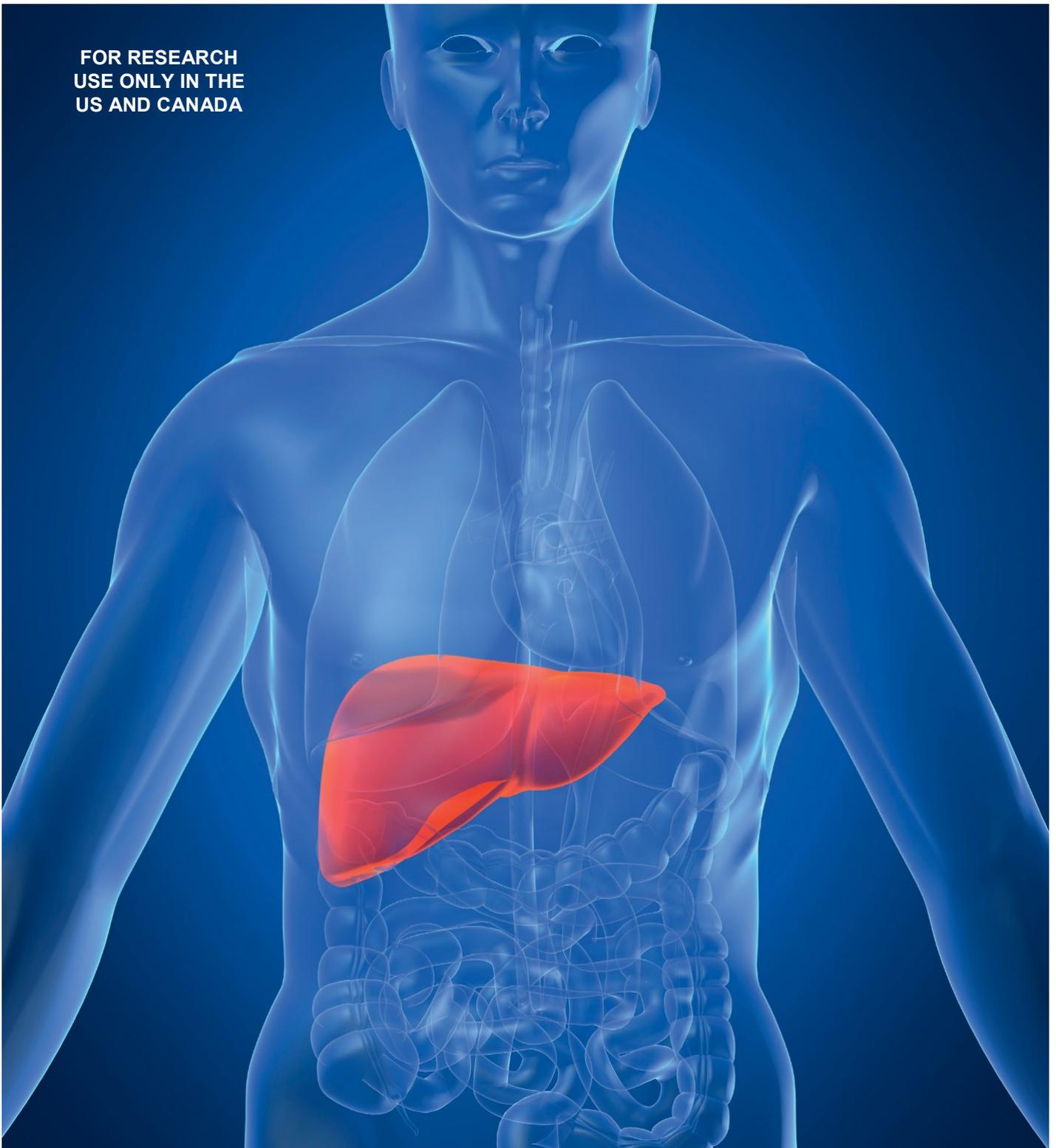


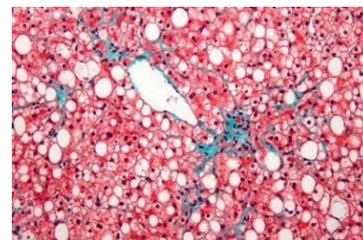
Novel Liver Injury Biomarkers

FOR RESEARCH
USE ONLY IN THE
US AND CANADA



Novel Liver Injury Biomarkers

Drug-induced liver injury (DILI) is a leading cause of liver failure and transplantation in western countries. In order to reduce the risk of failure of new medicines, predicting which drugs will prove toxic to the liver is an important aspect of the drug development process. As a result, groups in both the US and Europe are focused on validating new, cost-effective liver injury biomarkers. The European Innovative Medicines Initiative (IMI) is evaluating biomarkers such as caspase-cleaved & total Keratin 18 and α -GST for both clinical application and regulatory decision-making in clinical and translational drug development. In the US, the Critical Path Institute's (C-Path) Predictive Safety Testing Consortium, a program run in partnership with the Food and Drug Administration (FDA), is evaluating α -GST for similar reasons as IMI.



