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Hepa-IC

XG003

ELISA Kit for the detection of Squamous Cell Carcinoma Antigen (SCCA) variants Immune Complexes in Hepatocellular Carcinoma (HCC)

Rev. 04/2010

INTENDED USE

Hepa-IC is an enzyme linked immunosorbent assay (ELISA) for the quantitative measurement of Squamous Cell Carcinoma Antigen (SCCA) variants immune complexes (SCCA-IgM).

SUMMARY AND EXPLANATION OF THE TEST

Hepa-IC is an innovative in-vitro diagnostic method based on the detection of SCCA variants as circulating Immune Complexes (IC). Hepa-IC is a highly specific and sensitive ELISA assay for HCC detection designed to measure SCCA-IgM in patient sera (1-15). The amount of SCCA-IgM is expressed in Arbitrary Units (AU/mL), using a specific calibrator as reference. The measurement of SCCA-IgM offers the possibility to remarkably increase HCC detection sensitivity without compromising specificity compared to the serum levels of α -fetoprotein (AFP) (1,8,9,11-15). The assessment of SCCA-IgM has also been found to be useful in the monitoring of HCC development in chronic hepatitis (CH) and cirrhotic (CR) patients (2-7,10).

PRINCIPLE OF THE TEST

Standard Calibrators and specimens are simultaneously incubated with anti-SCCA variant antibodies coated to the wells of a microtiter plate. The immune complexes SCCA-IgM are detected by the addition of an enzyme conjugated secondary antibody and an enzyme substrate (ABTS). The developed color is proportional to the amount of the analyte in the sample.

REAGENTS AND MATERIALS PROVIDED

XG003-PL: 96 wells multi-strip Assay-Plate, pre-coated with affinity purified rabbit anti-SCCA

XG003-Calibrator: Two vials of calibrator lyophilized from PBS. White powder. Exact concentration on label. Totally soluble.

XG-EA: 1.1 mL of Enzyme-conjugated goat anti-human IgM secondary antibody (Green cap) 10-fold concentrate in stabilizer solution. The solution contains Proclin as preservative.

XG-CH4: Chromogen: ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid)); Buffer: phosphate citrate. Light green tablet + white powder lyophilized from 0.05 M phosphate-citrate buffer, pH 5. Totally soluble.

XG-SB: 200 μ L of Enzyme substrate: hydrogen peroxide solution 30% (w/w) in water. (Blue cap)

XG-DB5: Concentrated Dilution Buffer solution 5X, 10 mL. Once diluted, the working solution contains 1% BSA and 0.05% Tween 20 in PBS. The solution contain Proclin as preservative.

XG-WB2: Two tablets of lyophilized Washing Buffer. Once diluted, the working solution contains 0.05% Tween 20 in PBS. Totally soluble.

MATERIAL AND EQUIPMENT REQUIRED

Precision pipettes with disposable tips
Microplate washer
Microplate readers with a 405 ± 20 nm filter
Distilled or deionized water

STORAGE CONDITIONS

Storage at 4°C:

XG003-PL, XG-CH4[†], XG-SB, XG-DB5^{*}, XG-WB2^{*}

Storage at -20°C:

XG-EA, XG003-Calibrator[§].

Avoid repeated freeze and thaw cycles

(*) Must be used within one month of reconstitution

(§) Must be reconstituted just before the use

(†) Must be stored in a dark location

EXPIRATION DATE

Expiration date printed on the kit indicates limits of stability.

WARNINGS - POTENTIAL BIOHAZARDOUS MATERIALS

The **XG003-Calibrator** contains proteins of **human origin**. The reference material was tested using an approved method of evaluation for the presence of the antibodies to HIV, antibodies to the hepatitis C virus and hepatitis B surface antigens, and found to be negative. **Since no test method can offer complete assurance that HIV, hepatitis B virus, hepatitis C virus, or other infectious agents are absent, all human sourced materials should be considered potentially infectious.** It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens (16). Biosafety Level 2 (17) or other appropriate biosafety practices (18, 19) should be used for materials that contain or are suspected of containing infectious agents.

SPECIMEN COLLECTION AND PREPARATION

The use of serum samples are recommended for the Hepa-IC assay.



PRODUCT DATA SHEET

Serum specimens should be collected aseptically, avoiding hemolysis if possible.

Specimens should be stored at 2-8°C if the assay will be performed within 24 hours after collection. Specimens should be stored frozen if testing will occur after 24 hours.

If frozen, specimens should be mixed thoroughly after thawing to ensure consistency in the results. Avoid repeated freezing and thawing. Specimens showing particulate matter, erythrocytes, or turbidity must be clarified by centrifugation before testing.

