



**Pharma**

## **Biomarker Assay List**

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**t**

## **TNO expertise and innovation in Biomarkers :**

- Design and advice of their use in(non-)clinical studies
- Biomarker discovery and biological validation
- From animal disease models to man (and vice versa)
- Bio-analytical validation
- Sample analysis
- Biological interpretation

## Preface

### **Biomarkers and their significance in drug development**

The rising costs of drug development are putting considerable demands on efficiency in the selection of suitable drug candidates. An effective strategy in improving the selection process in R&D is the proper selection and application of biomarkers for efficacy and safety during the different stages of the drug development pipeline. A biomarker has been defined as “*a characteristic that can be measured and evaluated as an indicator of normal biologic processes, pathologic processes or pharmacologic responses to therapeutic intervention*” (NIH Biomarker Definitions Working Group, 1998).

Other definitions have since evolved and the discussion on what biomarkers should be and where to apply them continues. Biomarkers are currently being used in various areas, including disease identification, target discovery and validation, volunteer/patient inclusion and stratification during clinical studies, drug efficacy and safety and prediction of drug response.

*Much more than just analysis of biomolecules and metabolites.*

The risk of publishing a list of analytes is that too much emphasis is laid on analysis. Not only are there many more candidates than mentioned in this list, including several “non-chemical” biomarkers, but the true value in our opinion lies in the strength of data analysis and biological interpretation. Indeed, we are keen to analyse virtually every bio-molecule that has ever been described and help you identify the unknown remainder.

We have a long-standing tradition in assay development and the analysis of bio-molecules, and our laboratories enable us to combine various biochemical and molecular platforms with state-of-the-art analytical instrumentation (LC-MS/MS, GCxGC-TOF-MS, MALDI-TOF and LT-Q-FTMS). But there is more to it than that. Data analysis, pathology, pharmacology and toxicology are also areas of our expertise. Our expertise and scientific track record in specific areas of disease, including cardiovascular, metabolic and bone/joint diseases as well as inflammation, enables us to help partners put together an optimum package of biomarkers and interpret the results.

### **About this publication**

The aim of this publication is to provide *an impression* of what is possible in terms of analysis of endogenous biomolecules and metabolites. The term biomarker may also be a little confusing here, since many of the molecules are not specific biomarkers in themselves. We have summarised the validated and non-validated assays/methods that are available at TNO.

Depending on the purpose of the study or the specific needs, additional validation of an assay (for example in a specific species or matrix) may be necessary prior to sample analysis. Please note that new assays will be added to this list on a regular basis. For this reason we invite you to contact us for any question on biomarkers or endogenous compounds to be analysed.

### **About TNO Pharma**

As part of TNO, with 5,000 employees one of the largest independent research organizations in Europe, TNO Pharma serves the pharmaceutical industry in the following areas: disease models, toxicology, kinetics & metabolism, pharmacology safety, drug delivery and analysis.

# 1 A brief introduction to biomarkers

## 1.1 Definitions and concepts

Biomarkers are by no means new in the life sciences and have been an essential and continuously expanding part of clinical diagnosis for ages.

Later, methods to describe and quantify drug response and side effects as well as clinical chemistry and hematology became integral parts of the drug development process.

In 1998, the NIH Biomarker Definitions Working Group defined a biomarker as “*a characteristic that can be measured and evaluated as an indicator of normal biologic processes, pathologic processes or pharmacologic responses to therapeutic intervention*”.

The working group also addressed the relationship between biomarkers, clinical endpoints and surrogate markers. A *clinical endpoint* is in fact the most credible indicator of drug response and is defined as “*a characteristic or variable that reflects how a patient feels, functions or survives*”.

During clinical trials, clinical endpoints should in principle be used, unless a biomarker is available that has risen to the status of a *surrogate endpoint* and is “*is expected to predict clinical benefit (or harm, or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic or other scientific evidence*.” The NIH working group points out that probably only a few biomarkers are likely to achieve a consensus surrogate endpoint status.

In addition to the NIH definition, other groups have proposed definitions and classifications. One example is that of the WHO International Programme on Chemical Safety (IPCS) which defines biomarkers in a broader sense as “*any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease. Biomarkers can be classified into markers of exposure, effect and susceptibility*.” A further discussion of the definitions falls beyond the scope of this publication.

It should be realised that biomarkers and surrogates can be either physiologic endpoints, such as blood pressure, bone density and QT interval, or they can be laboratory endpoints, such as blood glucose, cholesterol, PSA, and viral RNA.

## 1.2 Biomarker identification and selection

There are two basic approaches to the identification and selection of biomarkers. The classical method involves hypothesis and is based on *a priori* knowledge of a process or mechanism. However, non-biased approaches are becoming increasingly applied.

Genomics-based methods (transcriptomics, proteomics and metabolomics) combined with multivariate (pattern recognition) statistics form the basis for the discovery of novel biomarkers and biomarker patterns. Multivariate data analysis techniques such as Principal Component Analysis (PCA) and PCA coupled with discriminant analysis (PCDA) are very powerful tools in analysing data and detecting clusters of similar datasets as well as differences between datasets.

In addition to the transcriptome and the proteome, the *metabolome* has become very relevant in this respect. Metabolomics involves the identification and quantification of large sets of metabolites from cells or biological fluids and their changes due to physiological and non-physiological processes. Ideally, easily accessible matrices, such as blood or urine, are studied.



The molecular structural and abundance diversity of the metabolome is significant and some metabolite groups, like lipids, are present in concentrations ranging from picograms to milligrams per litre. Therefore, we are now using multi-platform analytical strategies which enable us to maximise coverage of the metabolome.

In addition to NMR, LC-MS and GC-MS play a central role. Other technology and combinations (ICP-MS for trace elements, FT-MS, CE, etc.) are used for specific or low abundance classes of compounds. In this area, TNO Pharma is working closely together with Beyond Genomics (Waltham, MA, USA, <http://www.beyondgenomics.com/>).

Beyond Genomics applies Systems Biology to discover novel biomarkers and develop biological pathway knowledge for detecting disease and measuring drug response. Systems biology uses an integrated approach to study and understand the function of biological systems, and how perturbations of such systems, for example the administration of a drug, affect their function [example in ref 1].

Bio-informatics and mathematics are the key elements in systems biology to enable data-integration and -interpretation.

### 1.3 The importance of biomarker profiles

It is a well-known physiological fact that any organism will try to maintain a situation of homeostasis for as long as possible, using various compensation mechanisms when its system is disturbed. Therefore, it is quite understandable that single biomarkers are often unable to provide sufficient information and specificity and that *biomarker profiles* are necessary. In addition, it is becoming increasingly apparent that early markers for disease will differ from markers in the later stages of disease progression.

### 1.4 Biomarker validity

It is not the intention of this document to make any statements or claims on the validity of an individual biomarkers or sets of biomarkers. Validity is a complex characteristic and subject to much discussion. Validity describes the extent to which a biomarker reflects a designated event in a biological system. Validity requires not only analytical but also biological and sometimes epidemiological knowledge as well as knowledge of the (many) interference factors. Our experts will be happy to discuss these issues with you.

## 2 Bone and cartilage degradation products

### *Biomarkers for joint disease*

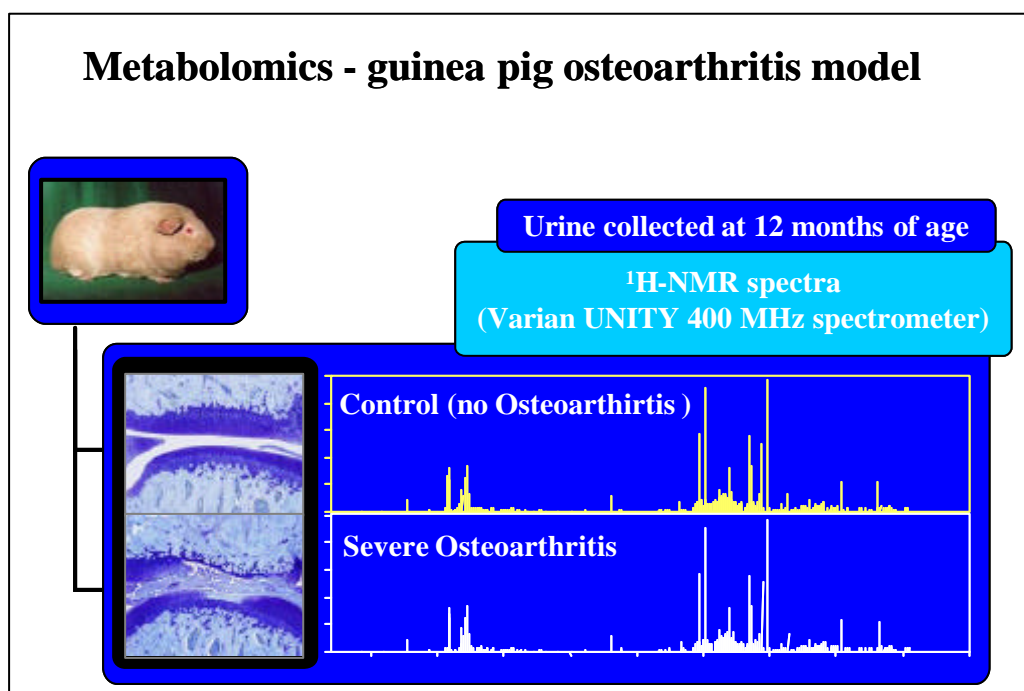
Cartilage, bone and synovial tissue are the major tissues involved in degenerative (e.g. osteoarthritis) and inflammatory (e.g. rheumatoid arthritis) joint diseases. Detailed understanding of the etiology and pathophysiology of these debilitating conditions is still lacking and imaging tools such as radiology currently do not (yet) provide sufficient means to diagnose the disease, monitor disease progression and effect of therapy and/or predict outcome of the disease. Since these diseases are multi-factorial and involve multiple tissues and processes panels of biomarkers are used to detect (the severity of) the disease. These include biomarkers for (aberrant) tissue synthesis, degradation as well as inflammation [ref 2].

