# DEVELOPMENT AND OPTIMIZATION OF REFERENCE MEASUREMENT PROCEDURES

**Summary of PhD Thesis**

**Patricia Kaiser, Dipl.-Ing.**

INSTAND e.V.

Society for Promotion of Quality Assurance
in Medical Laboratories

Düsseldorf, Germany

and

Department of Biochemistry
Albert Szent-Györgyi Medical Center
University of Szeged, Hungary

**2006**

## 1. INTRODUCTION

The most accurate procedures to assign reliable target values for quality control samples are the reference measurement procedures.

The main characteristics of reference measurement procedures are described in the ISO Standard 15193: 2005 "In vitro diagnostic medical devices- Measurement of quantities in samples of biological origin- Presentation of reference measurement procedures". In the ISO Standard a definition is given for the use of reference measurement procedures:

"*Reference measurement systems are needed for producing useful and reliable results of measurement, whether in science, technology, or routine service so as to be comparable and ultimately traceable to measurement standards of the highest metrological level.*"

The traceability of routine methods should be directly related to reference materials and reference measurement procedures and finally to the SI units. The traceability concept is applied by using primary and secondary standards, reference measurement procedures and certified reference materials (CRM). The manufacturers should calibrate their analytical systems with reference measurement procedures and/or certified reference materials, thus getting a link between internal and external quality assessment.

Based on the Article 11 of the "Directive 98/79/EC of the European Parliament and of the Council on in vitro Diagnostic Medical Devices" a mandated European Standard EN 14136 was developed, where again the criteria for the evaluation of kits and instruments in EQAS (external quality assessment schemes) have been defined. In paragraph 6.1.3 of the EN 14136 the assigning of values of survey samples should be traceable to a specific metrological level (as shown in EN ISO 17511: 2002 In vitro diagnostic medical devices- Measurement of quantities in samples of biological origin- Metrological traceability of values assigned to calibrators and control materials).

Descriptions, definitions, and classifications of reference materials are given in the international Standard ISO 15194: 2005- "In vitro diagnostics medical devices- Measurement of quantities in samples of biological origin- Description of reference materials".

According to ISO 15194 reference materials are "*standards of highest metrological level* "and "*are used to obtain this traceability, both through time, distance, and different measurement procedures.*"

Reference materials can be used to establish the "*unbroken chain of comparisons",* required in ISO Standards. The requirements for reference laboratories to fulfil the criteria of the reference measurement system are defined in the ISO Standard 15195: 2003 - "Laboratory Medicine- Requirements for reference measurement laboratories".
Reference measurement procedures should be used

1) for the determination of target values for internal and external quality control samples
2) for calibration of kits and instruments
3) to get identical reference ranges for a defined population in all laboratories
4) for evaluation of the performance of in-vitro diagnostic medical devices
5) to establish a basis for the comparability of results of epidemiological studies

According to the ISO Standards 17025: 2005 the development of reference measurement procedures is a dynamic process with continuous quality improvement. For reference measurement procedures it is desirable to use measurement systems of highest quality and performance. Therefore, established reference measurement procedures should be continuously evaluated and optimized or replaced by a new method, if better analytical equipments, procedures or materials are available. This continuous process of improvement in analytical quality is a basic principle of quality management in medical laboratories and also the aim of this study.
In the ISO Standard 15193: 2005 a reference measurement procedure is defined as a "*thoroughly investigated measurement procedure shown to yield values having an uncertainty of measurement commensurate with its intended use, especially in assessing the trueness of other measurement procedures for the same quantity and characterizing reference materials"*.
An essential requirement of an accepted reference measurement procedure is that the procedure is confirmed by international intercomparison studies, like JCTLM and IFCC Working Group on Standardization of $HbA_{1c}$.
In contrast to reference measurement procedures routine methods used in the clinical laboratories are mostly designed for a high throughput of analyses combined with a short turnaround time at acceptable costs and performance. The continuous process of

improvement in analytical quality is also an important strategy for routine measurement procedures.

Reference measurement procedures are used for the determination of target values in the EQAS in several countries. The fundamental difference of this evaluation procedure from the "consensus value principle" is that the same target values with the same acceptability ranges are set for all laboratories and by this way comparable results are collected in laboratory medicine.

The reliability of the reference measurement procedure must be permanently controlled and continuously evaluated with the effect, that the reference measurement procedures are dynamic, because of this continuously evaluation and improvement process.

In Germany the "Guideline of the Federal Medical Council for Quality Assurance of Quantitative Analyses in Laboratory Medicine" requires the use of reference measurement procedures for setting target values for many analytes, to ensure the comparability of results in laboratory medicine. The Federal Medical Council of Germany favours the development of reference measurement procedures. This strategy promoting the comparability of laboratory analyses at the highest level of accuracy supports the high quality of patient care and reduces costs in the health care system.

## 2. AIMS AND SCOPE

The aim of this study was to improve the quality of the medical laboratory performance by providing reference measurement procedures for several analytes for internal and external quality assessment. The study is focused on the implementation, improvement, and evaluation of existing reference measurement procedures and the development of new reference measurement procedures using GC-MS and LC-MS.

