Non-invasive Detection of Liver Injury and Fatty Liver Disease

Use of the Apoptosis Specific Biomarker Cytokeratin-18 (ccK18) to detect non-alcoholic steatohepatitis (NASH) and predict progressive liver disease.

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**Note:** K18 = Keratin 18, also known as Cytokeratin 18 (CK18); ccK18 = caspase-cleaved K18.
1 INTRODUCTION

Liver fibrosis, cirrhosis, inflammation and steatosis are major features of acute and chronic liver diseases, such as viral hepatitis (HBV, HCV), autoimmune or metabolic liver diseases, fatty liver disease and alcoholic or non-alcoholic steatohepatitis (ASH/NASH). With the increasing prevalence of obesity and Metabolic Syndrome an increase of non-alcoholic fatty liver disease (NAFLD) is obvious; a trend reflected in the increasing number of scientific publications on NAFLD and NASH.

NAFLD encompasses a wide spectrum of conditions associated with over accumulation of fat in the liver ranging from nonalcoholic fatty liver disease (NAFL(D)) or simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. Although NAFL typically follows a benign non-progressive clinical course, NASH is a potentially serious condition; as many as 25% of patients may progress to cirrhosis and experience complications of portal hypertension, liver failure, and hepatocellular carcinoma.

Patients with insulin resistance and other symptoms of metabolic syndrome should therefore be screened for NAFLD and its progressive and chronic form NASH (non-alcoholic steatohepatitis) [33]. In the USA, already 7% of all liver transplants are based on diagnosis of NASH [35]. The diagnostic challenge is to predict NAFLD patients that are likely to progress into severe liver disease, initiate therapy and life style changes and to monitor the efficacy of the measures.

Liver biopsy is currently the gold standard for the detection of early liver injury. This invasive examination method is limited by sample errors and the risk of clinical complications. Biopsies are ordinarily carried out under stationary conditions, which contribute toward the high costs of the examination [1]. They can also be refused by patients requiring frequent biopsies to monitor therapy. Furthermore, this technique is limited by the sample variability, such as for example inhomogeneous liver damage and by the variability of the same or different examiner(s) [3]. Particularly in children, it has been reported that liver biopsy is a burden with a 6.8% incidence of overall complications, a 2.4% incidence of major complications [4, 5].

Conventional liver biomarkers liver transaminases (AST/ALT), frequently provide incorrect information about liver injury. For example, it could be shown that up to 25-30% of the patient with fibrosis liver damage have normal transaminase levels. [6,7]. Patients with chronic HCV infection had normal transaminase levels despite inflammatory and fibrotic liver damage [8-10]. Moreover, falsely elevated ALT levels can be caused by of barbiturates, narcotics, methotrexate, chlorpromazine salicylates (aspirin), and other drugs that affect the liver. Thus, there is a strong need for new and non-invasive methods for the assessment of liver injury. Hepatocyte cell death, specifically hepatocyte apoptosis, is considered to play a crucial role in the formation of liver fibrosis or liver cirrhosis. Numerous studies have demonstrated that hepatocyte apoptosis can be specifically assessed by means of caspases cleaved fragments of Keratin 18 (ccK18), a major intermediate filament protein, expressed by hepatocytes. Caspase-cleaved K18 fragments (ccK18) are stable to further proteolysis and are subsequently released into the blood stream. Caspase cleavage of K18 generates a neo-epitope that is specifically detected by the monoclonal antibody M30. Ample evidence is provided that the M30 Apoptosense® ELISA allows the assessment of hepatocyte apoptosis. The biomarker ccK18 as determined by the M30 Apoptosense® ELISA allows prediction of the level of fibrosis (staging), diagnosis of NASH, and predict whether NAFLD patients are likely to progress into NASH. The assay can furthermore improve decisions on therapeutic regiments for patients.