### *Relevance as biomarkers*

To fully chart the processes occurring during joint disease a combination of biomarkers is needed that describes anabolic (e.g. CPII, 846 epitope, PICP, PINP, Osteocalcin) as well as catabolic (e.g. COMP, hydroxyproline, pyridinolines crosslinks HP and LP, glycosylated HP, CTX-I, NTx, pentosidine) and also inflammatory (e.g. hsCRP) processes in cartilage, bone, and tissue.

Within TNO, these biomarkers are routinely measured *in vitro* and *in vivo* in projects related to joint diseases. In addition to clinical studies they are used as biomarkers in the various disease models.

The biomarkers are mostly determined using, chromogenic and fluorogenic enzyme activity measurements, immuno-assays and HPLC methods. Most recently, we have developed NMR methodology to fingerprint the aberrant urinary metabolite composition in osteoarthritis [ref 3]. Other techniques, including immuno blotting, *in situ* hybridisation and Q-PCR are in use to localise enzymes etc. See also the sections on inflammation and oxidative stress as well as our information on *in vitro* and *in vivo* (osteo) arthritis disease.



Analyte	Information	Assay Principle
Hydroxyproline	Collagen degradation marker (connective tissue)	HPLC
HP (PYD)	Collagen crosslinks (bone and cartilage)	HPLC
LP (DPD)	Collagen crosslinks (bone)	HPLC
NTx	Collagen crosslinks (bone)	ELISA
CTx	Collagen crosslinks (bone)	ELISA
Glyc-HP	Synovial inflammation	HPLC
Pentosidine	Tissue degradation	HLPC
COMP	Cartilage degradation marker	ELISA
Osteocalcin (Bone GLA protein, BGP)	Rate of bone turnover	RIA
Osteocalcin carboxylation degree	Rate of bone turnover	RIA
PICP	Collagen type I synthesis marker (bone)	ELISA
PINP	Collagen type I synthesis marker (bone)	ELISA
CPII	Collagen type II synthesis marker (cartilage)	ELISA
846 epitope	Proteoglycan synthesis marker (cartilage)	ELISA
Calcitonin	Bone resorption, Ca and P resorption	RIA
C2C	Cartilage resorption	ELISA
C1, 2C	Cartilage and bone resorption	ELISA
Vitamin K1	Bone health	HPLC-fluorescence
25-hydroxy vitamin D	Bone health	RIA
1,25-dihydroxy vitamin D	Bone health	RIA
24,25-dihydroxy vitamin D	Bone health	RIA

### 3 Proteolytic Enzymes

#### Role of proteases

Proteases play a major role in many pathological conditions. Proteases are the effector molecules responsible for degradation of extracellular matrix in diseases involving tissue degradation, invasion or migration of cells (rheumatoid arthritis, (diabetic) nephropathy, tumor growth and metastasis, fibrosis, atherosclerosis, wound healing). Furthermore proteolytic activity is involved in cellular signalling, apoptosis and virulence of micro organisms and viruses (viral and bacterial infections).

Analysis of proteolytic activity provides new diagnostic and prognostic opportunities. Modulation of proteases is a promising approach for novel therapeutic intervention.

#### Relevance as biomarkers

Analysis of proteolytic activity provides new diagnostic and prognostic opportunities. Modulation of proteases is a promising approach for novel therapeutic intervention; analysis of proteolytic activity will give information about disease state and efficacy of therapy.

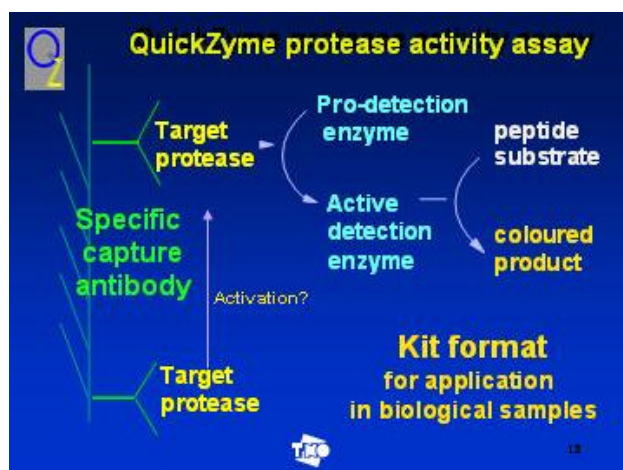
#### What we offer

We provide our customers with biomarker assays, and with tools and solutions facilitating development of therapeutics and diagnostics for protease-related diseases.

TNO Pharma offers its customers a variety of protease assays either as standard service (a list of assays is available on request) or custom developed. Apart from assays based on standard technology TNO Pharma offers its proprietary technology (QuickZyme™; see box) for both an increasing number of ready-to-use protease assays and for *custom assay development*. If required assays can be performed according to GLP regulations

#### Characteristics QuickZyme™ protease assays

- High sensitivity (low pM range)
- Chromogenic or fluorescent read-out
- Protein-based substrates
- Applicable for *any* protease target



Analyte	Information	Assay Principle
MMP-1	Biol.sampl* + screening, *F+* C	Immunologic / activity
MMP-2	Biol.sampl + screening, F+C	Immunologic / activity
MMP-3	Biol.sampl + screening, F+C	Immunologic / activity
MMP-8	Biol.sampl + screening, F+C	Immunologic / activity
MMP-9	Biol.sampl + screening, F+C	Immunologic / activity
MMP-13	Biol.sampl + screening, F+C	Immunologic / activity
MMP-14	Biol.sampl + screening, F+C	Immunologic / activity
MMP activity	General / gelatinase, F	Activity
MMP-1	Biol.sampl + screening F	Activity
MMP-3	Biol.sampl + screening F	Activity
MMP-13 gelatinase	Biol.sampl + screening F	Activity
Granzyme B	Biol.sampl + screening, F+C	Immunologic / activity
Cathepsin K	Biol.sampl + screening, F+C	Immunologic / activity
Cathepsin V	Biol.sampl + screening, F+C	Immunologic / activity
TNF $\alpha$ converting enzyme (TACE / ADAM17)	Biol.sampl + screening, F+C	Immunologic / activity
Cathepsin S	Biol.sampl + screening, F+C	Immunologic / activity
Cathepsin L	Biol.sampl + screening, F+C	Immunologic / activity
ADAMTS-1	Screening assay, F+C	Immunologic / activity
ADAMTS-4	Screening assay, F+C	Immunologic / activity
ADAMTS-5	Screening assay, F+C	Immunologic / activity
Alzheimer secretase / BACE	Biol.sampl + screening, F+C	Immunologic / activity
Anthrax Lethal Factor	Biol.sampl + screening, F+C	Immunologic / activity
MBL/MASP	Biol.sampl + screening, F+C	Immunologic / activity

\*F= fluorescent read out;

\*C= chromogenic read out

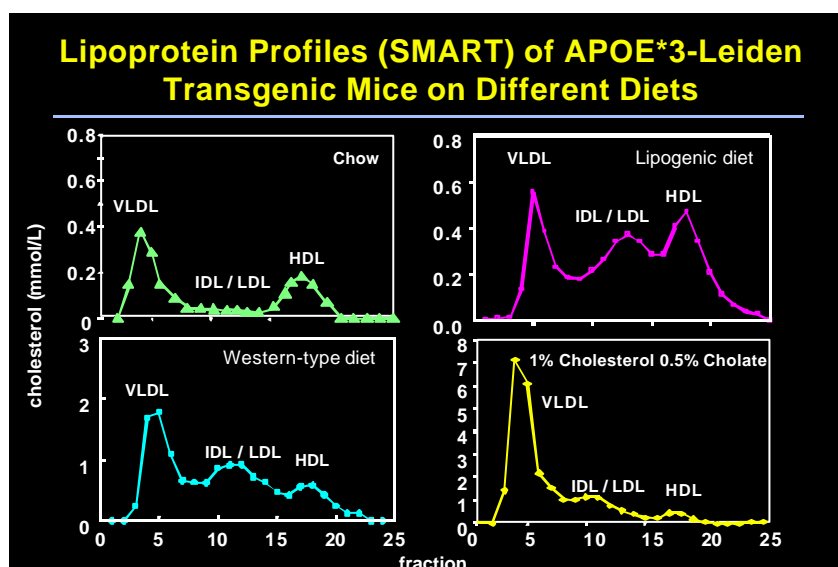
\*Biol samp = kit format, for measurement of biological samples

## 4 Lipids and apolipoproteins

The metabolism of cholesterol, other lipids and the serum profiles of these compounds are receiving considerable attention because of their association with amongst others cardiovascular disease (CVD) and the development of metabolic diseases. Until a few years ago many prevention- and therapeutic intervention strategies for CVD were aiming to reduce cholesterol or low density lipoprotein (LDL) cholesterol levels. It has now become clear that CVD is multi-factorial and that more processes, including inflammatory pathways, are involved in CVD. Statins for example were originally designed and developed to lower plasma cholesterol levels. It has now been shown that these compounds exhibit additional effects, independent of their cholesterol-lowering properties. Many of these beneficial pleiotropic effects appear to be related to protection of the vascular endothelium, and include anti-inflammatory effects. A recent study from our colleagues Kleemann *et al*, [ref 8] describes this phenomenon for Rosuvastatin.

A relatively new area in which we have become active is the use of lipid-related biomarkers to study hyperglycemia, insulin resistance and dyslipidemia due to antiretroviral (HIV-) therapy. TNO has a long-standing tradition in the field of lipid metabolism and apolipoproteins, in relation to atherosclerosis, inflammation and other diseases. For efficacy- and mechanistic studies [ref 9], TNO has developed the ApoE\*3 Leiden transgenic mouse model through the introduction of the human ApoE\*3 Leiden gene. The primary defect in the carriers of this dominant mutation is an impaired clearance of triglyceride rich lipoproteins (chylomicron- and VLDL-remnants).

