

**WHO/DIL/LAB/99.1**  
**ENGLISH ONLY**  
**Distr.: GENERAL**



**WORLD HEALTH ORGANIZATION**

**USE OF ANTICOAGULANTS IN DIAGNOSTIC  
LABORATORY INVESTIGATIONS**

**1999**

© World Health Organization (1999)

This document is not a formal publication of the World Health Organization (WHO), and all rights are reserved by the Organization. The document may, however, be freely reviewed, abstracted, reproduced or translated, in part or in whole, but not for sale or for use in conjunction with commercial purposes.

The views expressed in documents by named authors are solely the responsibility of those authors.

**WHO/DIL/LAB/99.1**  
**ENGLISH ONLY**  
**Distr.: GENERAL**



**WORLD HEALTH ORGANIZATION**

**USE OF ANTICOAGULANTS IN DIAGNOSTIC  
LABORATORY INVESTIGATIONS**

**List of authors:**

W. Ehret, F. da Fonseca-Wollheim, W.G. Guder, W. Heil, O. Müller-Plathe, G. Töpfer, H. Wisser and B. Zawta.

**List of contributors:**

G. Banfi, Milan, A. Deom, Geneva, C.G. Fraser, Dundee, P. Hagemann, Zürich, J. Henny, Nancy, A. Kallner, Stockholm, E.A. Leppänen, Helsinki, S. Narayanan, New York, M. Neumaier, Hamburg, M.A. Peça Amaral Gomes, Lisbon, R. Probst, Munich, Y. Schmitt, Darmstadt.

R. Zinck, Wiesbaden Delkenheim, K.-H. Büscher, P. Mikulcik, München, R. Hinzmann, München, A. Karallus/G. Linke, Heidelberg, M. Lammers, Marburg, M. Buchberger, Kremsmünster, J. Kukuk, Limburg, H. Gross, Hanau, D. Klahr, Tuttlingen, D. Kolpe, Nümbrecht-Elsenroth, H. Kitta, Usingen, G. Gunzer, Clare, O. Sonntag, Neckargemünd, S. Speidel, Mannheim, W. Brand, Nümbrecht, V.-J. Friemert, Deisenhofen, T. Kunert-Latus, Frankfurt/Main, G. Hoffmann, Grafrath

## CONTENTS

<b>1. INTRODUCTION</b> .....	4
<b>2. DEFINITIONS</b> .....	5
2.1 Whole blood .....	5
2.2 Serum .....	5
2.3 Plasma .....	5
2.4 Anticoagulants .....	5
2.4.1 Ethylene Diamine Tetraacetic Acid (EDTA) .....	5
2.4.2 Citrate .....	5
2.4.3 Heparinates .....	5
2.4.4 Hirudin .....	6
<b>3. PLASMA OR SERUM</b> .....	7
3.1 Advantages of using plasma .....	7
3.2 Disadvantages of plasma over serum .....	8
3.3 Analytical samples in the serological diagnosis of infectious diseases .....	8
<b>4. RECOMMENDATIONS</b> .....	9
4.1 Sample collection and transport time .....	9
4.2 Centrifugation .....	9
4.2.1 Background .....	9
4.2.2 Serum .....	9
4.2.3 Plasma .....	9
4.3 Storage .....	10
4.4 Evaluation of new analytical procedures .....	10
<b>5. Table: THE USE OF VARIOUS ANALYTICAL SAMPLES</b> .....	11
<b>6. REFERENCES</b> .....	26

## 1. INTRODUCTION

It is imperative that the *in vivo* state of the quantity in the body fluid under investigation be preserved unchanged for biochemical analysis to justify a medically valid conclusion. This is not always possible when measuring extracellular and cellular components of blood. Platelets and coagulation factors are activated when the blood vessels are punctured, and these processes continue when sample containers are used without an anticoagulant. Coagulation-related changes of some quantities can be largely avoided by using anticoagulants added to the sample containers. The types and concentrations of anticoagulants were defined for the preservation of venous blood samples and are now used for standardized collection of plasma around the world (1).

This recommendation compiles the findings described in the literature and those provided by the contributors to this document on the use of anticoagulants. The overview was developed during several meetings and after discussions with experts from the diagnostics industry.

## 2. DEFINITIONS

### 2.1 Whole blood

A venous, arterial or capillary blood sample in which the concentration and characteristics of cellular and extracellular quantities remain relatively unchanged when compared with the in vivo state.

### 2.2 Serum

The undiluted, extracellular portion of blood after adequate coagulation is complete.

### 2.3 Plasma

The virtually cell-free supernatant of blood containing anticoagulant obtained after centrifugation.

### 2.4 Anticoagulants

Anticoagulants are additives that inhibit blood and/or plasma to ensure that the quantity to be measured is changed as little as possible before the analytical process. Anticoagulation is achieved by either the binding of calcium ions (EDTA, citrate) or by the inhibition of thrombin activity (heparinates, hirudin). It is important that the blood be mixed with the following concentrations of solid or liquid anticoagulant immediately after sample collection:

#### 2.4.1 A salt of ethylene diamine tetra acetic acid (EDTA)

Dipotassium ( $K_2$ ), tripotassium ( $K_3$ ) (1) and disodium ( $Na_2$ ) are used as cations (2). Concentrations: 1.2 to 2.0 mg/mL blood (4.1 to 6.8 mmol/L blood) based on anhydrous EDTA.

