HEPA-IC

PRODUCT PROFILE

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

code XG003

Hepa-IC is an ELISA kit designed to determine the serological levels of Squamous Cell Carcinoma Antigen (SCCA)-IgM immune complex. Applications include:

**Risk assessment and diagnosis of HCC**
In cirrhotic patients, the increase of SCCA-IgM levels over time is prognostic of HCC evolution, to the extent that SCCA-IgM determination may be employed in cancer surveillance programs [1-3].

**Prognosis of HCC patients**
In patients with HCC, higher levels of SCCA-IgM are associated with shorter survival [4].

**Prediction of HCC treatment outcome**
The decrease of SCCA-IgM serological levels in HCC patients who receive locoregional or Sorafenib treatment is associated with a positive response to therapy [5].

**Assessment of liver disease evolution in patients with chronic hepatitis**
In patients with untreated chronic hepatitis B or C, the progressive increase over time of serum levels of SCCA-IgM is associated with worsening of the disease and liver tissue deterioration [6].

**Prediction of therapeutic outcome in HCV treatments**
Antiviral treatment with pegylated interferon and ribavirin induces a significant decrease of circulating SCCA-IgM levels only in HCV patients with a sustained virological response (based on HCV-RNA negativity at 24 weeks of follow-up) [7, 8].

**Identification of NASH in HCV-positive patients**
Patients with HCV infection and NASH have higher serological levels of SCCA-IgM than patients without NASH [9, 10].
An ELISA kit for the detection of SCCA-IgM immune complexes in serological samples

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**SCCA-IgM: A NOVEL BIOMARKER OF LIVER DISEASE**

Hepatocellular carcinoma (HCC) is one of the most common fatal cancers worldwide, the fourth one for incidence rate. Mortality index for this tumor is very high: most patients with HCC die within three years after diagnosis, and less than 5% survive to five years [11, 12]. Infection with Hepatitis B (HBV) and Hepatitis C virus (HCV), exposure to Aflatoxin B, excessive intake of alcohol, and cirrhosis are the major risk factors for HCC development. Worldwide, 240 and 150 million people are HBV or HCV infected, respectively [13]; every year, about 0.5% of these patients (about 2 million people) develops HCC; in addition, 1-8% of cirrhotic patients develops liver cancer every year [14]. In the Western world, 90% of HCC arises from a cirrhosis background, therefore surveillance of cirrhotic patients becomes crucial to identify the cancer at an early stage and improve the chances of successful therapy and survival [14].

The latest European practice guidelines [14] state that surveillance of patients with cirrhosis should be based on ultrasound (US) examination performed every six months. US are non-invasive, with no risks, well accepted by the patients, and relatively low cost; the specificity of this technique is >90% for advanced HCC, while the sensitivity can be 58-89% for early HCC. The use of computer tomography (CT) or magnetic resonance imaging (MRI) for surveillance is not suggested due to their cost, the tendency to give a high rate of false positives and their lack of cost-effectiveness.

In the past, the dosing of serum alpha-fetoprotein (AFP) was recommended for the non-invasive diagnosis of HCC; although this biomarker is still widely used, it is not suggested by guidelines anymore, since it increases the detection rate of US only by 6-8%, and it raises the number of false positives, resulting in an 80% increase of the cost for each liver cancer diagnosis.

Non-invasive diagnosis of HCC should be confirmed by one imaging technique (CT or MRI) for nodules with >2 cm diameter, or by two coincidental techniques (CT and MRI) for nodules with a diameter of 1-2 cm that show contrast uptake in the arterial phase and washout during the venous/late phase [14]. Only in inconclusive or atypical cases a biopsy should be performed.

Similarly to Europe, American guidelines also recommend surveillance of cirrhotic patients with US every six months, HCC diagnosis based on imaging techniques, and eventual biopsy in dubious cases [15]. Serological AFP is not suggested any longer because of its low sensitivity and specificity [15].

A comparison of different methods for HCC diagnosis is presented in Table 1.