ApoE\*3 Leiden mice thus exhibit elevated plasma cholesterol and triglyceride levels, mainly confined to the VLDL-IDL-LDL (apoB-containing) lipoproteins. Furthermore, APOE\*3-Leiden mice display a human-like lipoprotein profile and develop lesions that are comparable to their human counterparts with respect to morphological, histological and immuno-histochemical characteristics. A full biological analysis of the ApoE\*3 Leiden mouse, applying a Systems Biology approach, has been published recently together with our partners from Beyond Genomics Inc. [ref 1].



See also the sections on inflammation and vascular function. TNO was one of the first laboratories to propose and introduce C-reactive protein as biomarker for CVD,

Analyte	Information	Assay Principle
Cholesterol	Total	EIA
Cholesterol	Total	EIA
Cholesterol	Total	EIA
Cholesterol	Free	EIA
Cholesterol-ester	Esterified	Calculation
HDL-cholesterol		
HDL-cholesterol direct		
Phospholipids		
Lipoprotein separation	HDL2/HDL3/LDL/VLDL	
Lipoprotein separation	Selected: HDL2/HDL3	
Lipoprotein separation	Reduced: HDL/LDL/VLDL	
Lipoprotein separation		Seq. UCF
Lipoprotein separation		Agarose Elphor
LDL particle size		Ag-staining
Density		Densitometer
Lipids complete	TC, HC, TTG; LC	Package
LP composition	C,CE,TG,PL,Prot	Divers
Triglycerides	Total (Gross or Net)	EIA
Triglycerides	Total (Gross or Net)	EIA
Lipid sep. after Folch ext.		HPTLC
Lipid sep. after B.&Dyer ext.		HPTLC
Free fatty acid	Total	EIA
Free fatty acid	Total	EIA
Fatty acid profile		Esterizing
Free fatty acid profile (C14-C24)	FAME	GC
Total fatty acid profile (C8-C24)	FAME	GC
MDA-TBARS		
MDA	TBARS reagens and with HPLC separation	HPLC
Peroxides		Colorimetric
(V)LDL oxidation parameters	Lagtime,prop.rate,Vmax	Cu-oxidation
Protein conc. Lowry		Chemical
Protein conc. Bradford		Chemical
Bile acids	Total	EIA
Bile acid profile		Silylation
Phytosterols/ stanols	Latho-, lano-, sito-, campe	Silylation
Methylsterols		Silylation
Faecessterols		Silylation
Phytosterols/ stanols	Latho-, lano-, sito-, campe	GC

Analyte	Information	Assay Principle
Apo A-I		Antibody
Apo A-II		Antibody
Apo B-100		Antibody
Lp(a)		Antibody
Apo's A1, B100, Lp(a)	Package	Antibody
? 1-Glycoprotein		Antibody
Apo E		Antibody
Apo E-pheno-/genotyping		
LDL receptor genotyping		
HbA1c		Antibody
Glycated LDL		Antibody
Glycated Albumin		Antibody

## 5 Inflammation

### *Markers of Inflammation*

Inflammation represents a mechanism that is required as a first line of defence taking place immediately after tissue destruction. Usually inflammatory processes mediated by granulocytes, monocytes and lymphocytes resolve as soon as the job is done and tissue damage is under control. However, under certain conditions inflammation may become an uncontrolled entity that gives rise to a chronic disease stage.

TNO-Pharma has a strong background in inflammation that is traditionally based on fundamental and applied research in the field of multiple sclerosis, arthritis and atherosclerosis. More recently, the portfolio was expanded with models of Inflammatory Bowel Disease, Psoriasis and Atopic Dermatitis. Our ever-growing knowledge of these animal models of human disease is employed to identify and develop novel therapeutic strategies. TNO-Pharma has also developed considerable experience with respect to the inflammatory processes that play a role in human disease, largely based on cross-sectional and longitudinal studies. Based on this experience we are frequently involved as a partner in the designation of clinical trials as well as the identification and assessment of biomarkers during their monitoring phase.

Our expertise covers different levels of inflammatory diseases

- Human diseases;
- Animal models;
- Cell-based assays (proliferation, cytokines, COX-1, transcription factors, etc.);
- Activation markers on blood cells;
- Plasma levels of cytokines, chemokines, acute phase proteins.

Analyte	Information	Assay Principle
C-reactive protein (ultra-low)		Immunologic / EIA
LPS-induced TNF-alfa production in blood	Whole blood stimulation test	
SAA		Immunologic / EIA
s-PLA2		Immunologic / EIA
CD40-Ligand		Immunologic / EIA
IFN-gamma		Immunologic / EIA
TNF $\alpha$	High sensitive	Immunologic / EIA
Interleukin-1 $\beta$	High sensitive	Immunologic / EIA
Interleukin-4	High sensitive	Immunologic / EIA
Interleukin-6	High sensitive	Immunologic / EIA
Interleukin-8	High sensitive	Immunologic / EIA
Interleukin-10		Immunologic / EIA
Interleukin-12		Immunologic / EIA
Interleukin-18		Immunologic / EIA
TGF- $\beta$ 1		Immunologic / EIA
TGF- $\beta$ 2		Immunologic / EIA
NF-kappaB		Immunologic / EIA

Analyte	Information	Assay Principle
I-kappaB		Immunologic / EIA
NOx		EIA
MCP-1		EIA
RANTES		EIA
GCP-2		EIA
COX-1		Activity
COX-2		Activity

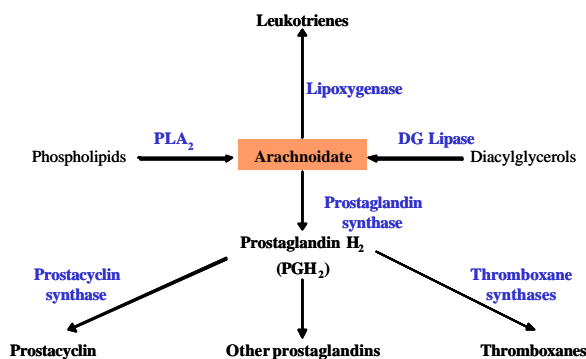
## 6 Eicosanoids

### *Eicosanoids*

Eicosanoids include prostaglandins, leukotrienes and the intermediate hydroperoxyeicosatetraenoic (HPETE) and hydroxyeicosatetraenoic (HETE) acids. The prostaglandins and leukotrienes act as paracrine and autocrine regulators through a family of transmembrane receptors. They regulate many cell functions and play crucial roles in a variety of physiological and pathophysiological processes, including regulation of smooth muscle contractility and various immune and inflammatory functions.

### *Relevance as biomarkers*

Due to their different roles, the eicosanoid metabolic pathways and their key regulatory enzymes are important pharmacological targets. The best known are probably the isoforms of PGH<sub>2</sub> Synthase, designated COX-1 and COX-2 (Cyclooxygenase 1 & 2), which are targeted by the NSAIDs.



Recent clinical studies indicate that urinary and plasma levels of isoprostanes (in particular 8-iso-prostaglandin (PG) F<sub>2</sub>α) are increased in clinical conditions where oxidative stress is playing a role. Airway inflammation in asthma is associated with cysteinyl leukotriene and prostaglandin D<sub>2</sub> production. Measurement of urinary metabolites of these eicosanoids (such as by leukotriene E<sub>4</sub> (LTE<sub>4</sub>)) may be useful biomarkers in certain asthma patients. Thromboxanes are involved in platelet activation and the urinary excretion of 11-dehydrothromboxane (TX) B<sub>2</sub> is used as biomarker here. In addition to body fluids we are analysing certain eicosanoids in tissue samples (e.g. gastric mucosa) and isolated cells from man and experimental animals

Within TNO, eicosanoids are routinely measured *in vitro* and *in vivo* in projects related to inflammation, immuno- modulation and side-effects of NSAIDs. In addition to clinical studies they are used as biomarkers in the various disease models

Immuno-assays are mostly applied for the analysis of one or just a few compounds at a time. Most recently, we have developed GC-MS/MS and LC-MS/MS methods for single or multiple molecules. Other techniques, including immuno blotting, *in situ* hybridisation and PCR are in use to localise enzymes etc.

See also the sections on inflammation and oxidative stress as well as our information on disease models.

Analyte	Information	Assay Principle
6-keto-PGF <sub>1a</sub>	Prostacyclin route	Antibody
Dinor-6-keto-PGF <sub>1a</sub>	Prostacyclin route	Antibody
TXB <sub>2</sub>	Thromboxane route	Antibody
Dinor-TXB <sub>2</sub>	Thromboxane route	Antibody
PGD <sub>2</sub>		Antibody
PGE <sub>2</sub>		Antibody
Bicyclo-PGE		Antibody
PGF <sub>2a</sub>		Antibody
11β-PGF		Antibody
13,14-dihydro-15-keto-PGF		Antibody
11-dehydro-TXB <sub>2</sub>		Antibody
8-isoprostane metabolites	Whole Body lipid peroxidation	GC-MS
NO	Whole Body NO production	GC-MS
3-nitro-tyrosin	Protein peroxides	GC-MS
LTB		Antibody
LTC		Antibody
LTE		Antibody
D4,7aketo PGF <sub>1a</sub>		Antibody
PGE2		RIA
8-iso-PGF2-alpha (urine)		GC-MS

## 7 Vascular function markers

Analyte	Information	Assay Principle
s-E-Selectin		Immunologic / EIA
s-Thrombomodulin		Immunologic / EIA
s-VCAM		Immunologic / EIA
s-ICAM		Immunologic / EIA
t-Fibronectin		Immunologic / EIA
Von Willebrand factor		Immunologic / EIA
Endothelin-1		EIA
NOx		EIA
Total Homocystein		HPLC
SAA		EIA

## 8 Coagulation and fibrinolysis

Haemostasis and tissue repair involve formation of the temporary protein matrix fibrin by coagulation, while its proteolytic dissolution is achieved by fibrinolysis.