Finally, assessment of hepatocyte apoptosis or total liver cell death by measurement of both caspase-cleaved and intact K18 molecules (using the M30 and M65 ELISAS), seems a promising tool to identify individuals with Drug Induced Liver Injury (DILI) or injury caused by toxic components like vinyl chloride.
Liver injury can also be assessed using other biomarkers. A list of some recently described liver damage biomarkers is shown in Table 1.

<table>
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<th>Liver injury biomarker</th>
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<tr>
<td>Caspase-cleaved Keratin 18 (ccK18: M30 ELISA)</td>
<td>Hepatocyte apoptosis</td>
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<tr>
<td>Keratin 18 (cleaved and uncleaved: M65 ELISA)</td>
<td>Hepatocyte apoptosis and necrosis (total cell death)</td>
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<td>Alpha Glutathione S-Transferase (α GST)</td>
<td>Hepatocyte damage</td>
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<td>Pi Glutathione S-Transferase (π GST)</td>
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<td>Collagen IV (serum)</td>
<td>Increased collagen deposition and liver fibrosis</td>
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<tr>
<td>Hyaluronic acid (HA)</td>
<td>Liver fibrosis and loss of liver function</td>
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</table>

Table 1 Type of information provided by various liver injury biomarkers.

2 THE M30 APOPTOSENSE® ELISA

2.1 Principle of the M30, M65 and M65 EpiDeath® assays

The M30 Apoptosense® ELISA is based on the monoclonal antibody M30. This antibody recognizes a neo-epitope on K18 which is exposed after caspase cleavage at residue Asp396. M30 detects only caspase-cleaved fragments of K18 (ccK18; K18Asp396) but not the native protein. Antigen generation is strictly dependent on caspase activation (activity is inhibited by broad-spectrum caspase inhibitors). A number of different caspases are able to cleave K18 at Asp396, primarily caspase-3, -7 and -9; activity is therefore not dependent on the action of a specific caspase. The presence of ccK18 in plasma therefore shows epithelial cell apoptosis.

The M65 & M65 EpiDeath® ELISAs (for clinical and toxicology testing, respectively) measure total soluble K18 released from dead cells (necrotic and apoptotic). M65 measurements will therefore represent the total epithelial cell death by any cause.

2.2 Normal subjects versus patients with liver damage

Patients with confirmed chronic hepatitis C (HCV patients) and persistently normal ALT levels were compared to healthy controls and HCV patients with elevated ALT [8,17]. HCV Patients with normal ALT had significantly higher serum levels of ccK18 (as measured by the M30 Apoptosense® ELISA) than healthy controls and significantly lower levels than HCV patients with elevated ALT.

Figure 2
Quantification of serum ccK18 levels in healthy controls and patients with chronic hepatitis C virus (HCV) infection. ccK18 levels were quantified in serum by the M30 Apoptosense® ELISA. Median ccK18 in 18 in healthy controls (n = 19). HCV-infected patients with persistently normal alanine aminotransferase (ALT) levels (n = 27) or elevated ALT levels (n = 72) are indicated by the horizontal line. The vertical lines indicate the range, the horizontal boundaries of the boxes represent the first and third quartile. Dots indicate extreme values. Data adapted from Kronenberger et al [8].

Figure 1
The principle of the M30 and M65 assays, measuring apoptosis and total cell death (necrosis + apoptosis) respectively.
This study indicates that also patients with chronic hepatitis C but normal ALT have an increased hepatocyte loss by apoptosis and demonstrates that the M30 Apoptosense® ELISA can be used for detecting liver damage in patients with chronic hepatitis C.

2.3 Staging liver fibrosis

ccK18 levels, as measured by the M30 Apoptosense® ELISA, are associated with the extent of fibrosis. In a study by Kronenberger [8], the highest levels of ccK18 were observed in patients with chronic hepatitis C and cirrhosis. ccK18 levels were also correlated with progression rate of fibrosis. This demonstrates that the M30 Apoptosense® ELISA can be used for liver fibrosis staging.