<table>
<thead>
<tr>
<th>METHOD</th>
<th>PROS</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP DETERMINATION</td>
<td>Not invasive; Cheap</td>
<td>High rate of false positive and false negative results</td>
</tr>
<tr>
<td>ULTRASOUNDS (US)</td>
<td>Not invasive; Cheap</td>
<td>May not detect small nodules (&lt;1cm diameter)</td>
</tr>
<tr>
<td>COMPUTERIZED TOMOGRAPHY (CT SCAN)</td>
<td>Not invasive</td>
<td>High rate of false positive results; Expensive</td>
</tr>
<tr>
<td>MAGNETIC RESONANCE IMAGING (MRI)</td>
<td>Not invasive</td>
<td>High rate of false positive results; Expensive</td>
</tr>
<tr>
<td>LIVER BIOPSY</td>
<td>Confirms HCC diagnosis</td>
<td>Invasive; Expensive; Prone to risks</td>
</tr>
</tbody>
</table>

Early detection of HCC is difficult due to the lack of adequate biomarkers to clearly and accurately differentiate the tumor from benign liver lesions.

Although it is not suggested by international guidelines any longer, the most widely used serologic marker to detect HCC is still AFP. As already mentioned, the use of this biomarker is controversial, since its levels are elevated (>20 ng/mL) in a large number of HCC patients (30-60%), but with low specificity (70-80%). In fact, many patients with chronic liver disease may have AFP levels in the range of 20-200 ng/mL [16, 17]. In addition, AFP serum levels in patients with cirrhosis and HCC often overlap, so higher AFP cut off values (>100 ng/mL) have been used to increase specificity, but this has reduced sensitivity to extremely low values (5-15%) [18].

Given the high heterogeneity of HCC [19], other biomarkers have been found over-expressed in the liver and/or serum of patients. Des-y-carboxy prothrombin (DCP) levels are elevated in 35-53% of HCC patients [20, 21] as a result of an acquired defect in the post-translational carboxylation of the prothrombin precursor in neoplastic cells [22]. While some studies reported the utility of this marker compared to AFP [23], others found no improvement over AFP determination and thus recommended a combination of both biomarkers to improve sensitivity and specificity [24]. Liver over-expression of Glypican-3 (GPC3) mRNA levels is observed in 75% of HCCs and in only 3.2% of healthy patients [25]. Immunohistochemistry analysis confirmed the occurrence of GPC3 protein in 72% of HCC cases [26], while serological GPC3 protein has been detected only in 40-53% of patients with HCC [26, 27].

Other markers proposed for HCC surveillance, including lectin-reactive AFP, p53 auto-antibodies, carbohydrate-deficient-transferrin, hepatitis B virus
Molecular biomarkers for HCC.

In regard to prognosis, many molecules have been proposed to monitor HCC therapy response, tumor recurrence and patient survival (Table 2); they include proliferation markers, cell cycle/apoptosis regulators, adhesion molecules, and angiogenesis promoters. However, none of these biomarkers shows good diagnostic performance [29].

**Table 2**: Comparison of the prognostic relevance of various molecular biomarkers for HCC.

<table>
<thead>
<tr>
<th>MARKER</th>
<th>PROS</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation markers</td>
<td>Malignant grade evaluation; Recurrence time prediction; Long-term survival prediction</td>
<td>Low sensitivity and specificity</td>
</tr>
<tr>
<td>(PCNA, Ki-67, Mcm2, Mib-1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear morphology markers</td>
<td>Tumor stage evaluation; Recurrence prediction; Progression prediction</td>
<td>Low sensitivity and specificity</td>
</tr>
<tr>
<td>(AgNOR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell Cycle regulators</td>
<td>Recurrence time prediction; Long-term survival prediction</td>
<td>Low sensitivity and specificity</td>
</tr>
<tr>
<td>(CyclinE, Cdc2, p27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor promoters</td>
<td>Recurrence time prediction; Progression prediction</td>
<td>Low sensitivity and specificity</td>
</tr>
<tr>
<td>(Ras, c-myc, c-erbB-2, EGF-R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptosis regulators</td>
<td>Recurrence time prediction</td>
<td>Low sensitivity and specificity</td>
</tr>
<tr>
<td>(Fas, Fas L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adhesion molecules</td>
<td>Tumor stage evaluation</td>
<td>Low sensitivity and specificity</td>
</tr>
<tr>
<td>(E-cadherin, ICAM-1, CD44 isoforms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer invasion markers</td>
<td>Recurrence time prediction; Long-term survival prediction</td>
<td>Low specificity</td>
</tr>
<tr>
<td>(MMP, uPA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiogenesis promoters</td>
<td>Long-term survival prediction</td>
<td>Low sensitivity Low specificity</td>
</tr>
<tr>
<td>(VEGF, bFGF)</td>
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</table>

