. When absorbance is read at 620 nm, extremely high samples may change the colour of the substrate from blue to greenish. This may lead to a falsely low absorbance that may fall within the calibration curve range and noticed as a hook. Such a hook effect at 620 nm has been noticed for samples containing more than 2000 µg/L.

In order to avoid reporting misleadingly low results due to apparent hook effect when absorbance is read at 620 nm it is recommended to use the Optional assay procedure and determine absorbance at 405 nm in patients analysed for the first time or in patients where very high CEA values may be expected.

Linearity

Patient samples were serially diluted with CEA Calibrator 0 and analysed. The obtained values were between 90–120 % of the expected values.

Specificity

The CanAg CEA EIA is based on two mouse monoclonal antibodies, the catching MAb 12-140-10 against Gold epitope IV and the detecting MAb 12-140-1 against Gold epitope V (4, 5). The NCCLS guideline EP7-P (8) was followed to determine possible sources of interference. The following substances and concentrations were tested and found not to interfere with the test.

	Concentration with no significant (± 10 %) interference
Lipemia (Intralipid®)	10 mg/mL
Bilirubin, unconjugated	0.6 mg/mL
Hemoglobin	5 mg/mL

Method comparison

The CanAg CEA EIA was compared to the Wallac Delfia CEA kit. Seventy-seven human serum samples ranging in values from 0-790 µg/L were measured and linear regression analyses of the results yielded:

CanAg CEA = 0.90 x Delfia CEA + 0.53 r =1.00

WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by Fujirebio Diagnostics AB may affect the results, in which event Fujirebio Diagnostics AB disclaims all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

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