Coagulation and fibrinolysis are evaluated frequently together to understand balance and dysbalance. Both systems are cascade processes of activation of proteolytic enzymes, co-factors and (pro)-inhibitors.

Evaluation is relevant for bleeding and thrombosis and for abnormal tissue repair and remodeling.

In recent years TNO has evaluated coagulation and fibrinolysis frequently to document thrombosis risk of interventions such as with estrogens, bleeding risk for thrombolytics, and efficacy of anti-thrombotic drugs, and pleiotrophic effects of anti-lipid and anti-inflammatory drugs and foods.

TNO has developed (a) knowledge to advise about selection of coagulation and fibrinolysis markers (b) technology to obtain proper blood samples for analysis including training and quality control.

Principles of assays involves antigen assays for levels of factors, activity assays for levels of factors, functional assays for parts of the process, assays for molecular markers of action in the systems.

### Coagulation

Analyte	Information	Assay Principle
PT (including INR)		Clotting
APTT		Clotting
Fibrinogen (clotting)		Clotting / Clauss
Fibrinogen(antigen)		Immunologic / EIA
Prothrombin		Chromogenic
Factor VIIc	Clottable Factor VII	Clotting
Factor VII mass	Total Factor VII	Chromogenic
Factor VIIa		Clotting
Factor VIIIc		Clotting
Factor X antigen		EIA
Anti Xa activity		Clotting
Anti II a activity		Clotting
Heptest		Clotting
APC resistance (APTT, global)		Clotting
APC resistance (APTT, specific)		Clotting
APC resistance (extrinsic, chromogen)		Chromogenic
Protein C antigen		EIA
Protein C activity		Clotting
Protein S antigen		EIA
Protein S activity		Clotting
Thrombin generation		Chromogenic

Analyte	Information	Assay Principle
TFPI (total)		EIA
TFPI (free)		EIA
C4-b binding protein		EIA
Antibrombin		Chromogenic
Fragment 1 + 2		EIA
Thrombin-antithrombin (TAT)		EIA
Soluble fibrin		EIA
Intact soluble fibrin		EIA
Beta thromboglobulin (urine/plasma)		EIA
Platelet factor 4		EIA
6-keto PGF1a+2,3 dinor 6-Keto-PGF1a(urine)		GCP
TXA2 + 11-dehydro-TXB2 (urine)		GCP
Creatinin		Ektachem
Vitamin K1		HPLC-fluorescence

## Fibrinolysis

Analyte	Information	Assay Principle
Plasminogen		Chromogenic
Plasmin antiplasmin (PAP)		Immunologic / EIA
Plasmin inhibitor		Chromogenic
t-PA antigen		Immunologic / EIA
t-PA activity		Immunologic / BIA
PAI-1 antigen		Immunologic / EIA
PAI-1 activity		Immunologic / BIA
t-PA:PAI-1 complex		Immunologic / EIA
Scu-PA and u-PA antigen		Immunologic / BIA / EIA
Prokallikrein		Chromogenic
Kallikrein generation test		Chromogenic
Factor XIIa		Immunologic / EIA
Fibrin degradation products (FbDP)		Immunologic / EIA
Fibrinogen degradation products (FgDP)		Immunologic / EIA
D-dimer		Immunologic / EIA
TAFI antigen		Immunologic / EIA
Elastase degradation products of fibrin		Immunologic / EIA

## 9 Hormone assays

Analyte	Information	Assay Principle
ACTH		RIA
Glucagon		RIA
Ghrelin (total or active)		RIA
Leptin		RIA
CCK		RIA
PTH	Intact PTH	RIA
Motilin		RIA
Renin		RIA
Testosterone		RIA
Estradiol		RIA
Aldosterone		RIA
Adiponectin		RIA
GLP-1		EIA
Corticosterone		RIA
Cortisol		RIA
Insulin		RIA
C-peptide		RIA
IGF-1		RIA
PYY		RIA
Resistin		EIA
Luteinising hormone (LH)		Immuno-assay
Follicle Stimulating Hormone (FSH)		Immuno-assay
Insuline (IRI)		Immuno-assay
Prolactin (PRL)		Immuno-assay
$\beta$ -Human Chorionic Gonadotropin ( $\beta$ -HCG)		Immuno-assay
Human Chorionic Gonadotropin (HCG)		Immuno-assay
Oestradiol (E2)		Immuno-assay / GCMS
Progesterone (Prog)		Immuno-assay / GCMS
Free Thyroxine (FT4)		Immuno-assay
Thyroid Stimulating Hormone (TSH)		Immuno-assay
Triiodothyronine (T3)		Immuno-assay
Total thyroxine (T4) (human)		Immuno-assay
Prostate Specific Antigen (PSA)		Immuno-assay
$\beta$ -2 microglobulin (BMG)		Immuno-assay
Human Growth hormone		Immuno-assay
Cortisol		Immuno-assay/LCMS

## 10 Oxidative stress markers

Chronic diseases like diabetes, heart disease, arthritis, obesitas and cancer have been linked to "oxidation" of "cellular molecules" such as proteins, lipids and DNA. This is where damaging modified oxygen molecules attach to molecules in cells and can cause damage and inflammation. This is why compounds with a suggested antioxidant activity might help to prevent or remove the damaging oxygen molecules from interacting with cellular molecules before causing damage and lead to disease.

TNO has developed and validated a large set of biomarkers to establish the efficacy of compounds or food products on inflammatory related diseases. These biomarkers can be applied in *in vitro* systems as well as in animal studies and clinical trials. Combination of these inflammatory markers with biomarkers of oxidative damage (e.g. damage to proteins, lipids and DNA) offers the possibility to establish effects of single compounds or whole food products on development of inflammatory mediated diseases or the complications of these inflammations.

*See also the sections on inflammation and eicosanoids*

Analyte	Information	Assay Principle
8-iso-PGF2-alpha	Lipid damage	GC-MS
MDA	TBARS, with separation	HPLC
8-hydroxy-dG	DNA damage	LC-MS
Protein carbonyls	Protein damage	ELISA
I-kappaB	Activation of inflammation pathway	ELISA
NF-kappaB	Activation of inflammation pathway	ELISA
3-nitrotyrosine (free and/or total)	Protein damage	GC-MS

## 11 Nutritional Status

Analyte	Information	Assay Principle
Vitamin A		HPLC
Vitamin B1		HPLC
Vitamin B2		HPLC
Vitamin B3		HPLC
Vitamin B6		HPLC
Vitamin B12		RIA
Vitamin C		HPLC
25-hydroxy vitamin D		RIA
1,25-dihydroxy vitamin D		RIA
24,25-dihydroxy vitamin D		RIA
Vitamin E		HPLC
Vitamin K1		HPLC-fluorescence
Folate		RIA
Pantothenic acid		Microbiological
Biotin		Microbiological
Amino Acid profile		Ion exchange chromatography
Carotenoids		HPLC
Flavonoids		HPLC
Free fatty acid profile (C14-C24)	FAME	GC
Total fatty acid profile (C8-C24)	FAME	GC
Minerals		ICP-AES
Trace elements		ICP-MS
Mercapturic acids	exposure to isothiocyanates	LC-MS

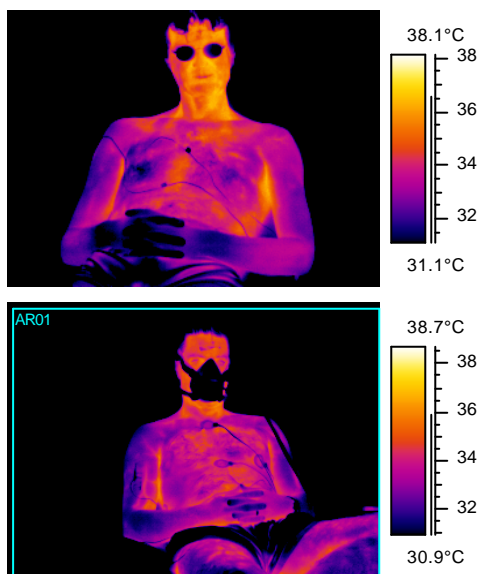
## 12 Biomarkers for satiety and satiation

The principal causes of the epidemic of overweight and obesity are sedentary lifestyles and high-fat, energy-dense diets, resulting in increased energy intake and decreased energy expenditure. Energy intake is predominately determined by two processes: satiety and satiation. We start eating when we get hungry (= absence of satiety) and stop when we feel full (satiation).

In response to the greater numbers of people with severe overweight (BMI > 30), the Pharma industry is developing therapeutic approaches that -combined with other measures – can be used to control overweight. There is a considerable need to evaluate efficacy and safety of these approaches in the clinical setting, and the development of suitable biomarkers is an important part of this.

Until recently, there were no validated biomarkers of satiety: Information on satiation and satiety could only be assessed by subjective introspection, measuring the interval until the spontaneous request of the next meal (satiety), or measuring the energy intake from the meal (satiation). We have set up - and are constantly extending - clinical protocols and sets of biomarkers according to what we call a “zoom-in” and a “zoom-out” principle.

With zoom out we refer to the use of explorative techniques to identify new (groups of) parameters related to appetite (i.e. genomics, fMRI) The zoom-in approach focusses on parameters known to be involved in the regulation of food intake (i.e. gastrointestinal hormones) and thermo-physiological measurements. In the latter case we set-up and validate methods and assays, and investigate the physiological meaningfulness of these in relation to satiety and satiation.



*Measurement of (radiant) skin temperature*

### *Subjective parameters*

Subjective measurements of hunger and satiety (Visual Analogue Scales, VAS), as well as intermeal interval and meal size are still highly important parameters in these studies, provided that they are highly standardized, validated and used by experienced investigators.