Squamous Cell Carcinoma Antigen (SCCA) is a serine protease inhibitor physiologically found in the spinous and granular layers of normal squamous epithelium; it is also expressed by neoplastic cells of epithelial origin [30]. Both SCCA isoforms SCCA1 and SCCA2 [31] protect neoplastic cells from apoptotic death induced by several types of stimuli, and in vivo experiments demonstrated that SCCA1 can promote tumor growth [32, 33]. SCCA variants are not expressed in normal hepatocytes and healthy liver, but they are over-expressed in 93% of surgically resected HCC (Figure 1) [34]. SCCA has been detected also in the liver of 50% of patients with chronic hepatitis C and in 75% of cirrhotic patients (Figure 1) [35].

In patients with liver cancer, serological free-SCCA is not detectable; however, immune-complexes formed by SCCA bound to immunoglobulin-M (SCCA-IgM) are found: the majority of HCC cases (70%) are strongly reactive for SCCA-IgM (mean±SD = 2568.5±6797.3 AU/mL; AU: Arbitrary Units), whereas healthy controls are negative (<120 AU/mL) (Figure 2) [35]. SCCA-IgM is detected also in 26% of cirrhotic patients and in 18% of patients with chronic hepatitis C, albeit at lower levels (mean±SD = 147.5±348.3 AU/mL and 39.5±89.7 AU/mL, respectively) (Figure 2) [35]. The serological concentration of SCCA-IgM parallels the extent of SCCA over-expression in the liver tissue, as detected by immunohistochemistry [35].

Table 3 shows a comparison of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) between SCCA-IgM and AFP levels in patients with different liver disease and healthy controls [35].

**Table 3**: Comparison of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of SCCA-IgM and AFP in differentiating patients with HCC (n=50) from those with cirrhosis (CR, n=50), chronic hepatitis (CH, n=50), and healthy subjects (n=50).

<table>
<thead>
<tr>
<th>BIOMARKER</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCA-IgM 120 AU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCC vs Control</td>
<td>70</td>
<td>100</td>
<td>100</td>
<td>77</td>
</tr>
<tr>
<td>HCC vs CR</td>
<td>74</td>
<td>73</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>HCC vs CH</td>
<td>82</td>
<td>80</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>HCC vs Control</td>
<td>70</td>
<td>100</td>
<td>100</td>
<td>77</td>
</tr>
<tr>
<td>HCC vs CR</td>
<td>74</td>
<td>73</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>HCC vs CH</td>
<td>82</td>
<td>80</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>HCC vs Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>77</td>
</tr>
<tr>
<td>HCC vs CR</td>
<td>74</td>
<td>73</td>
<td>71</td>
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<td>HCC vs CH</td>
<td>82</td>
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<td>73</td>
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</tr>
<tr>
<td>HCC vs Control</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>77</td>
</tr>
<tr>
<td>HCC vs CR</td>
<td>72</td>
<td>74</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>HCC vs CH</td>
<td>82</td>
<td>82</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: A. SCCA expression in cirrhotic liver. B. SCCA score distribution in liver specimens showing SCCA reactivity in the different groups of patients. CH: chronic hepatitis; CR: cirrhosis; HCC: hepatocellular carcinoma.

Figure 2: A: Significantly elevated serum levels of SCCA-IgM and AFP in HCC, cirrhosis (CR), chronic hepatitis (CH), and in controls as detected by ELISA. B: Box plot for SCCA-IgM (top) and AFP (bottom) values in the four groups of subjects. The box indicates the lower and upper quartile and the middle line indicates the median. Boxes are notched at the median with the lengths of the notches representing the 95% confidence interval. A dotted-line connects the observations within 1.5 inter-quartile ranges (IQRs) of the lower and upper quartile. Crosses represent the observations between 1.5 and 3.0 IQRs from the quartiles and circles represent points beyond this.
CLINICAL APPLICATIONS OF SCCA-IgM IN HEPATOCELLULAR CARCINOMA

