News

CE Mark
All Chromsystems products will shortly carry CE marking.

Chromsystems on the move!
In May 2002 we are moving to new premises in Munich with significantly extended laboratory, production and warehouse facilities. Fully equipped, modern seminar rooms will be available for customer workshops and training courses.

Look back

Medica 2001 – a tremendous success.
In Düsseldorf in November Chromsystems exhibited its product range at the Medica 2001 Exhibition. Customers from all over the world were able to gain first hand knowledge of the following new product highlights:

> Monitoring of oxidative stress
> Optimised Metanephrine testing
> Therapeutic Drug Monitoring of Levetiracetam (Keppra®)
> New internal standard for Vitamin A/E analysis
> Haemoglobin variant controls

Dates

In the first half of 2002 Chromsystems will attend the following exhibitions:

21.–24. March 2002:
Tüyap Ekspomed Labtek, Istanbul

23.–26. April 2002:
Analytica 2002, Munich

21.–24. May 2002:
Focus 2002, Glasgow

In addition, a series of practical workshops will again be held in our application laboratory in Munich (dates on request).

Planned international HPLC workshops:
Mid April 2002: Teheran, Iran
May/June 2002: Dubai, UAE

Subjects to alteration.

CHROMSYSTEMS Instruments & Chemicals GmbH
Fraunhoferstraße 22
D-82152 Martinsried/ München

Phone
+49 (89) 89 97 92-20
Fax
+49 (89) 89 97 92-25

eMail:
mailbox@ chromsystems.de
Internet:
www.chromsystems.de

1/2002

DIGEST

2002/Holzner & Lengnick

An increased occurrence of free radicals or reactive oxygen species (ROS) in an organism can lead to considerable cellular damage (oxidation of lipids, proteins and DNA), so-called oxidative stress. These impaired oxidation processes in the long-term lead to early cell ageing and "anti-ageing" therapies are therefore applied for cell regeneration. There is no specific indicator for oxidative stress; rather, the occurrence or aggravation of multiple symptoms are indicative: cardiovascular, gastrointestinal, immunological or dermatological diseases, infections, carcinogenesis, mutagenesis, neurological diseases, arthrosis et al.
A healthy organism is able to cope with free radicals by means of antioxidants and enzymatic mechanisms of detoxification.

Monitoring Oxidative Stress

There are basically three possibilities for the screening of oxidative stress in the clinical laboratory. First, the antioxidative supply, i.e. the level of specific antioxidative substances in the organism, can be quantified; second, the activities of detoxifying enzymes; and, last but not least, oxidative damage can be measured. Oxidative damage parameters are metabolites occurring typically under oxidative stress.

Antioxidative Supply

Antioxidants are compounds that scavenge free radicals and thus protect the organism from oxidative damage.

MONITORING OXIDATIVE STRESS

Antioxidative Supply	Detoxifying Enzymes	Oxidative Damage
Vitamin E	Superoxide dismutase	Malondialdehyde
Vitamin C	Catalase	8-OH-Deoxyguanosine
β-Carotene	Glutathione peroxidase	
Coenzyme Q_{10}		
Glutathione		

Vitamin E
A main task of vitamin E is the protection of LDL cholesterol and unsaturated fatty acids in the phospholipids of cellular membranes. Lipid peroxidation is prevented by reaction of free radicals with vitamin E to vitamin E radicals within the membranes. This vitamin is therefore also known as a radical scavenger.
Lack of vitamin E leads to an increased lipid peroxidation. This process leads to increased malondialdehyde serum levels which can be detected in the laboratory. Vitamin E can be regenerated from the vitamin E radicals with vitamin C, glutathione, and coenzyme Q_{10}.