#### 2.4.2 Citrate

Trisodium citrate with 0.100 to 0.136 mol/L citric acid. Buffered citrate with pH 5.5 to 5.6:84 mmol/L trisodium citrate 21 mmol/L citric acid. 0.109 mol/L (3.2%) was recommended (3). WHO and NCCLS recommend 3.2% since differences have been noticed between 3.2% and 3.8% when reporting INR using respective reagents (4,5). The International Society for Thrombosis and Hemostasis (ISTH) recommend the use of Hepes buffer for all investigations of hemostatic functions (3). A mixture of 1 part citrate with 9 parts blood is recommended for coagulation tests (1,5). One part citrate is mixed with 4 parts blood to determine the erythrocyte sedimentation rate (1).

#### 2.4.3 Heparinates

12 to 30 international units (IU)/mL of unfractionated sodium, lithium or ammonium salt of heparin with a molecular mass of from 3 to 30 kD is recommended to obtain heparinized plasma (1). Calcium-titrated heparin at a concentration of 40-60 IU/mL blood (dry heparinization) and 8-12 IU/mL blood (liquid heparinization) is recommended for the determination of ionized calcium (6).

#### **2.4.4 Hirudin**

An antithrombin extracted from leeches or prepared by a genetic engineering process. Hirudin binds thrombin to form a 1:1 hirudin-thrombin complex. It is used in a concentration of 10 mg/L (7).

The **colour codes** of tubes containing anticoagulants according to ISO 6710 are: EDTA = purple; citrate 9:1 = light blue; citrate 4:1 = black; heparinate = green; no additives (for serum) = red (1).

### 3. PLASMA OR SERUM

#### 3.1 Advantages of using plasma

The following aspects support the preferential use of plasma versus serum in laboratory medicine:

1. Time saving: Unlike serum, in which coagulation is not complete until after 30 minutes, plasma samples can be centrifuged directly after sample collection.
2. Higher yield: 15 to 20% more plasma than serum can be obtained from the same volume of blood.
3. Prevention of coagulation-induced interferences: Regardless of coagulation-induced changes in blood (see below) anticoagulants prevent coagulation of blood or plasma in the primary and secondary container after centrifugation that could cause analytical interferences (e.g., clogged sample needle in the analytical system).
4. Prevention of coagulation-induced changes: The coagulation process changes the concentrations of numerous quantities in the extracellular fluid beyond the maximum allowable limit (8,9). This is induced by the following mechanisms:
  - Increase in the number of platelet components in serum as compared with plasma (e.g. potassium, phosphate, magnesium, aspartate amino transferase, lactate dehydrogenase, serotonin, neuron-specific enolase, zinc). Release of ammonia from fibrinogen induced by Factor XIII.
  - A decrease in the concentration of quantities in serum as a result of the coagulation process (total protein, platelets, glucose).
  - Activation of the cell lysis of erythrocytes and leukocytes in uncoagulated blood (free haemoglobin, cytokines, receptors).

Due to coagulation-induced changes some determinations yield valuable results only when plasma is used (e.g. neuron-specific enolase, serotonin, ammonium).

#### 3.2 Disadvantages of plasma over serum

The addition of anticoagulants can interfere with certain analytical methods or change the concentration of the quantities measured:

- Contamination with cations: ammonium, lithium, sodium, potassium.
- Assay interference caused by metals binding to EDTA and citrate (e.g. inhibition of alkaline phosphatase activity by zinc binding, inhibition of metallo-proteinases, inhibition of metal-dependent cell activations in function tests, binding of calcium (ionized) to heparin (6).
- Interference by fibrinogen in heterogenous immunoassays (9).
- Inhibition of metabolic or catalytic reactions by heparin: e.g. Taq polymerase in the polymerase chain reaction (PCR) (10).

- Interference in the distribution of ions between the intracellular and extracellular space (e.g.  $\text{Cl}^-$ ,  $\text{NH}_4^+$ ) by EDTA, citrate (8).
- Serum electrophoresis can be performed only after pretreatment.

The table below provides information on the utility of anticoagulants for the measurement of analyses in whole blood, plasma or serum.

### **3.3 Analytical samples in the serological diagnosis of infectious diseases**

A variety of methods are used for serological diagnosis of infectious diseases. They include immunodiffusion, immunoprecipitation, counterimmuno-electrophoresis, agglutination of bacteria, haemagglutination and agglutination inhibition, particle-enhanced agglutination complement fixation, indirect immunofluorescence (IFA), enzyme-linked immunoassay (ELISA), radio immunoassay (RIA), neutralization of toxins or virus-activity, immunoblot (Westernblot) and others.

In general, serum is used for the serological diagnosis of infectious diseases; serum must be used for some immunological techniques such as complement fixation or bacterial agglutination tests; for others, such as some haemagglutination tests, ELISAs or immunoblots, serum and plasma can be used likewise. Usually, the commercially available tests are adapted to the use of serum only.

## 4. RECOMMENDATIONS

The table below indicates materials that are recommended for a specific test. The table also contains information on the utility of other sample materials as long as the measured results by that method do not exceed the maximum allowable deviation of measurement (11). A maximum deviation of 10% is taken as acceptable if the quantity is not included in the current list. In this case, other samples can also be used for the analysis.

### 4.1 Sample collection and transport time

The following procedure is recommended to avoid contamination when filling tubes (8): blood culture, serum [avoid serum as first tube when electrolytes are to be measured (19)], citrate, heparinate, EDTA, tubes containing additional stabilizers (e.g. glycolysis inhibitors).