Risk assessment and diagnosis of HCC
SCCA-IgM can be used to monitor patients with cirrhosis evolving to HCC. Indeed, a retrospective longitudinal study has shown that the biomarker increases over time in cirrhotic patients developing liver cancer, while it does not increase in patients without disease progression (Figure 3) [1]. These results were confirmed by a prospective study, which also showed that baseline SCCA-IgM serological levels were significantly higher in patients who developed HCC than in those who did not develop cancer (Figure 4) [3]. Another retrospective simulation also tested the performance of SCCA-IgM measured 12 months before HCC diagnosis and found that, when tumor incidence is 3%, the immune complex can identify HCV-infected cirrhotic patients with a negative predictive value (NPV) of 98.8% - that is, SCCA-IgM can segregate patients with an extremely low risk of developing HCC in the following year [2]. Based on this evidence, the biomarker SCCA-IgM may be used for risk assessment and early detection of HCC.

Figure 3: SCCA-IgM levels in the serum increase over time in patients who develop HCC.

Figure 4: Serological levels of SCCA-IgM at baseline are higher in patients who develop HCC than in those who do not develop liver cancer.

Prognosis of HCC patients
Increased levels of SCCA-IgM are associated with shorter survival in HCC patients (Figure 5) [4]. More specifically, patients with serological levels of the biomarker >130 AU/mL had a median survival of 26 months, while those with levels <130 AU/mL had a median survival of 48 months [4].

Figure 5: Higher levels of SCCA-IgM are associated with shorter survival in HCC patients.
Prediction of HCC treatment outcome
A study performed on 168 patients with HCC who were followed for two years showed that those with a positive response had significantly lower serological levels of SCCA-IgM compared to patients who had a negative response to treatment (median value: 126.7 vs. 165.1 AU/mL) [5]. This observation was independent from the type of therapy that was provided (locoregional therapy or Sorafenib). Therefore, the assessment of SCCA-IgM levels in patients undergoing HCC treatments may be helpful in monitoring and predicting the outcome of therapy.

CLINICAL APPLICATION OF SCCA-IgM IN HEPATITIS

Assessment of liver disease evolution in patients with chronic hepatitis
Studies have shown that in patients with chronic hepatitis (due to HBV or HCV infection) the serological levels of SCCA-IgM increase over time only in those with a progressive disease and histological deterioration - that is, in patients who develop fibrosis (Figure 6) [6]. Patients with no disease progression showed stable levels of the biomarker during the same time frame [6]. Authors concluded that monitoring SCCA-IgM behaviour over time could be useful for predicting disease outcome in individual patients with chronic hepatitis.

Prediction of therapeutic outcome in HCV treatments
The serological levels of SCCA-IgM were quantified in a group of HCV-positive patients with compensated cirrhosis who were treated with PEG-interferon plus ribavirin. The biomarker was analysed at baseline and after six and 12 months after the end of treatment. In patients with a sustained virological response (SVR) the levels of SCCA-IgM significantly decreased at every time point, while there was no significant variation in non-responders (Figure 7) [8]. These results were confirmed in another study, in which patients with HCV chronic infection were monitored before and during anti-viral treatment (PEG-interferon plus ribavirin): the profile of reduction of SCCA-IgM levels was different between patients with or without SVR [7]. In particular, a significant decrease in SCCA-IgM concentration was observed after four weeks of therapy in SVR patients, but not in null responders; the authors demonstrated that this decrease was an independent prognostic factor of therapeutic response [7]. Based on this evidence, SCCA-IgM may be used in the clinical setting to monitor or predict the therapeutic outcome of antiviral treatment in patients with HCV infection.

![Figure 6](image1.png)

*Figure 6: SCCA-IgM levels increase over time only in chronic hepatitis patients with progression of the disease. T1=baseline; T2= after a median period of six years.*

![Figure 7](image2.png)

*Figure 7: In HVC patients treated with PEG-interferon plus ribavirin, SCCA-IgM levels decrease in patients with SVR, and remain stable in non responders.*
Identification of NASH in HCV-positive patients

A study conducted on patients with HCV infection showed that there is a significant association between higher levels of SCCA-IgM and severe grades of steatosis and histological presence of non-alcoholic steatohepatitis (NASH) (Figure 8) [9]. Based on this finding, a novel diagnostic algorithm has been developed: serum SCCA-IgM determination was combined with other clinical parameters (glucose, insulin, ferritin, hemoglobin, smoke habits and HCV genotype) to improve the prediction of NASH in HCV patients. The diagnostic score showed a success for NASH diagnosis of 88-97% [10].