Micrograph showing a fatty liver

Liver fibrosis, cirrhosis, inflammation and steatosis are major features of acute and chronic liver injury. These liver injury manifestations are further associated with an increased risk of developing hepatocellular carcinoma. For individuals affected by liver damage, studies of fibrosis and steatosis are particularly valuable.

The most common traditional biomarkers of drug-induced liver injury, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), have several limitations. In testing a therapeutic for its potential to cause liver injury, AST/ALT increases are commonly observed even in the absence of evidence of tissue injury, and, conversely, they sometimes do not increase when tissue injury is observed. In clinical settings, AST/ALT levels frequently do not increase even when liver injury is evident. It has been reported that up to 25–30% of patients with fibrosis liver damage may have normal transaminase levels. Liver biopsy is still the gold standard for the detection of liver damage though this invasive examination method is limited by sample errors (e.g. inhomogeneous liver damage) and the risk of clinical complications.

Novel liver injury biomarkers provide useful information about the nature of the liver damage and have practical utility in following research situations:

Drug Development and Pre-clinical Studies

- Research of liver fibrosis and cirrhosis.
- Research or studies of NAFLD and NASH.
- Studying of NAFLD progression into NASH (with risk of developing liver cirrhosis and hepatocellular carcinoma).
- Measuring subjects with insulin resistance and other symptoms of metabolic syndrome for NAFLD and NASH.
- Research of hepatocellular carcinoma.
- Investigation of polycystic ovary syndrome subjects with NASH.
- Estimation of liver fibrosis in HCV and HBV-infected subjects.
- Research of graft versus host disease and evaluation of response to immunosuppressive agents.
- Environmental toxicant-induced liver injuries
- Drug development toxicity studies using liver cell culture models.
- Preclinical toxicity studies in animals.
- Toxicity studies in humans.

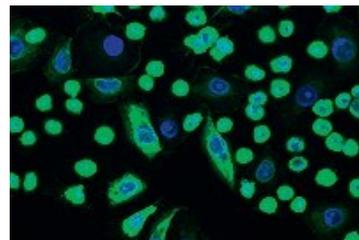
Liver injury biomarker	Indicative for
Caspase - cleaved Keratin 18 (ccK18: M30 ELISA)	Hepatocyte apoptosis
Keratin 18 (cleaved and uncleaved: M65 ELISA)	Hepatocyte apoptosis and necrosis
α -Glutathione S-Transferase (α -GST)	Hepatocyte damage
Collagen IV (serum)	Increased collagen deposition and liver fibrosis
Hyaluronic acid (HA)	Liver fibrosis and loss of liver function

Type of information provided by various liver injury biomarkers

Liver Injury Biomarker tests

Caspase - cleaved Keratin 18 (ccK18)

- Keratin 18 (K18) is an intermediate filament required for maintenance of the cytoskeletal architecture.
- Expressed by epithelial cells like hepatocytes and cleaved by apoptosis enzymes called caspases.
- ccK18 serves as a highly specific marker for hepatocyte apoptosis, which is considered to play a crucial role in the progression of liver disease.



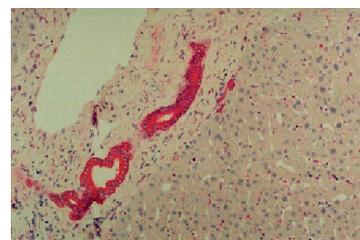
ccK18 in apoptotic cells

Keratin 18 (cleaved and uncleaved)

- Keratin 18 (K18) is an intermediate filament required for maintenance of the cytoskeletal architecture.
- Expressed by epithelial cells like hepatocytes and cleaved by apoptosis enzymes called caspases.
- K18 serves as a highly specific marker for hepatocyte cell death (necrosis and apoptosis).

α -GST (α -Glutathione S-Transferase)

- Constitutively expressed cytosolic protein involved in cellular detoxification.
- Localized to hepatocytes.
- Rapidly released (LEAKAGE) when hepatocytes are damaged.



α -GST in Hepatocyte

Collagen IV

- Structural protein of extra cellular matrix, abundant component of the basement membrane.
- The first collagen to be deposited when membranes are deposited.
- Biomarker of active collagen deposition in liver.
- Excellent biomarker of liver scarring and fibrosis.