Tilt the tube repeatedly directly after filling to thoroughly mix the sample with anticoagulant (do not shake, avoid foaming). Leave the containers at room temperature for at least 30 minutes to separate serum from non-anticoagulated samples of whole blood. This period is shorter when coagulation is activated. The length of time to leave the sample at room temperature should not exceed the time recommended by the Working Group for the stability of the quantity in the sample matrix (12).

### 4.2 Centrifugation

#### 4.2.1 Background

Blood cell constituents can be rapidly separated from plasma/serum by centrifugation (relative centrifugal force, rcf). Rcf and rotations per minute (rpm) can be calculated by using the rotating radius  $r$  (the distance between the axis of rotation and the base of the container in mm). The following equation applies:

$$rcf = 1,118 \times r \{rpm/1000\}^2$$

It is recommended to centrifuge blood containers in 90° -swing-out rotors that the sediment surface forms a right angle to the container wall, thus preventing contact between the sampling needle in the analyser and the cell surface or separating gels.

#### 4.2.2 Serum

When coagulation is complete, the sample should be centrifuged for at least 10 minutes at a minimum speed of 1500 g.

#### 4.2.3 Plasma

To obtain cell-free plasma, centrifuge the anticoagulated blood (citrate, EDTA or heparinized blood) for at least 15 minutes at 2000 to 3000 g. When separating serum or plasma, the temperature should not drop below 15°C or exceed 24°C.

### **4.3 Storage**

Uncentrifuged samples can be stored at room temperature for the time as published in the recommendations on stability (12). After centrifugation, the serum or plasma from primary tubes should be analyzed within the time as indicated in the recommendations for whole blood, if the whole blood sample is stored at room temperature without the use of separating gels or a filter separator. If serum or plasma must be refrigerated or frozen for preservation or because of labile quantities, blood cells must first be separated. Avoid freezing whole blood samples before or after centrifugation, when polymer separating gels are used.

### **4.4 Evaluation of new analytical procedures**

Before using a new reagent or method, check the suitability of the procedure by comparing at least 20 blood samples with normal, and 20 with pathological concentrations of the quantity to be measured. It can be assumed that the criteria for biological and clinical interpretation (reference intervals, clinical decision limits) have changed, if the mean of the difference between the samples tested deviates by more than the maximum deviation allowed (11) (or by more than 10%).

## 5. Table: THE USE OF VARIOUS ANALYTICAL SAMPLES

⊕ recommended sample

+ can be used without changes of results

(+) can be used with limitations [see remarks; in case of citrate plasma this indicates the need to consider dilution by liquid citrate (20)].

Increased (↗) decreased (↘) results may be observed when compared to recommended samples.

A blank field indicates that no data was found from literature.

Greek letters refer to information provided by diagnostic companies.

Numbers in brackets indicate references.

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citrate Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Acetaminophen	+	+ α, β	+ α					
Acetylsalicylate	+	+ β	+ β	(+) β				
α <sub>1</sub> Acid glycoprotein (orosomuroid)	+	+ γ	+ γ	(+)				
Adenovirus-antibodies	+							complement fixation test ELISA IgG, IgM
Adrenocorticotrophic hormone (ACTH)		+	⊕					stabilized with aprotinin 400 kU/mL, mercaptoethanol 2μl/mL
Alanine aminotransferase (ALAT, ALT, GPT)	+	+	+	(+)				
Albumin	+	+*	+	(+)				* bichromatic assays recommended for colorimetric methods (13)
Aldosterone	+	+	⊕					
Alkaline phosphatase	+↗	⊕	-	(+)				EDTA effectively binds essential cofactor zinc
Aluminium	-	-	-	-				special tube
Amikacin	+	+	-β	(+)β				
Amiodarone	+	+	-					HPLC
Amitriptyline	+	+						HPLC
Ammonium	-↗	(+)↗	⊖	-	+			use serine 5 mmol/L and borate 2 mmol/L to prevent in-vitro ammonia formation (14). Do not use ammonium heparinate
Amphetamine	+	+	+					
Amylase, pancreatic	+	+	+	(+)				
Amylase, total	+	+	(+)↘	(+)				possible decrease due to Mg and Ca binding (25)

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citrate Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Androstendione	+							
Angiotensin converting enzyme (ACE)	+		-	-				
$\alpha_1$ -Antitrypsin	+	+ $\gamma$	+ $\gamma$					
Anticonvulsant drugs	+							see phenobarbital, valproic acid, phenytoine
Anti-staphylolysin	+	+ $\gamma$	+ $\gamma$					
Anti-streptodornase B	+							
Anti-streptokinase	+							
Anti-streptolysin	+	+ $\beta, \gamma, \delta$	+ $\beta, \gamma, \delta$					
Anti-thrombin III	-			$\oplus$			+*	*test by Pharmacia
Anti-thrombin III immunol.			+ $\delta$	(+) $\delta$				
APC-resistance (genotyping factor V Leiden)					$\oplus$	$\oplus$		
APC-resistance (functional screening test)				$\oplus$				high level of heparin may require addition of heparin-binding substances
Apolipoprotein A1	+ $\nearrow$	+ $\gamma, \delta$	$\oplus \gamma, \delta$	(+)				see NCEP guidelines
Apolipoprotein B	+ $\nearrow$	+ $\gamma, \delta$	$\oplus \gamma, \delta$	(+)				see NCEP guidelines
Aspartate aminotransferase (ASAT)	+ $\nearrow$	$\oplus$	+	(+)				
Aspergillus antibodies	+							
Aspergillus antigen-detection	+							
Aspergillus, culture isolation								blood culture bottle
<b>Autoantibodies</b>								
Anti-mitochondrial antibodies (AMA)	+							
Anti-neutrophil cytoplasmic antibodies (ANCA)	+							
Anti-nuclear antibodies (ANA)	+							
Cardiolipin antibodies	+							
Cold-autoantibodies					+?			Native blood in 37°C water bath
DNA antibodies	+							
Thrombocyte antibodies			+	+				