Vitamin C
Vitamin C has three important tasks:
1. It scavenges free radicals.
2. It is necessary for enzymatic detoxification as it hydroxylises and activates superoxide dismutase.
3. It has a synergistic effect with vitamin E as it regenerates vitamin E radicals and it helps to repair lipid peroxidations:

R-O-O-H + vitamin C →
R-O-H + H_2O + dehydroascorbic acid

β-Carotene
β-carotene has great antioxidative potential and can quench free radicals as well as the very aggressive singulett oxygen. Such reactive and toxic molecules play a role in inflammatory processes and occur together with increased levels of homocysteine, leading to damage of cellular membranes and the DNA. This is being discussed as a trigger factor for cardiovascular diseases. Several recent clinical studies show that a sufficient supply of β-carotene helps to protect the organism from arteriosclerosis and cancer.

Coenzyme Q_{10}
Coenzyme Q_{10} is the most frequent ubichinone. Additionally to its function as provider of cellular energy, it has antioxidative potential. Similar to vitamin C, it has synergistic effects with vitamin E and prevents lipid peroxidation.

Glutathione
Glutathione is a tripeptide and works as a most important buffer for redox potentials in the organism. It guarantees the reductive level in the cell plasma. This is achieved by changing the ratio of the oxidised and reduced form of glutathione (GSSG and GSH respectively). This ratio can be used for the diagnosis of the antioxidant status.
Another task of glutathione is the detoxification of hydroperoxides with the glutathione peroxidase (see capacity of detoxification).

Enzymatic capacity of detoxification

There are also enzymatic mechanisms for defence against free radicals and reactive oxygen species in the cell. The superoxide dismutase (SOD) catalyses the reaction of the superoxide anione radical to H_2O_2 and oxygen. The oxidative H_2O_2 is detoxified by the enzymes catalase and glutathione peroxidase (GPx) producing water and oxygen. GPx additionally regenerates peroxidised membrane lipids.

Oxidative Damage

If antioxidative supply or enzymatic detoxification capacity are no longer fully guaranteed oxidative damage of cell structures and tissues occurs. These reactions are mostly radical and yield typical metabolites. The amount of metabolites can be used as a marker for the extent of oxidative stress.

Malondialdehyde (MDA)
Malondialdehyde is formed by lipid peroxidation and is a marker for oxidative damage of cellular membranes.

8-Hydroxy-2′-deoxyguanosine (8-OHdG)
The attack of DNA by free radicals leads to specific changes of some components. Defective guanosine for example is set free to blood circulation as 8-OHdG by enzymatic repair mechanisms and then excreted by the kidney. 8-OHdG thus is a useful marker for oxidative changes of DNA in the nucleus and mitochondria.

Enzymatic capacity of detoxification

Superoxide dismutase (SOD):

$$O_2 \xrightarrow{e^-} O_2^{\cdot -}$$
Superoxidanion

$$O_2^{\cdot -} + O_2^{\cdot -} \xrightarrow[\text{Superoxide dismutase}]{2\,H^+} H_2O_2 + O_2$$

Catalase:

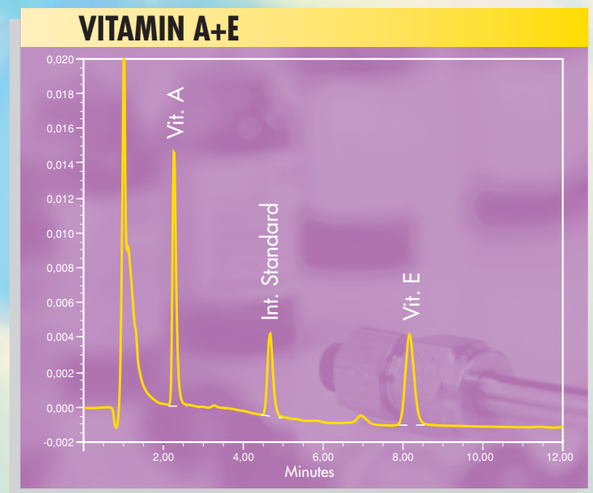
$$H_2O_2 + H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

Glutathione peroxidase:

$$2\,GSH + R\text{–}O\text{–}OH \longrightarrow GSSG + H_2O + ROH$$

Chromsystems Products for Monitoring of Oxidative Stress

All applications require only a simple isocratic HPLC system with UV detector. The sample preparations are based on fast and effective precipitation steps.