The Guideline of the Federal Medical Council for Quality Assurance of Quantitative Analyses in Laboratory Medicine of Germany requires reference measurement procedures for setting target values for selected analytes in internal and external quality control. Following the Guideline this study deals with the reference measurement procedures for $HbA_{1c}$, theophylline, digoxin, digitoxin, and additionally for glibenclamide, and immunosuppressive drugs.

A highly sensitive method for determination of glibenclamide has been developed in my diploma thesis and was used for drug monitoring in the German Diabetes Research Institute and for preclinical studies. On the basis of this method a reference measurement procedure is on the way to be established, which will then be used for setting target values in the new EQAS.

The reference measurement procedure for theophylline has been evaluated and established in our laboratory and is used in our EQA schemes for determination of the target values.

The reference measurement procedure for $HbA_{1c}$ had been developed in an international cooperation. This reference measurement procedure has been improved through extensive evaluation with different mass spectrometers. The use of this modified reference measurement procedure is demonstrated in international intercomparison studies and in EQAS.

For digoxin and digitoxin a new reference measurement procedure has been developed. With this method a new analytical principle in LC-MS/MS is introduced, by means of quantitative analysis of $Cs^+$ adducts, both in SIM and in MRM mode.

The measurement of $Cs^+$ adducts in LC-MS/MS described here is a new principle in the determination of immunosuppressive drugs (sirolimus, everolimus, tacrolimus, and cyclosporin A). This new method has been established in this study and will be a solid basis for the development of an accepted reference measurement procedure.

## 3. MATERIALS AND METHODS

### 3.1 HPLC measurements

#### 3.1.1 Measurement conditions for determination of glibenclamide

Chromatographic separation was performed with a LiChrospher Si 60 column, 4 x 250 mm, 10 µm. Isocratic elution was performed by using a mobile phase consisting of 1-octanol/2-methoxy-2-methylpropane/n-heptane (15:10:75; v/v/v). The flow rate was 2 ml/min. The fluorescence detector was set to the excitation wavelength of 450 nm and to the emission wavelength of 510 nm. The 4-methylcyclohexyl analogue of glibenclamide (N-4-beta-(2-methoxy-5-chlorobenzamido) -ethylbenzosulfonyl-N'- (4-methylcyclo-hexyl)-urea) was used as internal standard.

Blood sample preparation was performed by liquid-liquid extraction, followed by derivatization with 7-chloro-4-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl). For determination of glibenclamide in rat brain, the tissue was homogenized in perchloric acid, extracted with chloroform and derivatized as described for serum samples. The injection volume was 10 µl.

#### 3.1.2 Measurement conditions for determination of theophylline

Chromatographic separation was performed with a Nautilus C18 column 4.6 x 250 mm, 5 µm**.**

Isocratic elution was performed by using a mobile phase consisting of 0.02 mol/l acetate-phospate buffer, pH 3.0 containing 9.6 % v/v acetonitrile. The flow rate was 0.9 ml/min. The diode-array detector was set to 275 nm. 1,3-dimethyl-7-(2-hydroxyethyl)-xanthine was used as internal standard. For sample preparation serum samples were extracted by liquid-liquid extraction after protein precipitation. The injection volume was 10 µl.

### 3.2 GC-MS measurements

#### 3.2.1 Measurement conditions for determination of theophylline

Chromatographic separation was performed using a FS- Supreme-5 GC column with a length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 µm. The temperature programmes of the GC and the cold-injection system were optimized .The transfer line was set to 280 °C. 2-$^{13}$C ,1,3-$^{15}N_2$-theophylline was used as internal standard. In the mass spectrometer the detection mass (m/z) for theophylline was 237 amu and for 2-$^{13}$C ,1,3-$^{15}N_2$-theophylline 240 amu. For sample preparation serum samples were extracted by liquid-liquid extraction after protein precipitation, followed by derivatization with N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA). The injection volume was 2 µl.

### 3.3 LC-MS and LC-MS/MS measurements

#### 3.3.1 Measurement conditions for determination of $HbA_{1c}$

The original reference measurement procedure for the determination of $HbA_{1c}$ in human blood has been developed in collaboration with an international group of laboratories and has been described in detail in Jeppsson et al. "Approved IFCC reference method for the measurement of $HbA_{1c}$ in human blood" Clin.Chem.Lab.Med. **40**, 78-89 (2002). The principle of measurement of the IFCC reference measurement procedure is based on the determination of the ratio of glycated to non-glycated N-terminal hexapeptides of the β-chain of haemoglobin. For sample preparation the whole blood sample is hemolysed followed by a proteolytic cleavage with endoproteinase Glu-C to cleave the amino acid chain at the Glu-Glu positions. During the enzymatic cleavage of the β-chain of haemoglobin glycated and non-glycated ß- N-terminal hexapeptides are generated.