2.4 Reference ranges for ccK18 as measured by the M30 Apoptosense® ELISA

ccK18 values were measured in 200 apparently healthy Swedish blood donors. With the M30 Apoptosense® ELISA, from donors values showed a similar distribution and no age dependency. (see figures below). Based on the distribution following reference ranges have been set:

<table>
<thead>
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<th>Reference ranges</th>
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<tr>
<td>Healthy</td>
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</tr>
<tr>
<td>Slightly elevated</td>
<td>150 – 200 U/L</td>
</tr>
<tr>
<td>Elevated</td>
<td>&gt; 200 U/L</td>
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</table>

In case of suspected of fibrosis or fatty liver disease:
- ccK18 values between 150 and 200 U/L suggest an indication for mild fibrosis (for example NAFL).
- ccK18 values > 200 U/L are a strong indication for substantial liver disease (for example NASH).

Figure 3
Quantification of serum ccK18 levels in healthy controls and patients with chronic hepatitis C and different stages of fibrosis. Serum ccK18 levels were quantified by the M30 Apoptosense® ELISA. Median serum levels of ccK18 in healthy controls (n = 19), patients chronically infected with hepatitis C virus and no fibrosis (n = 4), fibrous portal expansion (n = 40), bridging fibrosis (n = 45) or cirrhosis (n = 8) are indicated by a horizontal line. The vertical lines indicate the range, the horizontal boundaries of the boxes represent the first and third quartile. Dots indicate extreme values. Data adapted from Kronenberger et al [8].

Figure 4
Distribution (A) and age dependency (B) of ccK18 normal values.
3 DETECTION OF NON-ALCOHOLIC STEATOHEPATITIS (NASH)

An increasingly common chronic disease is non-alcoholic fatty liver disease (NAFLD), ranging from nonalcoholic fatty liver disease (simple steatosis) to progressive non-alcoholic steatohepatitis (NASH). The prevalence of NAFLD and NASH are increasing, most probably linked to the increasing prevalence of metabolic syndrome and obesity. Patients with insulin resistance and other symptoms of metabolic syndrome should therefore be screened for NASH/NAFLD [17]. The diseases increasingly contribute to the health budget burden; it has been estimated that approximately 10–40 % of the adult population in Western developed countries has NAFLD and 2-5% NASH. Over a 10-year period, the frequency of liver transplantation for NASH has increased 5-fold to become the second most common indication for liver transplantation at some US centers in 2011 (Hepatitis C is first).

While most patients with steatosis tend to have a benign clinical course, a significant proportion of those with NASH have a progressive disease with a risk of developing liver cirrhosis and hepatocellular carcinoma [12]. In the USA, 7% of all liver transplants are based on diagnosis of NASH [35]. The diagnostic challenge is to predict NAFLD patients that are likely to progress into liver disease, monitor the progression and to decide whether a liver biopsy is really needed or a simple weight loss program is already effective [18].

3.1 Limitations of current diagnostic methods

The routine determination of transaminases (ALT/AST) is not suitable to assess liver fibrosis and steatosis or to differentiate between simple steatosis and NASH. A significant % of patients with normal (< 1x Upper limit of Normal (ULN)) or slightly elevated ALT levels (between 1-2 x ULN) have inflammatory and fibrotic liver damage. A decision for further investigation (e.g. liver biopsy) and treatment of patients solely based on ALT/AST would miss a significant number of patients with a significant liver problem.

3.2 Use of the M30 Apoptosense® ELISA for diagnosing NASH in adults and children

To identify NAFLD patients at increased risk of progressing liver disease, non-invasive parameters with higher sensitivity and specificity compared to the transaminases are needed. A growing number of publications demonstrate that the detection of ccK18 (by M30 Apoptosense® ELISA) is a very reliable tool for the diagnosis of NASH [11,13-19]. Figure 5 shows representative ccK18 (M30) data from recent studies for healthy individuals vs. patients with NAFLD or NASH. [23] Numerous studies have confirmed that NASH patients have significantly increased levels of ccK18 (M30) compared to healthy controls or individuals with simple steatosis [13], [14] and [18-22]).