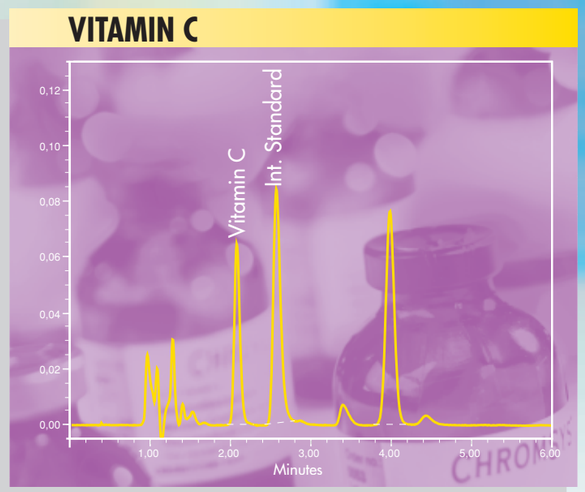


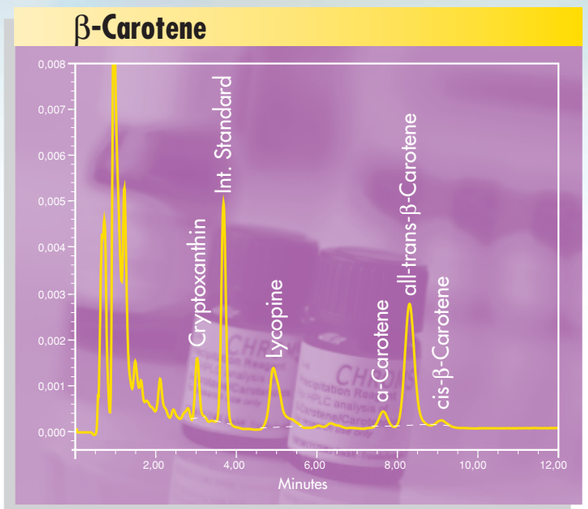
Vitamin A and E (Order no. 34000)

Sample volume and matrix:	200 µl serum/plasma
Injection volume:	50 µl
Flow rate:	1.5 ml/min
UV detection:	325 nm, after 4 min switch to 295 nm
Delivery date:	*available now*

Vitamin C (Order no. 65000)

Sample volume and matrix:	100 µl plasma
Injection volume:	20 µl
Flow rate:	1 to 1.5 ml/min
UV detection:	245 nm
Delivery date:	*expected February 2002*





β-Carotene (Order no. 32000)

Sample volume and matrix:	100 µl serum/plasma
Injection volume:	50 µl
Flow rate:	1.5 to 1.8 ml/min
UV/VIS detection:	453 nm
Delivery date:	*available now*

In progress:
> Coenzyme Q_{10}
> Glutathione
> Malondialdehyde

Delivery date: expected May 2002.

Clinical Relevance of the Determination of Mycophenolic acid Levels in Serum

Dr. M. Shipkova/Dr. PD. Niedmann/Universitätsklinik Göttingen, Zentrallabor, Germany

Mycophenolate mofetil (MMF) is the morpholine ethyl ester of the mycophenolic acid (MPA), which is the actual pharmacologically active substance. Mycophenolic acid is formed during the fermentation processes of some Penicillium species. The substance was developed in the 1960's as a drug with potential antibiotic, antineoplastic and antipsoriatic effect. It was first used in transplant medicine in the 1990's and goes back to A.C. Allison, who tried to find an immunosuppressive drug that selectively inhibits the proliferation of lymphocytes *(1)*. Today MMF is used in numerous imunosuppressive regimes for preventing graft rejection after renal, heart and liver transplantions. At the moment, concerning this application, intensive study is being carried out to see whether MMF can also be used in the transplantation of other solid organs, for other indications such as the prevention of graft versus host reaction in the case of haematopoietic stem cell transplantation or various autoimmune diseases. Another therapeutic approach aims at reducing the need for other immunosuppressants by co-administering MMF, thus minimising the occurrence of side effects, such as renal impairment triggered by cyclosporin A (CsA) *(2,3)*.