### *Hormones and other biochemical parameters*

Satiety and satiation are complex processes and far from fully understood. A wide range of biochemical parameters has been proposed as useful indicators here. Our experience indicates that some of these parameters are indeed valuable, whereas others are not. A detailed discussion falls beyond the scope of this text, but we will be happy to discuss this in more detail.

In principle, *satiation* can be followed by:

- Stomach fullness
- Cholecystokinin (CCK)
- Glucagon-like peptide 1 (GLP-1)
- Bombesin
- Somatostatin , and

*Satiety* by:

- Ghrelin
- Leptin (long-term, not short-term)
- PYY
- Glucose
- insulin
- Diet induced thermogenesis

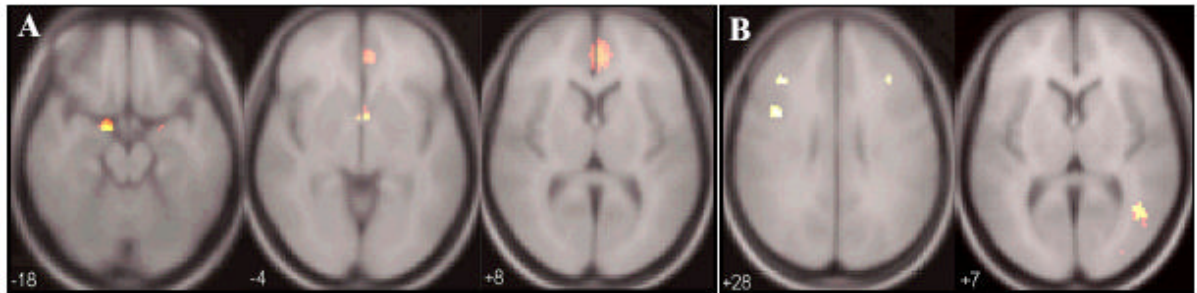
### *Physiological parameters include*

- Temperature (rectal, mean skin, mean body, IR-images)
- Ventilatory parameters (VO<sub>2</sub>, VCO<sub>2</sub>, V<sub>e</sub>, RQ, T<sub>e</sub>, Met.)
- Circulatory parameters (heart rate)
- Nervous parameters (thorax impedance, HRV)

### *Central biomarkers*

There is limited knowledge of how the brain contributes to the regulation of food intake in humans. After eating, the human brain senses a biochemical change and then signal satiation, but precisely when this occurs precisely is unknown.

With respect to CNS biomarkers of satiety and satiation, there have been a number of recent studies in literature using PET-scan techniques and f-MRI (functional-Magnetic Resonance Imaging). These studies have identified various regions in the brain that can distinguish between the state of extreme hunger and satiation. Other studies suggest that the brain responses to a meal differ between obese and lean persons. Together with the Utrecht Academic Hospital we have started to use fMRI for the studies on the regulation of appetite control.



## 13 Amino Acids

Amino acids form one of the major compound classes in life. They are the main building blocks for the proteins and peptides in the human body. A number of the amino acids are produced via our own biochemical pathways, others are acquired via nutrition, the so called essential amino acids. It is obvious that an optimal balance of amino acids is required for healthy function of the body. Hence, amino acids (-patterns) can serve as important biomarkers.

TNO has decades of experience with the analysis of amino acids. This results in broad skills in sample pre-treatment for various matrices, like serum, plasma, urine, faeces, intestinal content, tissue and biopts. Many methods are offered of the shelf.

Secondly, we have worked with, and made ourselves familiar with, all available state-of-the-art instrumental techniques.

Our sponsors can rely on us to asses which technique is appropriate for their project. On a regular basis, samples are analyzed to support the projects of our sponsors from the pharmaceutical- and biotech industry. In addition to this line of work, we analyze food supplements and raw materials on a contract basis.

Currently, most of the work is run on our JEOL AminoTac JLC-500/V amino acid analyzer. This system has integrated ion exchange chromatography with post-column derivatisation (ninhydrin or OPA) followed by colorimetric - or fluorescent detection. This system renders our sponsors clear and prompt answers by combining sensitivity, selectivity and reproducibility with quick run times.

The following services are offered:

- Group I amino acids (Cystine, Methionine, Aspartic acid, Threonine, Serine, Glutamic acid, Glycine, Alanine, Valine, Isoleucine, Leucine, Lysine and Arginine; optional: Proline, Ornithine and  $\gamma$ -Amino butyric acid)
- Group II amino acids (Aspartic acid, Threonine, Serine, Glutamic acid, Glycine, Alanine, Valine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, Lysine and Arginine; + optional: Proline, Ornithine en  $\gamma$ -Amino butyric acid)
- Tryptophan
- Complete amino acid composition
- Free amino acids
- Biogenous amines
- Specialties
- Individual amino acids can often also be analysed by GC-MS or LC-MS

*See for a list under endogenous compounds and metabolites*

## 14 Metal assays (and trace elements)

Analyte	Information	Assay Principle
Arsenic	serum/blood/urine	ICP-MS (and AAS)
Cadmium	blood/urine	ICP-MS (and AAS)
Chromium	urine	ICP-MS (and AAS)
Cobalt		ICP-MS (and AAS)
Copper	serum/urine	ICP-MS (and AAS)
Mercury	blood/urine	ICP-MS (and AAS)
Lead	blood/urine	ICP-MS (and AAS)
Manganese	urine	ICP-MS (and AAS)
Nickel	plasma/urine	ICP-MS (and AAS)
Selenium	serum/urine	ICP-MS (and AAS)
Vanadium	plasma	ICP-MS (and AAS)
Zinc	serum/urine	ICP-MS (and AAS)

## 15 Endogenous compounds and metabolites

Analyte	Information	Method of analysis
N(alfa)-Acetyl-L-ornithine	acyl amino acids	GCMS
N-Acetyl-L-glutamate	acyl amino acids	LCMS
N-Formyl-L-methionine	acyl amino acids	GCMS; LCMS
O-Acetyl-L-serine	acyl amino acids	GCMS; LCMS
(R,R)-2,3-Butanediol	alcohols/aldehydes/esters	GCMS
(S,S)-2,3-Butanediol	alcohols/aldehydes/esters	GCMS
1,3-Dihydroxyacetone	alcohols/aldehydes/esters	GCMS
Acetaldehyde (= ethanal)	alcohols/aldehydes/esters	GCMS
Acetoin	alcohols/aldehydes/esters	GCMS
Acetone	alcohols/aldehydes/esters	GCMS
D-Glyceraldehyde (=propanal, 2,3 dihydroxy)	alcohols/aldehydes/esters	GCMS
Glycerol	alcohols/aldehydes/esters	GCMS
Undecaprenol	alcohols/aldehydes/esters	GCMS
(3-Phosphatidyl)-ethanolamine (= cephalin)	alkylphosphates	LCMS
Acetylphosphate	alkylphosphates	LCMS
D-Glyceraldehyde-3-phosphate	alkylphosphates	GCMS
Dihydroxyacetonephosphate = DHAP = Glyceronephosphate	alkylphosphates	LCMS
Phosphatidylglycerol = 3-(3-sn-phosphatidyl)glycerol	alkylphosphates	GCMS; LCMS
sn-Glycerol-3-phosphate	alkylphosphates	GCMS; LCMS
Lipoamide	alkyl-sulfide + nitrogen compounds	GCMS
Allantoin	amines	GCMS
Cadaverine (= 1,5-Pentanediamine)	amines	GCMS
1-Amino-2-propanol (= threanine = isopropanolamine)	amines	GCMS
3-Amino-1,2-propanediol (= 1-Amino-2,3-propanediol)	amines	GCMS
4-Aminobutanal (=gamma-aminobutanal)	amines	GCMS
Spermidine (= N-(3-Aminopropyl)-1,4-butane-diamine)	amines	GCMS
Spermine (= N,N'-Bis(3-aminopropyl)-1,4-butanediamine)	amines	GCMS
Putrescine (= 1,4-Butanediamine = Tetramethylenediamine)	amines	GCMS
(4-Aminobutyl)guanidine (agmatine)	amino acid	GCMS
n-Butylamine	amino acid	GCMS
4-Aminobutyricacid (= ?-amino-butyric acid = GABA)	amino acid	GCMS
5-Aminolevulinic acid	amino acid	GCMS
Glycine	amino acid	GCMS / amino acid analyzer.
L-Alanine	amino acid	GCMS / amino acid analyzer

Analyte	Information	Method of analysis
L-Asparagine	amino acid	GCMS / amino acid analyzer
L-Aspartic acid	amino acid	GCMS / amino acid analyzer
L-Cystathionine	amino acid	GCMS
L-Cysteine	amino acid	GCMS / amino acid analyzer
L-Glutamate	amino acid	GCMS / amino acid analyzer
L-Glutamine	amino acid	GCMS / amino acid analyzer
L-Histidine	amino acid	GCMS / amino acid analyzer
L-Homocysteine	amino acid	GCMS / amino acid analyzer
L-Homoserine	amino acid	GCMS / amino acid analyzer
L-Isoleucine	amino acid	GCMS / amino acid analyzer
LL-2,6-Diaminoheptane-dioic acid (= LL-2,6-Diaminopimelic acid)	amino acid	GCMS
L-Leucine	amino acid	GCMS / amino acid analyzer
L-Lysine	amino acid	GCMS / amino acid analyzer
L-Methionine	amino acid	GCMS / amino acid analyzer
L-Ornithine	amino acid	GCMS / amino acid analyzer
L-Phenylalanine	amino acid	GCMS; LCMS
L-Proline	amino acid	GCMS / amino acid analyzer
L-Serine (=L-3-Hydroxy -alanine)	amino acid	GCMS / amino acid analyzer
L-Threonine	amino acid	GCMS / amino acid analyzer
L-Tryptophan	amino acid	GCMS; LCMS
L-Tyrosine	amino acid	GCMS / amino acid analyzer
L-Valine	amino acid	GCMS / amino acid analyzer
Lysine carboxylic acid	amino acid	GCMS
Selenomethionine	amino acid	GCMS
trans-4-Hydroxy -L-proline	amino acid	GCMS
2,3-Dihydroxybenzoicacid	aromatic compounds	GCMS; LCMS
4-Aminobenzoic acid (=p-aminobenzoic acid = PABA)	aromatic compounds	GCMS; LCMS
Arbutin	aromatic compounds	GCMS; LCMS
Menaquinone (=Vitamin K2)	aromatic compounds	GCMS
Salicin (= Salicoside)	aromatic compounds	GCMS; LCMS
3-Methylbut-2-enoyl-CoA (=3-methylcrotonyl-CoA)	CoA-esters	LCMS
Acetoacetyl-CoA	CoA-esters	LCMS
Acetyl-CoA	CoA-esters	LCMS
CoA-SH (Coenzyme A)	CoA-esters	LCMS
Crotonoyl-CoA	CoA-esters	LCMS
Crotonyl-CoA	CoA-esters	LCMS
Decanoyl-CoA	CoA-esters	LCMS
Dephospho-CoA	CoA-esters	LCMS