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citratd Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Thyreotropin receptor antibodies (TRAK)	+							
Barbiturates (see also phenobarbital)	+	+						
Bartonella spp. antibodies (cat scratch disease)	+							
Batroxobin time	-	-	-	⊕				
Benzodiazepines	+	+						
Bicarbonate	+	+			⊕			see also blood gases
Bilirubin, direct, total	+	+	+	(+)				
Blood cell surface markers (immunocytometry)					+	+		special stabilizer recommended (Cyfix II) (23)
Blood gases (CO <sub>2</sub> , O <sub>2</sub> )					⊕			heparinized titrated blood (6)
Bordetella pertussis antibodies	+							
Borrelia burgdorferi-antibodies (Lyme disease)	+							ELISA, Western Blot
Brucella antibodies (Brucellosis)	+							
CA 125	+	+ γ	+ γ	(+) γ				
CA 15-3	+	+ γ	+ β, γ	(+) γ				
CA 19-9	+	+ γ	+ γ	(+) γ				
CA 72-4	+	+ γ	+ γ	(+) γ				
Cadmium	-		⊖	-				special tube
Calcitonin	+	+						stabilized with aprotinin 400 kU/mL
Calcium	+	+	-	-	+			
Calcium ionized (free)	-	(+)	-	-	+			use calcium-titrated heparin (6)
Campylobacter jejuni/fetus antibodies	+							
Candida albicans antibodies	+							
Candida spp.; culture								blood culture bottle
Candida antigen detection	+							
Carbamazepine	+	+ β, γ, δ	+ β, γ	(+) β, γ				

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citratd Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Carbohydrate deficient transferrin (CDT)	+	+						method-dependent
Carcino-embryonic antigen (CEA)	+	+ $\beta$ , $\gamma$	+ $\beta$ , $\gamma$	+ $\gamma$				
Cardiolipin antibodies	+							
Catecholamines (epinephrine, norepinephrine)	-	$\oplus$	(+)	-				stabilized with EGTA and glutathione (26)
CD3/CD4 antigen					+	+		
Ceruloplasmin	+	+	+					
C1-esterase inhibitor	+		+ $\epsilon$	(+)				stabilize plasma by freezing
Chinidin	+	+ $\beta$	+ $\beta$	(+) $\beta$				
Chlamydia-antibodies (C. trachomatis, C. pneumoniae)	+							
Chloramphenicol	+	+ $\beta$	+ $\beta$					
Chloride	+	+	-	-	+			
Cholesterol	+	+	$\oplus$	(+)				
Cholesterol, HDL	+	+	$\oplus \delta$	-				
Cholesterol, LDL	+	+	$\oplus$	-				
Cholinesterase including dibucain number	+	+	+					
Circulating immune complexes	+							
Clostridium tetani toxin antibodies	+							
Coagulation factors II-XIII	-	-	-	$\oplus$				
Cocaine	+	+			+			
C3-complement	+	+	+ $\gamma$	(+) $\gamma$				
C4-complement	+	+	+	(+)				
Copper	+	+	-	-				special tube available
Corticotropin								see ACTH
Cortisol	+	+	+ $\gamma$					
Corynebacterium diphtheriae toxin antibodies	+							
Coxiella burnetii antibodies (Q-fever)	+							
Coxsackie-virus antibodies	+							

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citratd Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
C-peptide	+	+						
C-reactive protein (CRP)	+	(+) ** + $\alpha$ , $\epsilon$	(+) * + $\alpha$ , $\epsilon$	(+)				* method-dependent ** patient-dependent low results
Creatine kinase (CK)	+	+ $\beta$ , $\delta$	+ $\beta$ , $\delta$	(+)				
Creatine kinase MB, enzym	+	+	-	(+) $\delta$				
Creatine kinase MB, immunoassay	+	+ $\beta$ , $\gamma$	+ $\beta$ , $\gamma$ , $\delta$	(+) $\gamma$				
Creatinine	+	+	+	(+)				
Cryptococcus neoformans								blood culture bottle
Cyclosporin A	-	-	-	-		⊕		
CYFRA 21-1	+	+ $\gamma$	+ $\gamma$	(+) $\gamma$				
Cystatin C	+	+	⊕					
Cytomegalovirus antibodies	+	+ $\beta$	+ $\beta$	(+) $\beta$				
Cytomegalovirus antigen detection (pp65)	+					⊕		
Cytomegalovirus DNA-amplification						⊕		
D-Dimer	-	+	-	⊕				
Dehydroepiandrosterone-sulfate (DHEA-S)	+							
Dengue-virus antibodies	+							
Diazepam	+	+	+					
Differential leucocyte count						⊕		K <sub>3</sub> or K <sub>2</sub> EDTA, stability instrument-dependent
Digitoxin	+	+ $\alpha$ , $\beta$ , $\gamma$	+ $\gamma$					
Digoxin	+	+ $\alpha$ , $\beta$ , $\gamma$	+ $\beta$ , $\gamma$	(+) $\beta$				
Disopyramide	+	+	+	(+)				
DNA- and RNA-analysis by amplication					-	⊕	+	heparin inhibits Ta-qpolymerase and restriction enzymes (21), LiCl 1.8 mol/L eliminates this error (22)
Dopamine			+					
Echinococcus spp. antibodies	+							
ECHO-virus antibodies	+							
Elastase						+		