MMF acts as a non-competitive, reversible inhibitor of the inosine monophosphate dehydrogenase (IMPDH) *(4)*. This leads to the breakdown of de-novo purine synthesis in lymphocytes, which unlike other cells are dependent on this metabolic pathway. By reducing the intracellular pool to guanosine phosphates, the synthesis of DNA and RNA breaks down and the inhibition of the proliferation and function of the T-and B-lymphocytes follows. Furthermore the therapy with MMF causes a blocking of the transfer of mannose and fucose to adhesion molecules, thus preventing the accumulation of lymphocytes and macrophages in inflamed tissue regions of the graft. Moreover MPA is able to induce the apoptosis of activated T-lymphocytes and to reduce the formation of potentially damaging nitrogen oxide. Finally, in-vitro experiments have shown that the antiproliferative effect of MPA, even if less pronounced than in the case of lymphocytes, also occurs with fibroblasts, endothelial cells and smooth muscle cells. This pharmacodynamic property could have a favourable effect on the prevention of the chronic graft vasculopathy.

After MMF has been administered orally or intravenously it is very quickly hydrolysed to MPA *(2)*. The bioavailability of the active substance is around 94%. The maximum plasma concentration of MPA after being taken orally is reached in around 0.8 hrs. The average half-life for healthy people is 16 hrs. The main metabolite of MPA is the pharmacologically inactive 7-O-glucuronide (phenolic glucuronide, MPAG), which is mainly formed in the liver and of which up to 87% is excreted with the urine. The remainder is secreted into the bile and is subject to enterohepatic circulation. In the intestine deglucuronidation of MPAG to MPA takes place; this is reabsorbed resulting in a second MPA concentration maximum in plasma 6-12 hrs after the oral intake of MMF. In addition to MPAG, two other metabolites can be identified in humans. These are an acyl glucuronide, that is pharmacologically active in vitro like the parent substance, and a 7-O-glucoside. About 6% of the drug is eliminated unchanged with the stools. At clinically relevant concentrations MPA and MPAG are bound to albumin to 97% and 82% respectively *(2,5)*. In the blood, over 99.9% of the MPA is distributed extracellularly in the plasma. For this reason the plasma is the preferred medium for measuring the concentration of MPA.

The significance of therapeutic drug monitoring for optimisation of MMF therapy has not been fully clarified yet and is currently being studied intensively. Several clinical studies have shown that the pharmacokinetics of MMF are subject to great inter-individual variability. By monitoring the plasma MPA concentrations, the risk of an acute rejection after renal or heart transplantation can be reduced *(7,8)*. However monitoring the MPA-AUC (area under the curve) is more meaningful than determining trough concentrations before the next dose of the drug *(6-8)*. Furthermore renal and/or hepatic diseases can have an effect on the steady-state MPA-AUC value and on the free MPA concentration *(2,8)*. Because a significant correlation was observed between the AUC of the free MPA and the occurrence of severe infections *(7)*, monitoring the free MPA concentrations in the case of patients with altered protein binding is recommended in addition to the total MPA concentration. Changes in the MPA-AUC must also be taken into account when changing the co-medication. For example discontinuing steroids or interchanging CsA and tacrolimus. Other drugs such as metronidazole and antacids cause a reduction in the MPA-AUC levels *(2,8)*. In these cases, the MPA concentration in the plasma must be closely monitored.

The following provisional therapeutic ranges have been recommended for mycophenolic acid in the early phase after renal transplantation (triple therapy with ciclosporine, corticosteriods and MMF):

MPA pre-dose concentration:
1.0–3.5 mg/l
MPA-AUC-12 h interval:
30–60 mg x h/l

There is not yet a recommended therapeutic range for MPA in other therapeutic applications or for use as part of other therapy schemes.