![Figure 5](image)

Robust nonalcoholic fatty liver disease (NAFLD) staging with the cell death biomarker assay (M30-ELISA) [23]. (a) Left panel: the M30-ELISA allows the significant (**P < 0.01) discrimination between nonalcoholic steatohepatitis (NASH; n = 74) and nonalcoholic fatty liver (NAFL; n = 23) or healthy individuals (n = 23), and can even distinguish between NAFL and healthy controls. Right panel: the ELISA can significantly (**P < 0.01) distinguish between NAFL (NAS < 3; n = 23) and borderline NASH (NAS 3 – 4; n = 40) or NASH (NAS > 4; n = 34). (b) Significant discrimination (**P < 0.01) between minimal (≤ 10%; n = 69) and relevant (> 10% ; n = 52) liver steatosis by M30 Apoptosense® ELISA.
In a study by Feldstein et al. [31] performed with 139 patients with biopsy-proven nonalcoholic fatty liver disease (NAFLD) and 150 age-matched healthy controls, the ccK18 test was able to detect the presence of NASH with a specificity of more than 90%, or to exclude the presence of NASH with a sensitivity close to 80% by adopting different test thresholds.

In a recent pediatric study, Feldstein et al. [25] concluded that the ccK18 was an accurate biomarker to diagnose NASH in children with suspected NAFLD. Plasma ccK18 levels were significantly higher in children with NASH compared with those with hepatic steatosis and the test demonstrated excellent accuracy for diagnosing NASH on biopsy with AUC of 0.933. ccK18 levels correlated with the main histological features of NASH, NAS, and fibrosis stage. As in adults, ccK18 may become one of the most promising single non-invasive tests for diagnosing NASH in children (see also [26-30]).

Canbay et al. concluded that serological investigation, including the biomarker ccK18 can predict progression of NAFLD into NASH in obese patients [40] They also demonstrated that patients with polycystic ovary syndrome (PCOS) also have increased risk for developing non-alcoholic steatohepatitis (NASH). Due to this association they advise to investigate female NASH patients for PCOS and PCOS patients for NASH [21].

### 3.3 Diagnosing fatty liver disease and NASH in diabetes patients

In a recent study by Ramiljak S et al. [34] liver transaminases (ALT/AST) and liver damage biomarkers ccK18, K18 and α GST were measured in 32 diabetic patients and 36 healthy subjects. ALT/AST levels were elevated in only 22 (13%) of diabetic patients. In contrast, liver injury biomarkers were highly elevated in up to 65% of the cases and showed similar behavior in most patients (Figure 6), indicating ongoing liver damage. This pilot study supports the use of ccK18 as liver injury biomarker in diabetic patients to diagnose fatty liver disease and predict possible progression to NASH.

![Liver damage biomarkers in diabetic patients](image)

*Figure 6* Liver damage biomarkers in diabetic patients. For each diabetic patient, liver biomarker levels were expressed as a percentage of the upper limit of the normal range for the specific marker.
Finally, the M30 assay enables the monitoring of disease progression and thereby the assessment of progressing or improvement of disease activity in NAFLD patients.

3.4 Suggested new diagnosis management using the M30 Apoptosense® ELISA

A decision for further investigation (liver biopsy) and treatment of patients based on ALT/AST would miss a significant number of patients with an existing liver problem. Suggested new diagnosis management involves three options. Patients suspected of NAFLD are first tested for routine serum liver enzyme levels.