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citrate Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Electrophoresis								see lipoprotein or serum electrophoresis
Entamoeba histolytica antibodies	+							
Enterovirus-antibodies	+							
Epstein Barr heterophilic antibodies (Paul Bunnell test)	+							
Epstein-Barr-Virus-antibodies (anti-EBNA, -VCA, EA); IgG, IgM, IgA; ELISA	+							
Erythrocyte sedimentation rate (ESR)							⊕	1 part citrate, 4 parts blood
Erythrocytes					+	⊕	(+)	
Erythropoietin	⊕	+	+					
Estradiol (E <sub>2</sub> )	+	(+) γ	(+) γ	(+) γ				
Estriol (E <sub>3</sub> )	(+)	+						
Ethanol	+	+ β	+ β	(+) β, δ		+		
Ethosuximide	+	+	+					
Fatty acids, free	+	(+) * <sup>✓</sup>	(+) * <sup>✓</sup>					* activation of lipase by heparin
Ferritin	+	+ β, γ, δ	(+) * γ	(+) γ				* method-dependent
α <sub>1</sub> -Fetoprotein (AFP)	+	+ β, γ	+ β, γ	(+) β, γ				
Fibrin monomers	-	-	-	⊕				
Fibrin(ogen) degradation products	(+) *	-	-	(+) **				* special tube stabilized with 10 U thrombin and 150 AU kallikrein/mL blood (27) ** aprotinin or soybean trypsin inhibitor, special tube (27)
Fibrinogen, Clauss	-	-	-	⊕				
Fibrinogen immunol.	-	-	-	⊕				
Folate	+	+	+ β	(+) β		+ β		haemolysate prepared by 0.5 blood + 4.5 mL ascorbic acid (2 g/L). Na-heparinate shown to interfere with AxSYM test (β)
Follitropin (FSH)	+	+α, β, γ	+α, β, γ	(+) γ				

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citrated Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Francisella tularensis antibodies (tularemia)								
Fructosamine	+	+	+					
FSME virus antibodies	+							
Galactose 1-puridyltransferase (Beutler Test)						+		erythrocytes
Gastrin	+	⊕ *	+	(+)				* add aprotinin 2000 KIU/mL to stabilize
Gentamicin	+	+β, γ, δ	+β, γ, δ	(+) β				
Glucagon			+					stabilized with aprotinin (8)
Glucose Glucose capillary	- <del>↘</del> -	+ <del>↘</del>	+ <del>↘</del>		(+) (+)			use glycolysis inhibitor tube or whole blood with universal glycolysis inhibitor (mannose, fluoride)
Glutamate dehydrogenase	+	+	+					
γ-Glutamyl transferase IFCC/Szasz	+	+	+	(+)				
Glycated albumin								see fructosamine
Gold	+							
Glutamate oxaloacetate transaminase (GOT)								see ASAT
Glutamate pyruvate transaminase (GPT)								see ALAT
Growth hormone (STH; somatotropin)	⊕	-	-					
Haematocrit					+	⊕		
Haemoglobin A1c						⊕		haemolysate
Haemoglobin F (HbF)						+		
Haemoglobin in whole blood						⊕		
Haemoglobin plasma	(+) ↗	⊕	+					haemolysis during clotting
Hantavirus-antibodies	+							
Hantavirus-DNA-amplification					-	⊕	+	
Haptoglobin	+	+	+					
Helicobacter pylori-antibodies	+							ELISA; immunoblot detection of virulence factors (cag A and others)

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citratd Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Heparin (anti Xa)				⊕				
Hepatitis antibodies								
anti-HAV	+	+β	+β	(+)β				
anti-HBsAg	+	+β, δ	+β, δ	(+)β, δ				
anti-HBc	+	+β	+β	+β				
anti-Habe	+	+β	+β	(+)β				
anti-HCV	+	+β	+β	(+)β				
HBsAg	+	+δ	+δ	(+)δ				
HBeAg	+	+β	+β	(+)β				
Hepatitis B virus DNA						⊕		
Hepatitis C virus RNA-amplification, qualitative, quantitative (virus load)						⊕		
Hepatitis C virus genome typing						⊕		
Hepatitis D virus antibodies	+							
Hepatitis D virus RNA-amplification						⊕		
Hepatitis E virus antibodies	+							
Hepatitis E RNA-amplification (after stay in Asia)						⊕		
Heparin-associated thrombopenia; HIPA test	+							
Herpes simplex 1 or 2-virus antibodies	+							
HHV 6 antibodies (human herpes virus 6)	+							
HHV 6, 7, 8 -DNA-amplification						⊕		
H1 virus-1 and 2 antibodies (Human immunodeficiency virus)	+	+β	+β	(+)β				MEIA ELISA; immunoblot
H1 virus-1 RNA-amplification qualitative, quantitative (virus load)			+			⊕		
H1 virus-1 (provirus) DNA-amplification						⊕		
HLA-ABC typing					⊕			ammonium-heparinate blood
HLA-B27					⊕	⊕		“ ” “
HLA DR typing						⊕		