Analyte	Information	Method of analysis
Hexanoyl-CoA	CoA-esters	LCMS
Lauroyl-CoA (=dodecanoyl-CoA)	CoA-esters	LCMS
Malonyl-CoA	CoA-esters	LCMS
Myristoyl-CoA (=tetradecanoyl-CoA)	CoA-esters	LCMS
Octanoyl-CoA	CoA-esters	LCMS
Palmitoyl-CoA (=hexadecanoyl-CoA)	CoA-esters	LCMS
Propanoyl-CoA (= Propionyl-CoA)	CoA-esters	LCMS
Succinyl-CoA	CoA-esters	LCMS
Inositol (= myo-inositol = cyclohexitol)	cyclohexanols	GCMS
Prephenic acid	cyclohexanols	LCMS
Shikimic acid	cyclohexanols	GCMS; LCMS
Carbamide	inorganic compounds/ small organics	GCMS
Diphosphate	inorganic compounds/ small organics	GCMS
Orthophosphate	inorganic compounds/ small organics	GCMS
Phosphate	inorganic compounds/ small organics	GCMS
Pyrophosphate	inorganic compounds/ small organics	GCMS
Urea	inorganic compounds/ small organics	GCMS
Pyridoxal-5-phosphate	nucleotide derivatives: purines/pyrimidines + Ppi	GCMS; LCMS
Thiamine-diphosphate	nucleotide derivatives: purines/pyrimidines + Ppi	LCMS
Thiaminemonophosphate	nucleotide derivatives: purines/pyrimidines + Ppi	LCMS
Thymidine-5' -monophosphate	nucleotide derivatives: purines/pyrimidines + Ppi	LCMS
2' -Deoxyadenosine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS; LCMS
2' -Deoxycytidine	nucleotide derivatives: sugar+purine/pyrimidine	LCMS
2' -Deoxyguanosine	nucleotide derivatives: sugar+purine/pyrimidine	LCMS
2-Deoxyuridine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS; LCMS
5' -Deoxy -5'-(methylthio)adenosine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS; LCMS
5-Methylthioadenosine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS; LCMS
Adenosine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS; LCMS

Analyte	Information	Method of analysis
Cytidine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS; LCMS
Guanosine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS; LCMS
Inosine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS; LCMS
Pseudouridine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS
Riboflavin (= lactoflavin = Vitamin B2)	nucleotide derivatives: sugar+purine/pyrimidine	LCMS
Uridine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS; LCMS
Xanthosine	nucleotide derivatives: sugar+purine/pyrimidine	GCMS; LCMS
(9-D-Ribosylxanthine)-5'-phosphate	nucleotides	GCMS; LCMS
2'-Deoxyadenosine 5'-monophosphate (Damp)	nucleotides	GCMS; LCMS
2'-Deoxyadenosine 5'-triphosphate (Datp)	nucleotides	LCMS
2'-Deoxycytidine 5'-diphosphate (Dcdp)	nucleotides	LCMS
2'-Deoxycytidine 5'-monophosphate (Dcmp)	nucleotides	LCMS
2'-Deoxycytidine 5'-triphosphate (Dctp)	nucleotides	LCMS
2'-Deoxyguanosine 5'-monophosphate (Dgmp)	nucleotides	LCMS
2'-Deoxyguanosine 5'-diphosphate (Dgdp)	nucleotides	LCMS
2'-Deoxyguanosine 5'-triphosphate (Dgtp)	nucleotides	LCMS
2'-Deoxyuridine 5'-phosphate (Dump)	nucleotides	LCMS
2'-Deoxyuridine 5'-triphosphate (Dutp)	nucleotides	LCMS
2'-Deoxythymidine 5'-phosphate (Dtmp)	nucleotides	GCMS; LCMS
Dtdp	nucleotides	LCMS
Dttp	nucleotides	LCMS
3'-Phosphoadenylylsulfate (PAPS)	nucleotides	LCMS
5'-Inosinate = 5'-Inosine monophosphate	nucleotides	LCMS
Adenosine 5'-phosphosulfate = Adenylylsulfate = APS	nucleotides	LCMS
Adenylosuccinate = N6-(1,2-Dicarboxyethyl)-AMP	nucleotides	LCMS
Adenosine-5'-monophosphate (AMP)	nucleotides	GCMS; LCMS
Adenosine-5'-diphosphate (ADP)	nucleotides	LCMS
Adenosine-5'-triphosphate (ATP)	nucleotides	LCMS
Cytidine 5'-diphosphate (CMP)	nucleotides	LCMS
Cytidine 5'-diphosphate (CDP)	nucleotides	LCMS
Cytidine 5'-triphosphate (CTP)	nucleotides	LCMS
Inosine 5'-monophosphate (IMP, inosinic acid)	nucleotides	LCMS
Inosine 5'-diphosphate (IDP)	nucleotides	LCMS
Inosine 5'-triphosphate (ITP)	nucleotides	LCMS
Guanosine 5'-monophosphate (GMP)	nucleotides	GCMS; LCMS

Analyte	Information	Method of analysis
Guanosine 5'-diphosphate (GDP)	nucleotides	LCMS
Guanosine 5'-triphosphate (GTP)	nucleotides	LCMS
Thymidine 5'-monophosphate (Thymidylic acid = TMP)	nucleotides	LCMS
Thymidine 5'-diphosphate (TDP)	nucleotides	LCMS
Thymidine 5'-triphosphate (TTP)	nucleotides	GCMS; LCMS
Uridine 5'-monophosphate (= uridylic acid = UMP)	nucleotides	GCMS; LCMS
Uridine 5'-triphosphate (UTP)	nucleotides	LCMS
Xanthosine 5'-monophosphate (= Xanthylicacid = XMP)	nucleotides	GCMS; LCMS
NADP (= beta- Nicotinamide adenine dinucleotide 2-phosphate, oxidized)	nucleotides	LCMS
NADPH (= beta -Nicotinamide adenine dinucleotide 2'-phosphate, reduced)	nucleotides	LCMS
NAD (= beta-Nicotinamide adenine dinucleotide, oxidized)	nucleotides	LCMS
NADH (=beta-Nicotinamide adenine dinucleotide, reduced)	nucleotides	LCMS
Flavin adenine dinucleotide (FAD)	nucleotides	LCMS
Flavin mononucleotide (FMN = Riboflavin 5'-phosphate)	nucleotides	LCMS
Nicotinamide D-ribonucleotide	nucleotides	LCMS
Orotidine-5'-monophosphate (Orotidylic acid)	nucleotides	LCMS
®-Glyceric acid	organic acids	GCMS
®-Pantothenicacid	organic acids	GCMS; LCMS
(S)-3-Methyl-2-oxopentanoate	organic acids	GCMS; LCMS
4-Methyl-2-oxopentanoate	organic acids	GCMS; LCMS
(S)-Lactate	organic acids	GCMS
(S)-Malate	organic acids	GCMS; LCMS
1-Hydroxypropane-1,2,3-tricarboxylicacid	organic acids	GCMS; LCMS
2-Hydroxy -1,2,3-propanetricarboxylicacid	organic acids	GCMS; LCMS
2-Isopropylmalicacid	organic acids	GCMS
2-Keto-3-methylbutyricacid	organic acids	GCMS LCMS
2-Ketovaline	organic acids	GCMS LCMS
2-Oxobutanoicacid	organic acids	GCMS; LCMS
2-Oxobutyricacid (= 2-Ketobutyric acid)	organic acids	GCMS; LCMS
2-Oxoisocaproate	organic acids	GCMS; LCMS
2-Oxopropanoicacid	organic acids	GCMS
3-Carboxy -3-hydroxy -4-methylpentanoate	organic acids	GCMS
3-Methyl-2-oxobutanoate	organic acids	GCMS LCMS
3-Phenyl-2-oxopropanoate	organic acids	GCMS; LCMS
6-Carboxyhexanoate	organic acids	GCMS; LCMS
Acetate	organic acids	GCMS; LCMS
Allantoate	organic acids	GCMS