A. Patients with normal transaminase levels (< 1x ULN). To prevent missing a significant number of patients with a real liver problem (especially when other clinical information suggests a liver problem), serum ccK18 levels (M30) are measured to assess hepatocyte apoptosis. Elevated M30 levels are strong indicators for apoptotic cell death of injured hepatocytes and support further investigations, e.g., liver biopsy. Non-elevated M30 levels confirm the normal transaminase levels and justify no further investigation or to monitor the patient by a regular (for example yearly) M30 measurement.

B. Patients with slightly elevated transaminase levels (between 1-2 x ULN) are suspected for liver damage. To confirm the liver injury suspicion, serum ccK18 levels (M30) are measured to assess hepatocyte apoptosis. Elevated M30 levels confirm the suspicion for apoptotic cell death of injured hepatocytes and support further investigation by e.g., liver biopsy and further treatment. Non-elevated M30 levels justify no further investigation or to monitor the patient by a regular (for example yearly) M30 measurement.

C. Patients with high transaminase levels (> 2 x ULN). NASH can be assumed. M30 measurement can be considered as independent confirmation of apoptotic cell death of injured hepatocytes, followed by further investigation by liver biopsy and treatment. Elevated transaminase levels caused by drugs that affect the liver (e.g., barbiturates) should be excluded.

A decision for further investigation (liver biopsy) and treatment of patients based on ALT/AST would miss a significant number of patients with an existing liver problem. Suggested new diagnosis management involves three options. Patients suspected of NAFLD are first tested for routine serum liver enzyme levels.

3.5 Conclusions regarding the use of the ccK18 test in NAFL and NASH patients

- The apoptosis specific biomarker ccK18 is able to discriminate healthy persons or those with benign fatty liver (simple steatosis) from patients suffering from NASH in children and adults. ccK18 is a reliable test to diagnose NASH, with a cutoff value of > 200 U/L.
- In practice, ccK18 measurements seem a useful and objective non-invasive tool to assess NASH in patients suspected of NAFLD (for example obese) and predict progression of NAFLD to NASH.
- ccK18 can be used to identify an increased risk for developing NASH in PCOS patients.
- ccK18 may also be useful as a liver injury biomarker in diabetic patients to diagnose fatty liver disease and predict possible progression to NASH.
- ccK18 measurements can be used to monitor liver disease progression or improvement, following treatment or lifestyle interventions. ccK18 can also improve decisions on therapeutic regiments for patients.
4 DETECTION OF HEPATOCELLULAR CARCINOMA (HCC)

Hepatocellular carcinoma (HCC) is an increasing burden with very limited treatment options [37]. The majority of HCC cases develop in chronically inflamed livers due to chronic viral hepatitis, alcohol abuse and, with rapidly increasing incidence in patients with NASH [38]. Curative treatment options are confined to a small proportion of HCC patients [39], therefore, early diagnosis as well as estimation of survival and risk stratification is an important issue for HCC patients [36]. A commonly used laboratory screening parameter is alpha-Fetoprotein (AFP) [40]. However, it has low sensitivity and specificity and its use for HCC surveillance is not recommended anymore as ultrasound imaging has a more favorable efficiency [41]. However, dynamic imaging is a cost-intensive technique and therefore, new blood-based parameters with higher sensitivity and specificity for HCC diagnosis are desirable [36].

4.1 Detecting HCC and predicting survival

In a recent study by Kronenberger et al. [36] M30 and M65 cell death parameters were investigated in HCC patients. HCC patients showed higher serum levels of M30 and M65 than patients with liver cirrhosis without concomitant malignant disease; both markers were able to differentiate patients with HCC from cirrhotic patients. High M65 and macrophage activation marker sCD163 levels but not M30 serum levels were associated with overall mortality in patients with HCC. Accordingly, they found a strong correlation between M65 and sCD163, indicating that overall cell death, mostly caused by necrosis, is associated with macrophage activation.

In conclusion, the epithelial cell death marker K18, as measured by the M65 ELISA, has the potential to improve non-invasive diagnosis of HCC including early HCC. Elevated levels of M65 are associated with poor prognosis.