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citrated Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Homocystein	+↗	+	+	(+)		⊕		sample with EDTA/acidic citrate (0.5 mol/L) (17). Store blood at 0-4°C. Haemolyzed EDTA sample in detergent (18).
HTLV I antibodies (T-cell leukemia)	+							
HTLV I (provirus) DNA-amplification						⊕		
HTLV I RNA-amplification			+					
Human chorionic-gonadotropin (βhCG)	+	+ β, γ	+ β, γ	(+) γ				
Human growth hormone (HGH)	⊕	+						
3-Hydroxybutyrate					+			deproteinized blood
IgA	+	+ γ, δ	+ γ, δ					
IgD	⊕		- ↘, γ					
IgE antigen specific IgE	⊕ +	+ δ, ε	- ↘ + δ, ε	(+) ε				
IgG IgG subclasses	+ +	+ γ, δ	- ↘	-				
IgM	+	+ γ, δ	↘, γ, + δ					
Influenza virus ABC-antibodies	+							
Insulin	(+)↘	+	+					
Iron (Fe)	+	+	- ↘	- ↘				
JC polyoma virus-antibodies (progressive multifocal leukoencephalopathy, PML)	+							
JC polyoma virus DNA-amplification (PML)						⊕		
Lactate	- ä	- ä	- ä	-	(+)			use glycolysis inhibitor tube (fluoride/oxalate) if not immediately deproteinized in whole blood
Lactate dehydrogenase (LDH)	(+)↗	⊕	+	(+)				LDH platelet-dependent (8,9)
Lead (Pb)	-	-	+	-	(+)			use special tubes
Legionella - antibodies	+							

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citratd Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Leishmania spp. antibodies (Visceral leishmaniosis)	+							
Leptospira spp. antibodies (Leptospiroses)	+							
Leukocyte count					+	⊕	(+)	
Lidocaine	+	+β	+β					
Light chains (κ, λ)	+	+γ	+γ					
Lipase	+	+	-	-				EDTA binds calcium (activator)
Lipoprotein electrophoresis	⊕	-	-	-				
Lipoprotein (a)	+	+γ	+γ	-γ				
Listeria monocytogenes antibodies	+							
Listeria monocytogenes DNA-amplification						⊕		
Lithium	+	+*	-	-				* do not use Li-heparinate
Lupus anticoagulant	-	-	-	⊕				
Lutropin (LH)	+	+β	+β					
Lymphocyte subtypes					+	(+)		special stabilizer recommended (Cyfix II) (23)
Lymphocytic choriomeningitis virus (LCM) antibodies	+							
Lymphocytic choriomeningitis virus DNA-amplification						⊕		
Magnesium (Mg)	+↗	+	-	-↘	⊕			separate blood cells before analysis (16)
Measles virus antibodies	+							
Measles virus RNA-amplification						⊕		
Mercury (Hg)					+			special tube
Methadone	+	+						
Methotrexate	+							
α <sub>1</sub> -Microglobulin	+							
β <sub>2</sub> -Microglobulin	+	+γ	+γ	(+)γ				
Microfilaria					+	+		concentrated sample
Morbilli virus antibodies	+							
Morbilli virus DNA-amplification						⊕		

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citratd Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Mumps virus-antibodies	+							
Mycobacterium spp. DNA-amplification (tuberculosis and atypical mycobacteria)						⊕		
Mycoplasma pneumoniae antibodies	+							
Myoglobin	+	+ γ, ε	+ γ, δ, ε	(+) γ, ε				
Neisseria gonorrhoeae antibodies	+							
Netilmycin	+							
Neuron-specific enolase (NSE)	+ ↗	⊕						increased by thrombolysis
Nitrazepam	+	+ β	+ β	(+) β				
Opiates	+	+						
Osmolality	+	+						
Osteocalcin	+*	+*	⊕*					* stabilized with aprotinin 2500 kU/mL
Pancreas elastase	+							
Paracetamol	+	+ β	+ β	(+) β				
Parathyrin (PTH)	+		⊕					
Partial thromboplastin time activated (aPTT)	-	-	-	⊕				
Parvovirus B19 - DNA-amplification						⊕		
Phenobarbital	+	+ β, γ, δ	+ β, γ, δ	(+)β,γ,δ				
Phencyclidine	+							
Phenytoin	+	+ β, γ, δ	+ β, γ, δ	(+) β, γ				
Phosphate, inorganic	(+) ↗	⊕	+					platelet-dependent in serum (8, 9, 15)
Plasmodium spp.						⊕		microscopic examination of whole blood
Plasmodium antibodies	+							
Potassium	(+) ↗	⊕	-	-	+			platelet-dependent in serum (8, 9, 15)
Pneumococcus								blood culture
Prealbumin	+		+ γ					
Primidone	+	+	+	(+)				