Hyaluronic acid (HA)

- Large, linear, non-sulfated glycosaminoglycan (GAG) synthesized by fibroblasts and other connective tissue cells.
- Major component of connective tissues; about one fourth is found in the skeleton and its supporting structures like ligaments and joints.
- Measure serum levels of HA in subjects with liver fibrosis and cirrhosis

Novel Liver Injury Biomarkers

M30 Apoptosense® ELISA (PEVIVA®)

for Research Use Only

Measurement of caspase cleaved fragments of human intermediate filament protein Keratin 18 (cck18*) that contain the apoptosis specific M30 neo-epitope (K18Asp396-NE). Released from human epithelial cells following apoptosis.

Cat. No.:	P10010
Tests:	96
Method:	ELISA
Range:	75 – 1000 U/l (units are defined against a synthetic peptide standard containing the M30 and M5 epitopes; 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), 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. Mouse plasma can be used for measurement of human xenografts. Reference: Olofsson et al, Cancer Biomark , 2009 Cell lysates or cell culture supernatants from K18 positive (epithelial) apoptotic cells or tissues – For cell culture samples, the M30 Apoptosense® ELISA or the M30 CytoDeath™ ELISA can be used.
Sample preparation:	Fresh samples are stable for a maximum of 1 day at 2 – 8 °C, for at least 9 months at -20°C, and for at least 2 years at -80°C. Avoid repeated freeze-thawing.

Species: Human, non-human primates

Hook Effect: No high dose “hook effect” occurs until 50000 U/l which is well above concentrations of K18Asp396-NE (M30)-reactive material observed in cell culture samples.

Intended Use:

The M30 neo-epitope (K18Asp396-NE) is a sensitive and integrative indicator specific for epithelial cell death involving caspase -3, -7 or -9 activation.

Quantitative measurement of the apoptotic cell death biomarker K18Asp396-NE (“M30 antigen”) released from dying human epithelial cell lines in vitro or in vivo. The cells or tissues should be of human epithelial origin (e.g. kidney, gut, colon, lung or liver) expressing K18.

***Note:** caspase-cleaved K18 = cck18 previously Cytokeratin 18 (CK18/ccCK18)

M65 EpiDeath® ELISA (PEVIVA®)

for Research Use Only

Measurement of cleaved and uncleaved fragments of human intermediate filament protein keratin 18 (K18). Released from human epithelial cells following total cell death (apoptosis + necrosis).

Cat. No.:	P10040
Tests:	96
Method:	ELISA
Range:	200 – 5000 U/I (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/I = 1.24 pM).
Sensitivity:	25 U/I
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.

Species:	Human, non-human primates
Hook Effect:	No high dose “hook effect” occur until 200000 U/I which is well above concentrations of M65-reactive material observed in cell culture or serum/plasma samples.
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.

Intended Use:

Quantitative measurement of total soluble keratin 18 (K18) released from dead cells (necrotic and apoptotic). The cells or tissues should be of human epithelial origin (e.g. kidney, gut, colon, lung or liver) expressing K18.

The M65 EpiDeath® ELISA can be combined with the M30 Apoptosense® ELISA (PEVIVA Prod. No. 10010) for determination of cell death mode (apoptosis versus necrosis).

***Note:** caspase-cleaved K18 = ccK18 previously Cytokeratin 18 (CK18/ccCK18)

α -GST, Human serum & plasma

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Cat. No.:	TE1056
Tests:	96
Method:	ELISA
Range:	2.5 - 80 μ g/l
Sensitivity:	1.9 μ g/l
Assay time:	2 h
Sample volume:	100 μ l (Suggested initial dilution 1:5)
Sample type:	Serum & Plasma
Sample preparation:	Centrifuge within 3 hours from time of collection and transfer the sample from the original tube for storage. Samples can be stored at 20 – 25 °C for up to 48 hours, at 2 – 8 °C for up to 1 week, or at -20 °C for >1 year. Avoid repeat freeze- thaw cycles of samples.
Species:	Human
Reference values:	0 - 12 μ g/l
Cross reaction:	No cross-reactivity was observed with μ GST up to 500 μ g/l or π GST up to 500 μ g/l

Background:

α -GST is a member of the GST superfamily of small cytosolic proteins primarily involved in cellular detoxification reactions. α -GST is approximately 51kDa and comprises 2-5% of the soluble cytosolic protein content in hepatocytes. As a result of its constitutive nature, α -GST is quickly released into serum/plasma upon lysis of liver cells.