INSTRUCTIONS FOR USE

PROCEDURAL NOTES

- Allow samples and reagents to reach room temperature prior to testing. Do not use water baths to thaw samples or reagents.
- Mix samples and all reagents thoroughly before use.
- Avoid excessive foaming of reagents. Also avoid exposure of reagents to excessive heat or light during storage and incubation.
- Avoid handling the tops of the wells both before and after filling.
- Standards and samples should be assayed in duplicate.
- Run a separate standard curve for each assay.
- Use only coated wells from the same reagent batch for each assay. Also do not mix reagents from different kit lots.
- Perform incubations in a sealed box containing a wet paper towel in order to prevent evaporation.

REAGENTS PREPARATION

- Reconstitute XG-CH4 chromogen solution with 20 mL of deionized water.
- Reconstitute XG003-Calibrator with 440 µL of deionized water for each calibrator vial.
- Prepare the required amount of XG-DB5 dilution buffer by diluting 5-fold the concentrated solution in deionized water. If crystals appear upon refrigeration, warm the bottle to 37°C with mixing to dissolve.
- Reconstitute 1 tablet of XG-WB2 washing buffer in 500 mL of deionized water. Prepare 1 liter with two tablets of XG-WB2 washing buffer for the entire plate.
- Prepare the required amount of XG-EA enzyme-conjugated secondary antibody solution diluting 10-fold in reconstituted XG-DB5 dilution buffer.

ASSAY PROTOCOL

1. Prepare assay reagents as described above.
2. Set up the microtiter plate with sufficient wells to enable the running of all required standards and samples.
3. Remove excess microtiter plate strips from the frame and store in the re-sealable foil bag with the desiccant provided.
4. Wash the microtiter plate strips three times with XG-WB2 washing buffer (300 µL/well).
5. Dispense 100 µL/well of standard calibrators (in duplicate) starting from the reconstituted solution and performing in-plate 2-fold serial dilutions in order to obtain a five-point calibration curve. Use XG-DB5 dilution buffer as diluent (videoclip available at <http://www.xeptagen.com/elisains>). For exact concentration of the reconstituted calibrator please refer to the concentration value (AU/mL) indicated on the XG003-Calibrator vial. Also dispense 100 µL/well of XG-DB5 dilution buffer as blank, in duplicate.
6. Dispense 100 µL/well of eight fold (1:8) diluted sample (in duplicate). Use XG-DB5 dilution buffer as diluent.
7. Incubate 1 hour at room temperature.
8. Wash six times with XG-WB2 washing buffer (300 µL/well).
9. Add 100 µL/well of diluted XG-EA enzyme-conjugated secondary antibody solution.
10. Incubate 1 hour at room temperature.
11. Wash six times with XG-WB2 washing buffer (300 µL/well).
12. Prepare the required amount of chromogen-enzyme substrate solution adding 1 µL of XG-SB enzyme substrate solution per 3 mL of XG-CH4 chromogen solution. The chromogen-enzyme substrate solution must be used within 24 hours.
13. Apply 150 µL/well of freshly prepared chromogen-enzyme substrate solution. Allow color to develop for 20 min. at 37°C in the dark and measure OD values of each well using an ELISA plate reader set to 405 nm.
14. Elaborate OD values with Xerepro software (<http://www.xeptagen.com/software>) or plot the standard curve ΔOD values as described in the next section: Processing of the results.

PROCESSING OF THE RESULTS

Average the duplicate readings for each standard calibrator and sample, and subtract the zero standard optical density.

The standard calibrators may be used to construct a standard curve with values reported in AU/mL (see Fig. 1). This data deduction may be performed through computer methods using curve fitting routines or may also be manually deduced by plotting the absorbance



PRODUCT DATA SHEET

values of the standard on the y-axis versus concentration on the logarithmic x-axis and drawing the standard curve.

The immune complexes (SCCA-IgM) concentration in the biological sample can be calculated directly from the calibration curve by interpolation. The value obtained must be multiplied by the dilution factor.

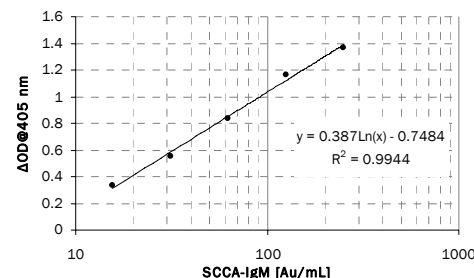


FIG. 1: Range of linearity of a typical standard curve for SCCA-IgM after 20 minutes of substrate incubation.

QUALITY CONTROL

The intra- and inter-assay coefficients of variation were determined on 4 typical standard curves and the results were less than 15 %.

For optimal performance, the absorbance of the zero standard should be < 0.2 OD405.

It is recommended that each laboratory assays appropriate quality control samples in each run to ensure that all reagents and procedures are correct.

INTERPRETATION

The SCCA-IgM cut-off value was 120 AU/mL for differentiating HCC from non-malignant chronic liver diseases (11-14).

SPECIFIC PERFORMANCE CHARACTERISTICS

The linear range of the assay is 12,5-200 AU/mL.

The sample with values above 200 AU/mL should be further diluted and re-measured.

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