en

Immunoassay

REF CMB1301 / CMB1302 / CMB1303 / CMB1304 / CMB1305

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

SCCA CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of SCCA in human serum.

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Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

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Introduction

Squamous cell carcinoma (SCC) is the second most common skin cancer. It is typically characterized by a red papule or plaque with a scaly or crusted surface [1], it may be suspected whenever a small, firm reddish-colored skin lesion, growth or bump appears on the skin, but it may also be a flat growth with a curly and crusted surface [2]. Most often these growths are located on the face, ears, neck, hands and/or arms, but they may occur on the lips, mouth, tongue, genitalia or other area.
Squamous cell carcinoma antigen (SCCA) is a glycoprotein with a molecular weight between 42 to 48 kDa [4]. It is a member of the serine protease inhibitor (serpin) family, is widely used as a serum marker in cancers of the uterine cervix, the head and neck, lung and esophagus. Total SCCA in the circulation comprises 2 nearly identical, approximately 45 kDa proteins, SCCA1 and SCCA2 [3]. They are co-expressed in normal and malignant squamous epithelium, but it is mainly the acidic isoform SCCA2 that is present in the circulation of cancer patients. In patients responding to initial therapy, an elevated SCCA2/SCCA1 mRNA ratio in the primary tumor predicted recurrence independent of clinical stage. The relative risk of developing a recurrence was 7.2 in patients with elevated vs. normal SCCA2/SCCA1 mRNA ratios. SCCA2/SCCA1 mRNA ratio in primary tumors could be useful for individual selection of treatment strategy for patients with head and neck cancer [5].

Measurement Principle

This assay is based upon the two-step sandwich method. In the first step, the sample and SCCA antibody coated microparticles are added. During the incubation, SCCA present in the sample binds to the antibodies coated microparticles. After the washing, in the second step, Enzyme Conjugate is added to the reaction mixture. During the incubation, a complex is generated among the microparticles, the SCCA within the sample and enzyme-linked antibodies by immunological reactions. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of SCCA in the sample.

Materials provided

1. Calibrators

6 vials each containing lyophilized calibrator A through F. The matrix is sodium citrate buffer containing bovine serum. Contains a selection of preservatives.
Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Invert the calibrator several times to mix it completely. Then allow the reconstituted material to stand for at least 30 minutes.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2

- Microparticles Solution

SCCA antibody coated microparticles in phosphate buffer solution (PBS) containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

HRP (horseradish peroxidase) labeled mouse monoclonal anti-SCCA in MES buffer containing bovine serum. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needle
7. Wash Buffer used in the washing procedure
8. Distilled or deionized water

Metrological Traceability of Calibrators

The measurand or analyte in the SCCA calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511. The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Conduct the assay away from bad ambient conditions. e. g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
9. Do not use reagents beyond the labeled expiry date.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended before loading it on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light. When stored as directed, all reagents are stable until the expiration date.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20 ℃, under which conditions the stability will be retained for 2 months. Avoid multiple freeze-thaw cycles, do not freeze-thaw more than 3 cycles.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot or red blood cells.
6. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
7. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
8. Avoid grossly hemolytic, lipemic or turbid samples.
9. Test the sample within 1 hour, for longer use samples should be capped and stored at 2-8 ℃ up to 24 hours. Or freeze the samples that need to be stored or transported at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
10. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
11. Notice that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
12. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
13. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample cups or tubes on the sample rack, 50 μL of samples for each test. But considering the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Move the sample to the set point
  - Load a reaction vessel into the process path
  - Aspirate and transfer sample into the reaction vessel
  - Add Microparticles Solution to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mix, incubate and wash the reaction mixture
  - Add Chemiluminescent Substrate
  - Measure chemiluminescent emission to determine the quantity of SCCA in the sample
  - Discard the used reaction vessel
  - Calculate the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrator information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

Measurement Results

The sample test results are determined automatically by the system software. The amount of SCCA in the samples is determined from the measured light production by means of the stored calibration data. Refer

to the Assay Analyzer's operation manual on reviewing sample results.

Control Procedure

The recommended control requirement for this assay is to purchase control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the acceptable ranges. When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. It is recommended that each laboratory establish its accepted range to ensure proper test performance.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Performance of this test has not been established with neonatal samples.
5. This assay was designed and validated for use with human serum or plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
6. This test measures concentrations within the range of 0.2-70ng/mL.

Biological Reference Interval

By testing serum samples from 156 individuals defined as normal by a clinician, results are as follow:
1.5 ng/mL (95^{th} percentile)
1.3-1.6 ng/mL (95% confidence interval of the percentile)
It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (ng/mL)	Within-run	Total
			%CV	%CV
1	80	1.49	6.38	8.00
2	80	2.93	7.08	8.46
3	80	18.63	5.41	7.60

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Limit of Blank: 0.2 ng/mL
Limit of Detection: 0.4 ng/mL
Limit of Quantitation: 0.8 ng/mL with a coefficient of variation of ≤ 20 %

3. Analytical Specificity

No interference with 25mg/L bilirubin, 500mg/dL of hemoglobin and 50mmol/L of triglyceride.

4. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and a SCCA Test which was already available on the market. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	363	0.6288	0.9506	0.9743

Literature References

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