Intended use:

The α -GST EIA provides a method for the quantitative determination of α -glutathione S-transferase (α -GST) in human serum and plasma. The α -GST EIA can also be used for the determination of α GST in human urine.

Serum & Plasma:

α -GST is expressed in hepatocytes and its increase in blood is a sensitive indicator to study acute hepatic injury. Increased α GST can be used to study hepatotoxicity.

Collagen IV, Human serum

for Research Use Only

Cat. No.:	TE1053
Tests:	96
Method:	ELISA
Range:	15.6 – 1000 µg/l
Sensitivity:	15.6 µg/l
Incubation time:	1 hour
Sample volume:	50 µl
Sample type:	Serum
Sample preparation:	Samples can be stored at 2 – 8 °C for one week Samples may be stored at -20 °C for 12 months. Repeated freeze-thawing of samples should be avoided.

Reference values: 99 ± 23 µg/l (Mean ±1 SD, N= 180)

Species: Human

Cross reaction: Cross reactivity is less than 2% with Collagen II and less than 0.5% with other forms of collagen.

Interference:

No significant interference has been observed in this assay with lipaemic, haemolytic or icteric samples.

- Lipaemia: Less than 10 % interference up to 1200 Formazine turbidity units.
- Haemolysis: Less than 10 % interference up to 3 g/l haemoglobin.
- Icteric: Less than 10 % interference up to 0.2 g/l bilirubin.

Intended use:

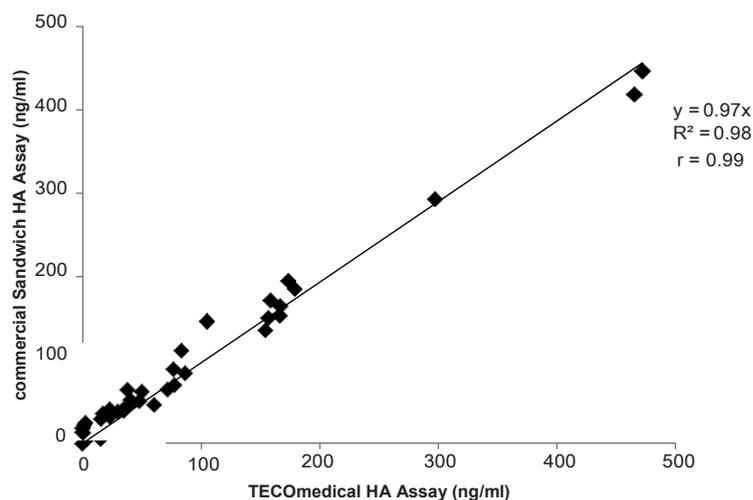
Collagen IV is a basement membrane protein found in the liver. Its deposition increases with liver fibrosis. As a consequence, serum/plasma levels can increase with progressing liver damage.

Hyaluronic Acid – Hyaluronan (HA) (TECO®)

for Research Use Only

Cat. No.:	TE1018-2
Tests:	96
Method:	ELISA
Range:	0 – 1000 ng/ml
LLOQ:	16 ng/ml
Incubation time:	3 hours
Sample volume:	100 µl (dilute 1:20)
Sample type:	Serum, EDTA-plasma and cell culture supernatant
Sample preparation:	Fasting blood collection. Serum or EDTA Plasma stable for 72 hours at 2–8 °C, 6 months at -20 °C, longer storage at -80 °C. Maximum 3 freeze- and thaw cycles.

Correlation: Between TECOmedical HA Assay and a commercial Sandwich HA Assay



Background:

Hyaluronic acid, also known as Hyaluronan (HA) is a large, linear, non-sulfated, glycosaminoglycan (GAG) with a molecular weight between 106 and 107 Da. It is a major component of connective tissues and thus distributed ubiquitously in the organism. About one-half of the body's entire hyaluronic acid is found in the skin and about one fourth in the skeleton and supporting structures like ligaments and joints. Hyaluronic acid is synthesized by fibroblasts and other specialized connective tissue cells. Hyaluronic acid is especially important for the structure and organization of extracellular matrices. The hyaluronic acid network acts as an osmotic buffer and is responsible for water homeostasis as well as it regulates protein distribution via the formation of flow and diffusion barriers. Additionally, hyaluronic acid interacts with proteins and cell surfaces and thus has a strong influence on cell proliferation, differentiation and tissue repair. Importantly, about 90% of all serum HA is cleared by the liver.

Intended use:

The Hyaluronic Acid ELISA provides a method for the quantitative determination of Hyaluronic Acid in plasma, serum and other biological fluids. Serum/plasma concentrations can increase as a result of liver damage.