Multiple blood collection, which is needed for the estimation of a full 12h MPA-AUC is very impracticable. Therefore various sampling schemas for an abbreviated AUC have been developed *(9,10)*.

To analyse the free mycophenolic acid, centrifugation of the plasma is carried out using ultrafiltration membranes (e.g. CENTRIFREE; Amicon/Millipore) followed by the measurement of MPA concentration in the filtrate *(5,11)*.

Literature

(1) Allison AC, Eugui EM (1994): Preferential suppression of lymphocyte proliferation by mycophenolic acid and predicted long-term effects of Mycophenolate Mofetil in transplantation. Transplant Proc 26(6): 3205-3210

(2) Bullingham RES, Nicholls AJ, Kamm BR (1998): Clinical pharmacokinetics of Mycophenolate mofetil. Clin Pharmacokinet 34(6): 429-455

(3) Mele TS, Halloran PF (2000): The use of mycophenolate mofetil in transplant recipients. Immunopharmacology 47(2-3): 215-245

(4) Allison AC, Eugui EM (2000): Mycophenolate mofetil and its mechanisms of action. Immunopharmacology 47(2-3): 85-118

(5) Nowak I, Shaw LM (1995): Mycophenolic acid binding to human serum albumin: characterization and relation to pharmacodynamics. Clin Chem 41(7): 1011-1017

(6) Shaw LM, Nicholls A, Hale M, Armstrong VW, Oellerich M, Yatscoff R, Morris RE, Holt DW, Vankataramanan R, Haley J, Halloran P, Ettenger R, Keown P, Morris RG (1998): Therapeutic monitoring of mycophenolic acid: a consensus panel report. Clin Biochem 31(5) 317-322

(7) Oellerich M, Shipkova M, Schutz E, Wieland E, Weber L, Toshoff B, Armstrong VW. (2000): Pharmacokinetic and metabolic investigations of mycophenolic acid in pediatric patients after renal transplantation: implications for therapeutic drug monitoring. German Study Group on Mycophenolate Mofetil Therapy in Pediatric Renal Transplant Recipients. Ther Drug Monit 22(1): 20-26.

(8) Shaw LM, Korecka M, DeNofrio D, Brayman KL. (2001): Pharmacokinetic, pharmacodynamic, and outcome investigations as the basis for mycophenolic acid therapeutic drug monitoring in renal and heart transplant patients. Clin Biochem 34(1): 17-22.

(9) Hale MD, Nicholls AJ, Bullingham RE, Hene R, Hoitsma A, Squifflet JP, Weimar W, Vanrenterghem Y, Van de Woude FJ, Verpooten GA. (1998): The pharmacokinetic-pharmacodynamic relationship for mycophenolate mofetil in renal transplantation. Clin.Pharmacol.Ther. 64 (6): 672-683.

(10) Schütz E, Armstrong VW, Andreeva M, Niedmann PD, Weber L, Tönshoff B, Oellerich M.(1997) Prediction of mycophenolic acid plasma AUC by three point model in pediatric kidney recipients. Ther Drug Monit. 19: 555 (Abstrakt)

(11) Shipkova M., Niedmann PD., Armstrong VW., Schütz E., Wieland E., Oellerich M. (1998): Simultaneous determination of mycophenolic acid and its glucuronide in human plasma using a simple high-performance liquid chromatographic procedure. Clin Chem 44(7): 1481-1488.