5 DRUG OR TOXIC CHEMICAL INDUCED LIVER INJURIES

Toxic liver injury can be caused by many drugs and toxic chemicals, and is always associated with liver steatosis, inflammation and fibrosis. Drugs causing liver damage are barbiturates, narcotics, methotrexate, chlorpromazine salicylates (aspirin). Newly developed drugs are always tested for Drug Induced Liver Injury (DILI).

Vinyl chloride (VC) is a potent liver toxic chemical widely used in the industry. VC workers exposed to VC on a regular basis may develop liver steatosis and fibrosis. Among highly exposed VC workers, the prevalence of steatohepatitis was 80% and 55% had fibrosis [24]. Although mean serum transaminases were normal in these patients, total K18 (M65 EpiDeath* ELISA), reflecting total cell death (apoptosis and necrosis), was elevated. However ccK18 (M30 Apoptosense* ELISA) reflecting apoptosis was not elevated. These observations are suggesting the presence of necrotic cell death.

SUMMARY

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<thead>
<tr>
<th>Assay</th>
<th>Description</th>
<th>Application</th>
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<tr>
<td>M30 Apoptosense* ELISA</td>
<td>Apoptosis</td>
<td>NASH / Liver Diseases</td>
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<tr>
<td>M65* ELISA</td>
<td>Apoptosis &amp; Necrosis: Total Cell Death</td>
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<td>M30 Apoptosense* / M65* ELISA</td>
<td>Apoptosis / Total Cell Death</td>
<td>Ratio of Cell Death Modes</td>
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<td>M65 EpiDeath* ELISA</td>
<td>Apoptosis &amp; Necrosis: Total Cell Death</td>
<td>Toxicology</td>
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### M30 Apoptosense® ELISA

The M30 Apoptosense® ELISA measures soluble human intermediate filament protein fragments of keratin 18 (K18) that contain the M30 neo-epitope (K18Asp396-NE) released from human epithelial cells. M30 detects only caspase-cleaved fragments of K18 (ccK18; K18Asp396) but not the native protein. Antigen generation is strictly dependent on caspase activation. The presence of ccK18 in plasma therefore shows epithelial cell apoptosis.

#### Reference values

For details, see chapter 2. ccK18 values were measured in 200 apparently normal Swedish blood donors. With the M30 Apoptosense® ELISA, male and female values showed a similar distribution and no age dependency. Based on the distribution the recommended cut-off value for elevated ccK18 has been set at > 200 U/L.

In case of suspected fibrosis or fatty liver disease: ccK18 values between 150 and 200 U/L suggest an indication for mild fibrosis (for example NAFL); ccK18 values > 200 U/L are a strong indication for severe significant liver damage (for example NASH).

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<tr>
<td>Sample type</td>
<td>Human serum or plasma (EDTA, citrate, heparin plasma), K18Asp396-NE (M30)-reactive material released from apoptotic K18 positive human cells. The same type of material, i.e. serum or plasma collected by one method, should be used for a specific project.</td>
</tr>
<tr>
<td>Sample preparation</td>
<td>Fresh samples are stable for up to maximum one day at 2 – 8 °C, for at least 9 months at -20 °C, and for at least two years when stored at -80 °C. Avoid repeated freeze-thawing.</td>
</tr>
<tr>
<td>Reference values</td>
<td>≤ 150 U/L Healthy</td>
</tr>
<tr>
<td></td>
<td>150 – 200 U/L Slightly elevated</td>
</tr>
<tr>
<td></td>
<td>&gt; 200 U/L Elevated</td>
</tr>
<tr>
<td>Species</td>
<td>Human, non-human primates.</td>
</tr>
<tr>
<td>Hook Effect</td>
<td>No high dose “hook effect” occurs until 50,000 U/L which is well above concentrations of K18Asp396-NE (M30)-reactive material observed in cell culture samples.</td>
</tr>
</tbody>
</table>
M65® ELISA

The M65 ELISA is optimized for clinical use and use in combination with the M30 Apoptosense® ELISA. It measures total soluble K18 released from dead cells (necrotic and apoptotic). M65 measurements will therefore represent the total epithelial cell death by any cause.