Analyte	Information	Method of analysis
alpha-Ketohydrocinnamicacid	organic acids	GCMS; LCMS
Butanedionicacid	organic acids	GCMS; LCMS
Cis-Aconiticacid	organic acids	GCMS; LCMS
Citrate	organic acids	GCMS; LCMS
D-Biotin	organic acids	GCMS; LCMS
Ethanedioicacid	organic acids	GCMS
Ethylenesuccinicacid	organic acids	GCMS; LCMS
Formate (=Methanoic acid)	organic acids	GCMS; LCMS
Fumarate	organic acids	GCMS; LCMS
Glyoxylate	organic acids	GCMS
Isocitrate	organic acids	GCMS; LCMS
keto-Phenylpyruvate	organic acids	GCMS; LCMS
Mercaptopyruvate	organic acids	GCMS
Oxalic acid	organic acids	GCMS
Oxoglutaricacid	organic acids	GCMS; LCMS
Phenylpyruvate	organic acids	GCMS; LCMS
Pimelate	organic acids	GCMS; LCMS
Propanoic acid	organic acids	GCMS
Pyroracemicacid	organic acids	GCMS
Pyruvate	organic acids	GCMS
Succinate	organic acids	GCMS; LCMS
Trans-butenedioicacid	organic acids	GCMS; LCMS
Urocanate	organic acids	LCMS
Glutathione reduced (gamma-L-Glutamyl-L-cysteinyl-glycine)	peptide	LCMS
Glutathione oxidized	peptide	LCMS
Homocystine	peptide	GCMS
L-Dicysteine	peptide	GCMS
D-Glycerate-2-phosphate (= 2-phosphoglyceric acid)	phosphoorganic acids	GCMS; LCMS
D-Glycerate-3-phosphate (= 3-phosphoglyceric acid)	phosphoorganic acids	GCMS; LCMS
Phosphoenolpyruvate (PEP)	phosphoorganic acids	LCMS
Phosphoglycolic acid	phosphoorganic acids	GCMS; LCMS
Porphobilinogen	porphorines	GCMS; LCMS
4-Methyl-5-(2'-hydroxyethyl)-thiazole	purines/pyrimidine	GCMS
(S)-4,5-Dihydroorotate	purines/pyrimidines	GCMS; LCMS
2-Amino-6-hydroxypurine	purines/pyrimidines	GCMS
3-Pyridinecarboxylicacid	purines/pyrimidines	GCMS; LCMS
5,6,7,8-Tetrahydrofolate	purines/pyrimidines	
Adenine	purines/pyrimidines	GCMS; LCMS
Cytosine	purines/pyrimidines	GCMS; LCMS

Analyte	Information	Method of analysis
Deoxythymidine	purines/pyrimidines	GCMS; LCMS
Dihydrofolate	purines/pyrimidines	LCMS
Dihydroneopterin	purines/pyrimidines	GCMS; LCMS
Dihydropteridine	purines/pyrimidines	GCMS; LCMS
Folate	purines/pyrimidines	GCMS; LCMS
Guanine	purines/pyrimidines	GCMS
Hypoxanthine (=Purine-6-ol)	purines/pyrimidines	GCMS
L(-)-5-Formyl-5,6,7,8-tetrahydrofolicacid (Folinic acid)	purines/pyrimidines	GCMS
Niacin	purines/pyrimidines	GCMS; LCMS
Nicotinamide (= Niacinamide = Vit B3 = Vit PP)	purines/pyrimidines	GCMS
Nicotinate	purines/pyrimidines	GCMS; LCMS
Nicotinicacidamide	purines/pyrimidines	GCMS
Orotate	purines/pyrimidines	GCMS
Pteroylglutamicacid	purines/pyrimidines	GCMS; LCMS
Pyridoxal	purines/pyrimidines	GCMS; LCMS
Pyridoxine	purines/pyrimidines	GCMS; LCMS
Pyridoxol	purines/pyrimidines	GCMS; LCMS
Tetrahydrofolicacid	purines/pyrimidines	
Thymidine	purines/pyrimidines	GCMS; LCMS
Uracil-6-carboxylicacid	purines/pyrimidines	GCMS
Urate	purines/pyrimidines	GCMS; LCMS
Xanthine	purines/pyrimidines	GCMS; LCMS
O-Succinyl-L-homoserine	succinyl amino acids	GCMS; LCMS
Ascorbic acid (Vitamin C)	sugar acids	LCMS
D-Glucaric acid	sugar acids	GCMS; LCMS
D-Gluco-hexonic acid	sugar acids	LCMS
D-Gluconic acid	sugar acids	LCMS
D-Glucosaccharic acid (=d-Saccharic acid)	sugar acids	GCMS; LCMS
Glucuronic acid	sugar acids	GCMS; LCMS
Chitosamine (=D-Glucosamine = 2-Amino-2-deoxy -D-glucose)	sugar amines	GCMS
Adenosine diphosphoglucose (= ADPglucose)	sugar nucleotides	LCMS
dTDP-D-glucose	sugar nucleotides	LCMS
UDP-D-galactose	sugar nucleotides	LCMS
UDP-D-glucose	sugar nucleotides	LCMS
UDP-N-acetyl-D-glucosamine	sugar nucleotides	LCMS
2-Deoxy -D-ribose 1-phosphate	sugar phosphates	GCMS
2-Deoxy -D-ribose 5-phosphate	sugar phosphates	GCMS; LCMS
6-Phospho-D-gluconate (= 6-phosphogluconic acid)	sugar phosphates	GCMS; LCMS
Alpha-D-Glucose 1-phosphate	sugar phosphates	LCMS

Analyte	Information	Method of analysis
Alpha-D-Glucose 6-phosphate	sugar phosphates	GCMS; LCMS
beta-D-Fructose 1-phosphate	sugar phosphates	GCMS; LCMS
beta-D-Fructose 6-phosphate	sugar phosphates	GCMS; LCMS
beta-D-Glucose 1-phosphate	sugar phosphates	LCMS
beta-D-Glucose 6-phosphate	sugar phosphates	GCMS; LCMS
D-Erythrose 4-phosphate	sugar phosphates	GCMS; LCMS
D-Glucosamine 6-phosphate	sugar phosphates	GCMS
D-Mannitol 1-phosphate	sugar phosphates	GCMS; LCMS
(Alpha-) D-Mannose 1-phosphate	sugar phosphates	LCMS
D-Mannose 6-phosphate	sugar phosphates	GCMS; LCMS
D-Ribose 5-phosphate	sugar phosphates	GCMS; LCMS
D-Ribulose 5-phosphate	sugar phosphates	GCMS; LCMS
N-Acetyl-D-glucosamine 6-phosphate	sugar phosphates	LCMS
N-Acetyl-D-glucosamine 1-phosphate	sugar phosphates	LCMS
Sucrose 6-phosphate	sugar phosphates	GCMS; LCMS
Trehalose 6-phosphate	sugar phosphates	GCMS; LCMS
D-fructose 1,6-bisphosphate	sugarbisphosphates	LCMS
1-alpha-D-glucopyranosyl-2-beta-D-fructofuranoside	sugars	GCMS
1-alpha-D-Glucopyranosyl-4-alpha-D-glucopyranose	sugars	GCMS
1-beta-D-Galactopyranosyl-4-alpha-D-glucopyranose	sugars	GCMS
2-Deoxy -beta-D-erythro-pentose	sugars	GCMS
Alpha, alpha-Trehalose	sugars	GCMS
Alpha-D-Glucose (alpha-D-Glucopyranose)	sugars	GCMS
beta-D-Fructose (= beta-D-Arabino-hexulose =beta-D-fructofuranose= D-Fructose = D-(-)-Fructose = D-Levulose = Fructofuranose)	sugars	GCMS
beta-D-Glucose	sugars	GCMS
Cellobiose	sugars	GCMS
D-Galactose	sugars	GCMS
D-Lyxulose	sugars	GCMS
D-Mannitol	sugars	GCMS
D-Mannose (=carubinose)	sugars	GCMS
D-Ribose	sugars	GCMS
D-Ribulose = D-Riboketose = D-Arabinoketose	sugars	GCMS
Sorbitol (=gulitol = glucitol)	sugars	GCMS
D-Tagatose (=lyxo -hexulose)	sugars	GCMS
D-Xylose	sugars	GCMS
D-Xylulose	sugars	GCMS
Gluconicacidlactone	sugars	GCMS
Gluconiclactone	sugars	GCMS

Analyte	Information	Method of analysis
Isomaltose (=brachiose)	sugars	GCMS
Lactose	sugars	GCMS
L-Arabinose	sugars	GCMS
Maltose	sugars	GCMS
Mannose	sugars	GCMS
Melibiose	sugars	GCMS
Saccharose	sugars	GCMS
2-Actamido-2-deoxy -D-mannose = N-Acetyl-D-mannosamine	acetyl sugar amines	GCMS

## 16 Miscellaneous assays and techniques

As already mentioned in the preface, this publication aims to provide an impression of what is possible in terms of analysis of endogenous biomolecules and metabolites. Yet, many other possible approaches exist. This final paragraph provides some alternatives and possible considerations.

*Please don't hesitate to enquire.*

### 16.1 *Ex-vivo human cell based assays.*

Human cells and tissues can serve directly for diagnostic purposes but also to analyse functional responses. An example of the latter application are stimulation-assays, where a response of certain tissue or cell-type to a standard (for example inflammatory-) stimulus is being analysed using different techniques. This approach can help to identify new biomarkers or to measure specific biomarkers.

TNO Pharma has built up experience with the following human cell types:

#### *Peripheral blood mononuclear cells*

- Monocytes
- Dendritic cells
- CD4+/CD8+ T cells
- B cells

#### *Macrovascular endothelial cells*

- Umbilical vein (HUVEC)
- Umbilical artery (HUAEC)
- Aorta (HAEC)
- Iliac vein (HIVEC)

#### *Microvascular endothelial cells*

- Foreskin (HFMVEC)
- Endometrium (HEMVEC)
- Brain capillaries (BCEC)

#### *Other cell types*

- Brain-derived astrocytes
- Brain-derived microglia
- Brain organotypic cultures
- Eye lenses
- Synovial fibroblasts (synoviocytes)
- Chondrocytes (bovine)
- Alginate-recovered chondrocytes (bovine)
- Cartilage explant cultures, also as co-cultures with leukocytes
- Fat explant cultures
- Hepatocytes (limited availability)
- Stromal cells of various tissues
- Smooth muscle cells
- Fibroblasts
- Myoblasts

*Gastro-intestinal tissues*

- Jejunum explants
- Ileum explants
- Colon explants

In addition we have collected biopsies for different tissues during clinical studies.