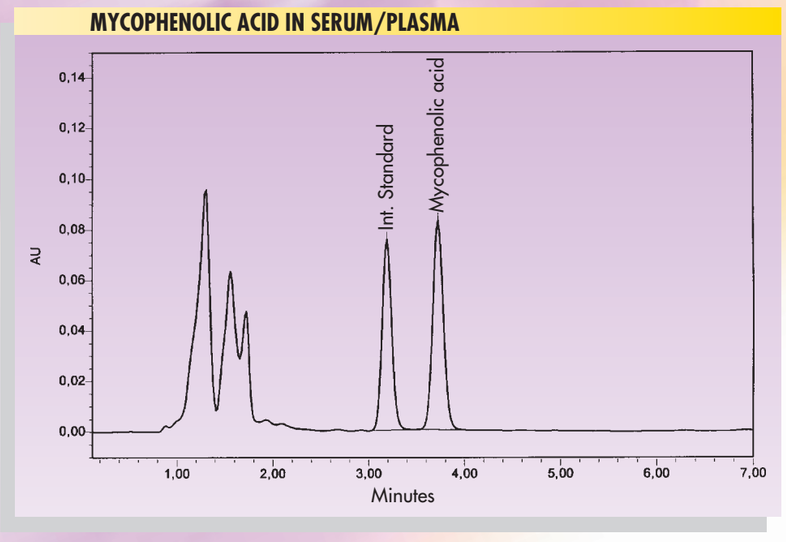
Chromsystems Reagent Kit for HPLC Analysis of Mycophenolic Acid in Serum

(Order no. 46000)

This reagent kit is designed for safe and reliable determination of Mycophenolic acid, the active form of the immunosuppressive drug mycophenolate mofetil in plasma/serum. By means of selective solid phase extraction interfering components are separated, the analyte is quantified by the inclusion of an internal standard. This kit combines fast sample throughput with high cost efficiency.

HPLC parameters:

Injection volume:	20-40 µl
Flow rate:	1 ml/min
Wavelength:	215 nm
Column temperature:	~ 25 °C



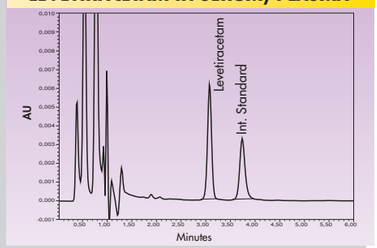
Completion of Antiepileptics Monitoring: Levetiracetam (Keppra®) in serum/plasma

Chromsystems now offers an HPLC reagent kit for the diagnosis of the new antiepileptic drug Levetiracetam (Keppra®) in serum/plasma. This kit completes the range of HPLC applications for antiepileptic drugs from Chromsystems.
This Chromsystems reagent kit is designed for the fast and reliable determination of Levetiracetam levels in serum/plasma. The sample preparation is based on solid phase extraction (SPE) which can be carried out very quickly and easily. Using the selectivity of SPE, all other antiepileptic drugs and their metabolites are removed. An internal standard is used to guarantee the exact quantification of Levetiracetam. The chromatographic separation is carried out on an RP column followed by UV detection.

HPLC parameters:

Injection volume:	10 µl
Flow rate:	1.5 ml/min
Wavelength:	210 nm
Column temp.:	~ 25 °C

LEVETIRACETAM IN SERUM/PLASMA

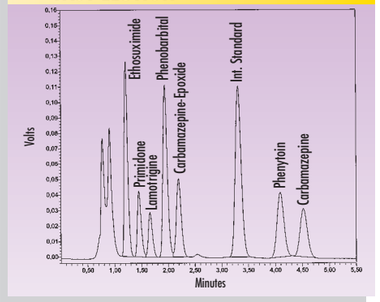


Already established in routine testing: Monitoring of important antiepileptics

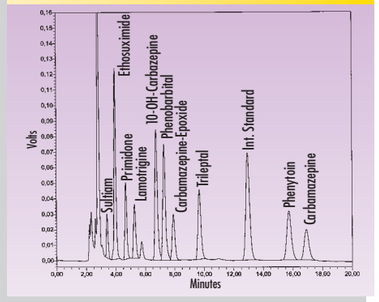
Specific monitoring of Ethosuximide, Primidone, Phenobarbital, Phenytoin, Carbamazepine, Lamotrigine, Trileptal and Sultiam in serum or plasma. In addition, the metabolites Carbamazepine-10, 11-epoxide and 10-OH-Carbazepine are assayed.

The sample preparation is based on a simple but efficient precipitation step. To quantify the analytes a high speed chromatography ("**Fast Elution**" in less than 5 minutes) and alternatively a "**High Resolution**" setup are available.