Reference values

Total soluble K18 (uncleaved and cleaved Keratin 18 representing total cell death (necrosis and apoptosis) was measured in >200 apparently normal Swedish blood donors. Male and female values showed a similar distribution and no age dependency. Based on the distribution the cut-off value for elevated K18 has been set at >450 U/L.

| Cat. No. | 10020 |
| Tests    | 96    |
| Method   | ELISA |
| Range    | 125 - 2000 U/L (The Units measured by the M65® ELISA are defined against a synthetic standard. 1 U/L = 1.24 pM.) |
| Sensitivity | 11 U/L |
| Incubation time | 2 hours 20 minutes |
| Sample volume | 25 µl |
| Sample type | Human serum or plasma (EDTA, Citrate, Heparin plasma), containing K18-reactive material released from K18 positive human cells. Multiple freeze-thaw cycles of samples are well tolerated. The same type of material i.e. serum or plasma collected by one method should be used for a specific project. |
| Sample preparation | Fresh samples are stable for up to two days at 2 – 8 °C; for at least 9 months at -20 °C; and for at least two years when stored at -80 °C. |
| Reference values | In serum from 222 Swedish blood donors, the median level was 264 U/L with a range between 136 – 480 U/L. The 95th percentile was 413 U/L. |
| Species | Human, non-human primates, bovine. |
| Specificity | The assay uses two monoclonal antibodies directed to epitopes in the 284 – 396 region of the K18 protein. Soluble full length K18 as well as K18 fragments and protein complexes that expose these epitopes will be detected by the assay. |
| Hook Effect | No high dose “hook effect” occurs before 70 000 U/L. |
M65 EpiDeath® ELISA

Measures total soluble K18 released from dead cells (necrotic and apoptotic). M65 measurements will therefore represent the total epithelial cell death by any cause.

Reference values

Total soluble K18 (uncleaved and cleaved Keratin 18 representing total cell death (necrosis and apoptosis) was measured in 200 apparently normal Swedish blood donors. Male and female values showed a similar distribution and no age dependency. Based on the distribution the recommended cut-off value for elevated K18 has been set at > 200 U/L.

| Cat. No. | 10040 |
| Tests   | 96    |
| Method  | ELISA |
| Range   | 67 – 5000 U/l (The units measured by the M65 EpiDeath® ELISA are defined against native antigen spiked into serum. Native antigen is calibrated against a recombinant protein standard. 1 U/l = 1.24 pM). |
| Sensitivity | 25 U/L |
| Incubation time | 4.5 hours |
| Sample volume | 25 µl |

Sample type

Human serum or plasma (EDTA, citrate, heparin plasma), K18-reactive material released from K18 positive human cells. Multiple freeze-thaw cycles of samples are well tolerated. The same type of material, i.e. serum or plasma collected by one method, should be used for a specific project. Cell culture supernatants from K18 positive (epithelial) cells or tissues.

Sample preparation

If the assay is to be performed the same day, the samples can be stored at 2 – 8 °C. Samples are stable for at least 9 months at -20 °C, and for at least two years when stored at -80 °C.

Reference values

≤ 150 U/l Healthy
150 – 200 U/l Slightly elevated
> 200 U/l Elevated

Species

Human, non-human primates

Specificity

The assay uses two monoclonal antibodies directed to epitopes in the 284 – 396 region of the K18 protein. Soluble full length K18 as well as K18 fragments and protein complexes that expose these epitopes will be detected by the assay.

Hook Effect

No high dose "hook effect" occur until 200,000 U/l which is well above concentrations of M65-reactive material observed in cell culture or serum/plasma samples.
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