**16.2 Specific assays, read-out methods and techniques**

In various situations it may be necessary to use methods other than bio-analytical analysis. To give a few examples on what is possible:

*Cell migration assays*

- 2D- and 3D migration assays

*Endothelial tube formation (angiogenesis) assays*

- 3D assays, incorporating tailor-made fibrin or collagen matrices

*Co-culture tube formation assays*

- Stromal cells – endothelial cells
- Tumour cells – endothelial cells
- Synovioyte migration and invasion assays
- cDNA micro-array-based gene profiling assays
- Real time PCR
- Various types of proliferation assays
- Histology and Immunohistochemistry
- In situ hybridisation

**16.3 Custom ELISA development**

We are regularly developing, validating and implementing ELISA's "from scratch" Starting from a protein (for example a proposed biomarker) or an antibody we set up the assay and optimise the conditions. So if you have a specific protein to be analysed, please inquiry

**16.4 "Routine"clinical chemistry, hematology and urine analysis**

Not included in this list are "standard" clinical chemistry-, haematology- and urinalysis parameters. These assays are routinely performed at TNO, often in combination with animal or human safety or efficacy studies.

**16.5 Physiological biomarkers**

Our clinical department is able to measure diagnostic parameters and functional responses from relatively simple to highly specialised. Please ask the TNO Pharma office for more information

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• MMP-14	7
• MMP-2	7
• MMP-3	7
• MMP-3	7
• MMP-8	7
• MMP-9	7
• Motilin	18
• Myristoyl-CoA (=tetradecanoyl-CoA)	28

## N

• N(alfa)-Acetyl-L-ornithine	26
• N-Acetyl-D-glucosamine 1-phosphate	33
• N-Acetyl-D-glucosamine 6-phosphate	33
• N-Acetyl-L-glutamate	26
• NAD (= beta-Nicotinamide adenine dinucleotide, oxidized)	30
• NADH (=beta-Nicotinamide adenine dinucleotide, reduced)	30
• NADP (= beta- Nicotinamide adenine dinucleotide 2-phosphate, oxidized)	30
• NADPH (= beta -Nicotinamide adenine dinucleotide 2'-phosphate, reduced)	30
• NF-kappaB	11
• NF-kappaB	19
• N-Formyl-L-methionine	26
• Niacin	32
• Nickel	25
• Nicotinamide (= Niacinamide = Vit B3 = Vit PP)	32
• Nicotinamide D-ribonucleotide	30
• Nicotinate	32
• Nicotinicacidamide	32
• 3-nitro-tyrosin	14
• 3-nitrotyrosine (free and/or total)	19
• NO	14
• NOx	12
• NOx	15
• NTx	5

## O

• Octanoyl-CoA	28
• Oestradiol (E2)	18
• Orotate	32
• Orotidine-5'-monophosphate (Orotidylic acid)	30
• Orthophosphate	28
• Osteocalcin (Bone GLA protein, BGP)	5
• Osteocalcin carboxylation degree	5
• O-Succinyl-L-homoserine	32
• Oxalic acid	31
• 2-Oxobutanoicacid	30
• 2-Oxobutyricacid (= 2-Ketobutyric acid)	30
• Oxoglutaricacid	31
• 2-Oxoisocaproate	30
• 2-Oxopropanoicacid	30

<b>P</b>
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• t-PA activity	17
• PAI-1 antigen	17
• PABA = 4-Aminobenzoic acid (=p-aminobenzoic acid )	27
• PAI-1 activity	17
• t-PA antigen	17
• t-PA:PAI-1 complex	17
• Palmitoyl-CoA (=hexadecanoyl-CoA)	28
• Pantothenic acid	20
• (R)-Pantothenic acid	30
• Pentosidine	5
• Peroxides	9
• PGD <sub>2</sub>	14
• PGE <sub>2</sub>	14
• PGE <sub>2</sub>	14
• 11β-PGF	14
• 6-keto PGF <sub>1a+2,3</sub> dinor 6-Keto-PGF <sub>1a</sub> (urine)	17
• 6-keto-PGF <sub>1a</sub>	14
• 8-iso-PGF <sub>2</sub> -alpha	19
• 8-iso-PGF <sub>2</sub> -alpha (urine)	14
• PGF <sub>2a</sub>	14
• 3-Phenyl-2-oxopropanoate	30
• Phenylpyruvate	31
• Phosphate	28
• (3-Phosphatidyl)-ethanolamine (= cephalin)	26
• Phosphatidylglycerol = 3-(3-sn-phosphatidyl)glycerol	26
• 3'-Phosphoadenylylsulfate (PAPS)	29
• 6-Phospho-D-gluconate (= 6-phosphogluconic acid )	32
• Phosphoenolpyruvate (PEP)	31
• 6-Phosphogluconic acid = (6-Phospho-D-gluconate )	32
• Phosphoglycolic acid	31
• Phospholipids	9
• Phytosterols/ stanols	9
• PICP	5
• Pimelate	31
• PINP	5
• s-PLA <sub>2</sub>	11
• Plasmin antiplasmin (PAP)	17
• Plasmin inhibitor	17
• Plasminogen	17
• Platelet factor 4	17
• Porphobilinogen	31
• Prephenic acid	28
• Progesterone (Prog)	18
• Prokallikrein	17
• Prolactin (PRL)	18
• Propanoic acid	31
• Propanoyl-CoA (= Propionyl-CoA)	28
• Prostate Specific Antigen (PSA)	18
• Protein C activity	16
• Protein C antigen	16
• Protein carbonyls	19

• Protein conc. Bradford	9
• Protein conc. Lowry	9
• Protein S activity	16
• Protein S antigen	16
• Prothrombin	16
• Pseudouridine	29
• PT (including INR)	16
• Pteroylglutamicacid	32
• PTH	18
• Putrescine (= 1,4-Butanediamine = Tetramethylenediamine)	26
• 3-Pyridinecarboxylicacid	31
• Pyridoxal	32
• Pyridoxal-5-phosphate	28
• Pyridoxine	32
• Pyridoxol	32
• Pyrophosphate	28
• Pyrroacemicacid	31
• Pyruvate	31
• PYY	18
• PYY	22

## R

• RANTES	12
• Renin	18
• Resistin	18
• Riboflavin (= lactoflavin = Vitamin B2)	29
• (9-D-Ribosylxanthine)-5'-phosphate	29

## S

• SAA	11
• SAA	15
• Saccharose	34
• Salicin (= Salicoside)	27
• Scu-PA and u-PA antigen	17
• Selenium	25
• Selenomethionine	27
• s-E-Selectin	15
• Shikimic acid	28
• s-ICAM	15
• Soluble fibrin	17
• Somatostatin	22
• Sorbitol (=gulitol = glucitol)	33
• Spermidine (= N-(3-Aminopropyl)-1,4-butane-diamine)	26
• Spermine (= N,N'-Bis(3-aminopropyl)-1,4-butanediamine)	26
• Succinate	31
• Succinyl-CoA	28
• Sucrose 6-phosphate	33

**T**

• TAFI antigen	17
• Testosterone	18
• 5,6,7,8-Tetrahydrofolate	31
• Tetrahydrofolicacid	32
• TFPI (free)	17
• TFPI (total)	17
• TGF- $\beta$ 2	11
• TGF- $\beta$ 1	11
• Thiamine-diphosphate	28
• Thiaminemonophosphate	28
• Threanine = isopropanolamine = 1-Amino-2-propanol	26
• Thrombin generation	16
• Thrombin-antithrombin (TAT)	17
• s-Thrombomodulin	15
• Thymidine	32
• Thymidine 5'-diphosphate (TDP)	30
• Thymidine 5'-monophosphate (Thymidylic acid = TMP)	30
• Thymidine 5'-triphosphate (TTP)	30
• Thymidine-5'-monophosphate	28
• Thyroid Stimulating Hormone (TSH)	18
• TNFa	11
• TNFa converting enzyme (TACE / ADAM17)	7
• Total fatty acid profile (C8-C24)	9
• Total fatty acid profile (C8-C24)	20
• Total Homocystein	15
• Total thyroxine (T4) (human)	18
• Trace elements	20
• Trans-4-Hydroxy-L-proline	27
• Trans-butenedioicacid	31
• Trehalose 6-phosphate	33
• Triglycerides	9
• Triglycerides	9
• Triiodothyronine (T3)	18
• TXA2 + 11-dehydro-TXB2 (urine)	17
• TXB <sub>2</sub>	14

**U**

• UDP-D-galactose	32
• UDP-D-glucose	32
• UDP-N-acetyl-D-glucosamine	32
• Undecaprenol	26
• Uracil-6-carboxylicacid	32
• Urate	32
• Urea	28
• Uridine	29
• Uridine 5'-monophosphate (= uridylic acid = UMP)	30
• Uridine 5'-triphosphate (UTP)	30
• Urine = 8-iso-PGF2-alpha	14
• Urocanate	31

**V**

• Vanadium	25
• s-VCAM	15
• Vitamin A	20
• Vitamin B1	20
• Vitamin B12	20
• Vitamin B2	20
• Vitamin B3	20
• Vitamin B6	20
• Vitamin C	20
• Vitamin D 1,25-dihydroxy	5
• Vitamin D 1,25-dihydroxy	20
• Vitamin D 24,25-dihydroxy	5
• Vitamin D 24,25-dihydroxy	20
• Vitamin D 25-hydroxy	5
• Vitamin D 25-hydroxy	20
• Vitamin E	20
• Vitamin K1	5
• Vitamin K1	17
• Vitamin K1	20
• Von Willebrand factor	15

**X**

• Xanthine	32
• Xanthosine	29
• Xanthosine 5'-monophosphate (= Xanthylicacid = XMP)	30

**Z**

• Zinc	25
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