Thus, the determination of SCCA-IgM may help the clinicians in the identification of NASH in patients who already have chronic liver disease.

Figure 8: SCCA-IgM is associated with histological features of NASH in HCV patients.

HEALTH TECHNOLOGY ASSESSMENT

About 5% of patients with cirrhosis develop HCC every year [36] and current guidelines suggest to monitor cirrhotic patients using ultrasounds (US) every six months [14]. Sensitivity of semi-annual US for early diagnosis of HCC is 70% [37]. It can be assumed that US identify each year 70% of the 5% cirrhotic patients who develop liver cancer, that is 3.5% early HCC. Assuming that each semi-annual US monitoring identifies half of the HCC developing that year, every six months US identify half of 3.5% - that is 1.75% HCC, while every four months US identify a third of 3.5% - namely 1.17% HCC.

The current cost of US for the Italian national health system is about €74 per patient. Based on the assumptions aforementioned, by monitoring 1000 cirrhotic patients with US every six months, 53 cases of early HCC will be identified every year at a total cost of €222,000 (Table 4).

Clinical data show that a serological value of SCCA-IgM >500 AU/mL is observed in patients with cirrhosis who develop HCC during the following 12 months [2]. Therefore, determination of SCCA-IgM can identify HCC up to 12 months in advance compared to US. On these basis, XEPTAGEN proposes a new monitoring system for cirrhotic patients that combines US with SCCA-IgM. In this system, cirrhotic patients who are negative for HCC at semi-annual US are analyzed for SCCA-IgM serological levels. If SCCA-IgM is less than 500 AU/mL, the patient continues the semi-annual monitoring with US; in case SCCA-IgM is higher than 500 AU/mL, the surveillance on the patient becomes more stringent and US is performed every four months (Figure 9).

Based on XEPTAGEN’s data from a wide population of patients with cirrhosis, about 20% of cirrhotic patients with HCC will have SCCA-IgM levels >500 AU/mL. Based on all these information, the monitoring of 1000 cirrhotic patients with US plus SCCA-IgM every six months will identify 59 cases of early HCC each year, with a total cost of €413,000 (Table 4). Compared to current guidelines, every 1000 cirrhotic patients XEPTAGEN’s protocol would cost an extra €191,000 and it would identify six extra patients with early HCC each year - patients who would be missed and diagnosed at a later stage otherwise.

The corrected survival at 5-years after HCC diagnosis with semi-annual US is 91% at the time of diagnosis and 75 and 60%, respectively, at 12 and 24 months post-diagnosis [38]. Assuming that use of US corresponds to 24 months post-diagnosis,
while use of US together with SCCA-IgM determination corresponds to 12 months post-diagnosis, the adoption of the biomarker could increase the 5-years survival from 60% to 75%. Therefore, by diagnosing HCC one year in advance, the 5-years survival would increase by 15%, which corresponds to an extra nine months of life. One year of human life has been valued at about $129,000 [39], equivalent to approximately €118,000. This value amounts to €9,830 for one month and to €88,500 for nine months of life. By multiplying €88,500 for the six extra patients identified in advance with US plus SCCA-IgM, we get a sum of €531,000 (Table 4). This value is almost three times higher than the additional cost of analyzing SCCA-IgM together with US (€191,000).

Table 4: Ecomonomical analysis of 1000 cirrhotic patients monitored with current guidelines or with XEPTAGEN's new protocol.

| Positive US | 53 | 59 | +6 |
| Total cost | €222,000 | €413,000 | +€191,000 |
| 5-years survival | 60% | 75% | +15% |
| Survival value | - | - | +€531,000 |

Figure 9: Scheme of XEPTAGEN’s new monitoring system for cirrhotic patients that combines US with SCCA-IgM.

REFERENCES
IgM compared to total SCCA-IgM for diagnosis of hepatocellular carcinoma. Gut. 57:A147. 2008


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