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citratd Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Procainamide	+	+ β	+ β	(+) β				
N-Acetyl-procainamide	+	+ β	+ β	(+) β				
Procalcitonin	+	+ δ						
Progesterone	+	+ β	+ β					
Prolactin	+	+ β, δ	+ β					
Propaphenone	+	+						
Propoxyphene	+	+						
Prostate specific antigen (PSA), free	+	+ γ	+ γ					
Prostate specific antigen (PSA), total	+	+ γ	+ γ	(+) γ				
Protein C	-	-	-	⊕				
Protein S	-	-	-	⊕				
Protein S 100	+							
Proteins, total	+ ~	⊕	+ δ	(+)				plasma results higher due to fibrinogen
Prothrombin time (Quick)	-	-	-	⊕				
Pyruvate	- ~	- ~			+			deproteinized blood
Q-fever antibodies	+							
Quinidine	+	+ β	+ β	(+) β				
Red blood cell count					+	⊕	(+)	
Renin	-	-	+	-				
Reovirus antibodies	+							
Reptilase time								see Batroxobin time
Respiratory syncytial virus (RSV) antibodies	+							
Rheumatoid factors (RF) subfractions IgA, IgG	+ +		+ γ	(+) γ				
Reticulocyte count					(+)	⊕		K <sub>2</sub> or K <sub>3</sub> EDTA, method-dependent
Rickettsia antibodies	+							
Rotavirus antibodies	+							
Rubellavirus antibodies	+	+ β	+ β	(+) β				
Rubellavirus DNA-amplification						⊕		
Salicylate	+	+ β, δ	+ β, δ	(+) β, δ				

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citratd Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Sandfly (pappataci-) fever antibodies	+							
Selenium (Se)	-	-	-	-		+		special tubes
Serum amyloid A (SAA)	+							
Serum electrophoresis	⊕	(+)						fibrinogen to be considered when using heparin plasma, may be eliminated
Sodium (Na)	+	+	-	-	+	*		* use 140 mM Na stabilized heparin 8-12 IU/mL blood (6)
Somatotropin (STH)								see growth hormone
Squamous cell carcinoma antigen (SCC)	+							
Staphylococcal antibodies; anti-staphylolysin	+							
Streptococcal antibodies; anti-streptolysin 0 anti-streptokinase	+							
Streptococcal antibodies; anti B DNase	+							
Streptococcal antibodies, anti-hyaluronidase	+							
Tacrolimus (FK 506)			-	-		⊕		
Testosterone	+	+ γ	+ γ	(+) γ				
Tetrahydrocannabinol - carbonic acid (THC)	+	+						
Theophylline	+	+ β, γ	+ β, γ, δ	(+) β, γ				
Thrombin time	-	-	-	⊕				
Thrombocyte function							⊕	special tube (23)
Thyreotropine (TSH)	+	+ β, γ	+ β, γ	(+) γ				spot blood in new borns
Thyroglobulin	+							
Thyroid antibodies	+							
Thyroxine (T4)	⊕	+ β, γ	+ β, γ	(+) γ				plasma difference method-dependent
Thyroxine, free (fT4)	+	+ β, γ	+ γ	(+) γ				
Thyroxine binding globulin (TBG)	+	+						

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citrate Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Tick born encephalitis-virus antibodies (FSME)	+							
Tobramycin	+	+ β, δ	+ β, δ	(+) β				
Toxoplasma gondii-antibodies (IgG, IgM)	+	+ β	+ β	+ β				
Transferrin	+	+	+ γ					
Treponema pallidum antibodies	+							TPHA, IFT, FTA, abs., VDRL, Immunoblot
Treponema pallidum DNA-amplification					¾			
Tricyclic antidepressants	+	+ β	+ β	(+) β				
Triglycerides	+	+	¾	(+)				
Triiodothyronine (T3)	⊕	(+) β, γ, δ						plasma difference method-dependent
Triiodothyronine, free (fT3)	+	+ β, γ	+ β, γ	(+) γ				plasma difference method-dependent
Troponin I	+	+	+		+			method dependent
Troponin T	+	+ γ	(+) γ					
Trypanosoma gambiense					(+)			ideally, blood film of capillary blood
T-Uptake	+	+ β, γ	+ β, γ	(+) γ				
Urea	+	+	+					do not use NH <sub>4</sub> heparinate
Urate	+	+	+ β	(+)				
Valproate	+	+ β, γ, δ	+ β, γ, δ	(+) β, γ				
Vancomycin	+	+ β	+ β	(+) β				
Varicella Zoster virus antibodies	+							
Varicella Zoster virus DNA-amplification					⊕			
Vasoactive intestinal polypeptide (VIP)			⊕*					* Aprotinine 500-2000 KIU/mL (27)
Vasopressin (ADH)	-		⊕					frozen plasma
Vitamin A (retinol)	+							
Vitamin B1 (thiamin)		+	+					
Vitamin B12 (cobalamin)	+	+ β	⊕ β					
Vitamin B2 (riboflavin)		+	+					
Vitamin B6 (pyridoxal phosphate)			⊕					

Analyte	Serum	Heparinized Plasma	EDTA Plasma	Citrated Plasma	Whole Blood			Remarks
					Hep.	EDTA	Cit.	
Vitamin C (ascorbic acid)		+						stabilized with 60 mg/L metaphosphate
Vitamin D, 1,25-dihydroxycholecalciferol	+							
25-hydroxycholecalciferol	+							
Vitamin E (tocopherol)	+		⊕					
Vitamin K (transphylochinone)								
Yersinia enterocolitica antibodies	+							
Zinc (Zn)	-	+	-	-				special tube, avoid contamination by stopper (24)

Legend:     $\alpha$  : Ortho: Vitros-systeme  
                $\beta$  : Abbott: Axsym  
                $\gamma$  : Roche: Hitachi, Elecsys  
                $\delta$  : Beckman: (partially different specifications) synchron LX/CX Immage Array, Access  
                $\epsilon$  : Dade Behring: Behring Nephelometer Analyzer