FAST ELUTION



HIGH RESOLUTION



IMMUNOSUPPRESSANTS CONTROLS (WHOLE BLOOD)

Levels I, II and, III cover the trough concentrations of the respective therapeutic ranges; Level IV is designed for quality control of peak levels immediately after drug administration.

Order no.	Product	Concentration range (dependent on batch)	Expiry date (unopened)	Stability (reconst.; +2-8 °C)	Stability (reconst., -20 °C)
0082	Lev. I	Ciclosporin A 80–100 µg/l Rapamycin (Sirolimus) 3–6 µg/l Tacrolimus (FK 506) 6–10 µg/l	2 years	7 days	min. 6 months
0083	Lev. II	Ciclosporin A 200–240 µg/l Rapamycin (Sirolimus) 8–10 µg/l Tacrolimus (FK 506) 9–12 µg/l	2 years	7 days	min. 6 months
0084	Lev. III	Ciclosporin A 400–500 µg/l Rapamycin (Sirolimus) 18–25 µg/l Tacrolimus (FK 506) 20–30 µg/l	2 years	7 days	min. 6 months
0085	Lev. IV	Ciclosporin A 1600–2000 µg/l Rapamycin (Sirolimus) 35–45 µg/l Tacrolimus (FK 506) 40–50 µg/l	2 years	7 days	min. 6 months

HEMOGLOBIN CONTROLS (WHOLE BLOOD)

The controls allow the additional identification of HbC.

0156	Hemoglobin A_{1c}, A_2, F	Hemoglobin A_{1c} 2–4 % Hemoglobin A_2 4–5 % Hemoglobin F 6–7 %	2 years	7 days (HbA_{1c}) 21 days (HbA_2, HbF)	min. 6 months
0157	Hemoglobin A_2, F, S, C	Hemoglobin A_2 2–3 % Hemoglobin F 0–1 % Hemoglobin S 8–9 % Hemoglobin C 7–8 %	2 years	21 days	min. 6 months

New!
HPLC Instruments from Chromsystems

HPLC Pump Chromsystems CLC 300

The HPLC Pump Chromsystems CLC 300 is a dual piston solvent delivery system. An affordable isocratic pump with excellent performance and reliability, it's ideally suited to routine clinical testing.

> **Stainless steel, analytical dual piston pump**
> **Dialogue programmable via menu-display**
> **RS 232C interface and analogue pressure output**

Electrochemical Detector Chromsystems CLC 100

The electrochemical detector Chromsystems CLC 100 has been specifically designed for HPLC analyses in a routine clinical environment. The combination of high sensitivity (with detection limits in the pictogram range) and easy handling are its particular features. Real potential display guarantees reliable and reproducible results. Depending on the polarity and strength of the working potential between the working and reference electrodes in the flow cell, single components of the sample are either oxidised or reduced. The resulting electron flow between the working and auxiliary electrodes is then registered as the signal.

> **Short equilibration time**
> **Simple handling of the cell**
> **Also suitable for narrow-bore columns**
> **AUTOZERO-Function**

Programmable Autosampler Chromsystems CLC 200

The Autosampler Chromsystems CLC 200 is an automatic sample processing system; the standard model can inject up to 120 samples. The analysis program can easily be adjusted to the individual application through the different menus.
The instrument can be used as a stand alone system or integrated into a system controlled via a PC.
The stepper motor driven syringe measures exactly the sample volume selected.

> **Programmable volume injection in increments of 1 µl**
> **Programming via stepwise dialogue through graphic display**
> **High sample loading capacity**
> **High reproducibility**

UV-VIS Detector Chromsystems CLC 400

The UV-VIS Detector Chromsystems CLC 400 is a high sensitivity, variable wavelength, single channel UV-VIS detector with diode array technology. Wavelength changes are achieved without any mechanical moving parts. The detector ideally meets the requirements of clinical routine testing.

> **Deuterium and Tungsten lamps for wavelengths from 190 to 720 nm**
> **Integrated peak detection**
> **Time and scan programmes**