**REFERENCES**

1. ISO 6710. Single use containers for venous blood specimen collection. Berlin: Beuth Verlag, 1996.
2. Goosens W, Van Duppen V, Verwilghen RL. K2 or K3-EDTA: The anticoagulant of choice in routine haematology? *Clin Lab Haemat* 1991; 13:291-5.
3. Polack B, Mossuz P, Barro C, Pernod G. Pre-analytical phase in haemostasis. Proceedings of the First Symposium on the impact of the pre-analytical phase on the quality of laboratory results in haemostasis; 1996 Oct 14; Montpellier (France). Becton Dickinson, 1996.
4. Adcock DM, Kressin DC, Martar RA. Effects of 3.2% vs. 3.8% citrate concentration on routine coagulation testing. *Am J Clin Pathol* 1997; 107:105-10.
5. Witt I, Beeser H, Müller-Berghaus G. Minimalanforderungen zur Gewinnung von Citratplasma für hämostaseologische Analysen. *Lab Med* 1995; 19:245-7.
6. Burnett RW, Covington AK, Fogh-andersen N, Külpmann WR, Maas AHJ, Müller-Plathe O, Siggard-Anderson O, van Kessel AL, Wimberley PD, Zijlstra WG. Approved IFCC recommendations on whole blood sampling, transport, and storage for simultaneous determination of pH, blood gases, and electrolytes. *Eur J Clin Chem Clin Biochem* 1995; 33: 247-53.
7. Engstadt CS, Guttenberg TJ, Posterut B. Modulation of blood cell activation by four commonly used anticoagulants. *Thrombosis and Hemostasis* 1997; 77: 690-6.
8. Guder WG, Narayanan S, Wisser H, Zawta B. Samples: from the patient to the laboratory. The impact of preanalytical variables on the quality of laboratory results. Darmstadt: GIT Verlag, 1996.
9. Voit R. Plasma-Serum Unterschiede und Lagerungsstabilität klinisch-chemischer Meßgrößen bei Verwendung von Plasmatrennröhrchen [dissertation] München (FRG): Ludwig-Maximilians-Universität, 1993.
10. Neumaier M, Braun A, Wagener C. Fundamentals of quality assessment of molecular amplification methods in clinical diagnostics. *Clin Chem* 1998; 44: 12-26.
11. Qualitätssicherung der quantitativen Bestimmungen im Laboratorium. Neue Richtlinien der Bundesärztekammer. *Dt. Ärztebl* 1988; 85: B517-32.
12. Guder WG, da Fonseca-Wollheim F, Heil W, Müller-Plathe O, Töpfer G, Wisser H, Zawta B. Stabilität der Meßgrößen in der Probenmatrix. *Mitt Dtsch Ges Klin Chem* 1995; 26: 205-24.
13. Hallbach J, Hoffmann GE, Guder WG. Overestimation of albumin in heparinized plasma. *Clin Chem* 1991; 37: 566-8.
14. Da Fonseca-Wollheim F. Deamidation of glutamine by increased plasma  $\gamma$ -glutamyltransferase is a source of rapid ammonia formation in blood and plasma specimens. *Clin Chem* 1990; 36: 1479-82.

15. Lutomski DM, Bower RH. The effect of thrombocytosis on serum potassium and phosphorus concentration. *Am J Med Sci* 1994; 307: 255-8.
16. Schwinger R, Antoni DH, Guder WG. Simultaneous determination of magnesium and potassium in lymphocytes, erythrocytes and thrombocytes. *J Trace Elem Electrolytes Health Dis* 1987; 1: 88-98.
17. Willems HPJ, Bos GMJ, Gerrits WBJ, Heijer M, Vloet S, Blom HJ. Acidic citrate stabilizes blood samples for assay of total homocysteine. *Clin Chem* 1998; 44: 342-4.
18. Probst R, Brandl R, Blümke M, Neumeier D. Stabilization of homocysteine concentration in whole blood. *Clin Chem*, 1998; 44:1567-9.
19. Leppänen E, Gräsbeck R. The effect of the order of filling tubes after venipuncture on serum potassium, total protein, and aspartate and alkaline aminotransferase. *Scand Clin Lab Invest* 1980; 46: 189-91.
20. Lammers M. Dilution of citrated plasma. *Eur J Clin Chem Clin Biochem* 1996; 34: 369.
21. Holodniy M, Kim S, Katzenstein D, Konrad M, Groves EW, Merigan TC. Inhibition of human immunodeficiency virus gene amplification by heparin. *J Clin Microbiol* 1991; 29: 676-9.
22. Jung R, Lübcke C, Wagener C, Neumaier M. Reversal of rt-PCR inhibition observed in heparinized clinical specimens. *Biotechniques* 1998; 23:24-8.
23. Ruf A, Patscheke H. Whole blood stabilization for the immunocytometric analysis of blood cells. Basel: FESCC Educational Program, 1997: 24.
24. Schmitt Y. Influence of preanalytical factors on the atomic absorption spectrometry determination of trace elements in biological samples. *J Trace Elem Electrolytes Health Dis* 1987; 1: 107-14.
25. IFCC method for  $\alpha$ -amylase (1,4- $\alpha$ -D-Glucan 4-Glucanohydrolase, EC 3.2.1.1.) *Clin Chem Lab Med* 1998; 36:185-203.
26. Boomsma F, Alberts G, van Eijkl, Mau in't FeldAJ, Schalenkamp ADH. Optimal collection and storage conditions for catecholamine measurement in human plasma and urine. *Clin Chem* 1993; 39:2503-8.
27. Narayanan S., Protection of peptidic substrates by protease inhibitors. *Biochim Clin* 1987; 11:954-6.

=====