Chromatographic separation of the hexapeptides was performed with a ZORBAX SB-CN column 2.1 x 150 mm, 5µm. Gradient elution was performed by using a mobile phase consisting of 0.07 % v/v TFA in water and 0.07 % v/v TFA in acetonitrile. The column temperature was 50 °C. The flow rate was 350 µl/min. In the positive ion mode of the mass spectrometer the detection mass (m/z) for the ß-N-terminal hexapeptide of $HbA_0$ was 348.3 amu and for the ß-N-terminal hexapeptide of $HbA_{1c}$ was 429.3 amu, using the Q1 multiple ion mode. Quantification was performed by external calibration. The injection volume was 2 µl.

#### 3.3.2 Measurement conditions for determination of digoxin and digitoxin

Chromatographic separation was performed using a LiChrospher RP-18 column (2 x 125 mm, 5 µm) equipped with a LiChrospher RP-18 pre-column (2 x 10 mm, 5 µm). Gradient elution was performed by using a mobile phase consisting of 0.1 % v/v formic acid in water + 100 µmol/l caesium hydroxide (adjusted to pH 7 with formic acid) and 0.1 % v/v formic acid in methanol + 100 µmol/l caesium hydroxide (adjusted to pH 7 with formic acid). The column temperature was 40 °C. The flow rate was 300 µl/min. Three fold stable isotope-labelled digoxin and digitoxin as internal standards were synthesized in our laboratory. In the positive ion mode of the mass spectrometer the detection mass (m/z) in the Q1 multiple ion mode for digitoxin was 897.3 amu, for digitoxin-$^2H_3$ 900.3 amu, for digoxin 913.3 amu and for digoxin-$^2H_3$ 916.3 amu. In the MRM mode the mass transition from the above mentioned Q1 detection mass in the Q1 multiple ion mode of each compound to a Q3 mass of 132.9 amu was measured. The injection volume was 10 µl.

### 3.3.3 Measurement conditions for determination of sirolimus, everolimus, tacrolimus and cyclosporin A

Chromatographic separation was performed using a phenyl-hexyl-RP column Luna®, 2 x 150 mm, 5 µm. The elution was performed by using a ternary gradient consisting of eluent A 0.1 % v/v formic acid in water + 100 µmol/l caesium hydroxide (adjusted to pH 7 with formic acid), eluent B 0.1 % v/v formic acid in methanol + 100 µmol/l caesium hydroxide (adjusted to pH 7 with formic acid) and eluent C 0.1 % v/v formic acid in acetonitrile + 100 µmol/l caesium hydroxide (adjusted to pH 7 with formic acid). Cyclosporin D, ascomycin and 32-desmethoxy-rapamycin were used as internal standards. The flow rate was 300 µl/min. The column temperature was set to 50 °C. In the positive ion mode of the mass spectrometer in the MRM mode the Q1 mass (m/z) of 936.9 amu for tacrolimus, 1046.8 amu for sirolimus, 1090.8 amu for everolimus 1335.0 amu for cyclosporine A 924.9 amu for ascomycin, 1016.5 amu for 32-desmethoxy-rapamycin, 1349.2 amu for cyclosporine D and a Q3 mass for each compound of 132.9 amu was measured. The injection volume was 10 µl.

## 4. RESULTS AND DISCUSSION

According to the ISO Standard 15193: 2005 "useful and reliable results of measurement" for reference measurement procedure are required which are "ultimately traceable to measurement standards of highest metrological order". Additionally, the uncertainty of measurement of the values has to be determined.

In this study the traceability of the results is given by reference measurement procedures using primary and secondary standards.

The trueness of measurements has been determined by using certified secondary reference materials. When no certified reference material was available, the trueness of measurements was determined by recovery studies.

The uncertainty of measurement was determined by repeated measurements of a sample. The measurement uncertainty is caused by different factors. These factors have been determined in our laboratory for the accreditation process. A main component of the total measurement uncertainty is the uncertainty of the primary or secondary reference material. Other components of the total uncertainty originate from several steps in sample preparation, such as weighing, or influences due to fluctuations in room temperature, humidity during sample preparation, or from peak integration. The uncertainty of measurement in this study is represented by the estimated relative standard deviation (coefficient of variation). When no

international acceptability criteria for accuracy and precision are given, in this study the criteria for the evaluation of the reference measurement procedure were derived from the "German Guideline of the "Federal Medical Council for Quality Assurance of Quantitative Analysis in Laboratory Medicine" (RiliBÄK). The requirements for precision and accuracy for reference laboratories using reference measurement procedures were set at 1/3 of those for precision and accuracy of the routine laboratories (see column 5 and 6 of the current RiliBÄK).

### 4.1 Determination of glibenclamide by HPLC and fluorescence detection

To improve the analytical quality of routine measurement of glibenclamide in patient samples a reliable, sensitive and specific analytical method by the use of HPLC and fluorescence detection was developed in my diploma thesis "Methoden-Entwicklung zur Bestimmung von Glibenclamid in biologischem Material mittels Hochdruckflüssigkeitschromatographie unter Verwendung eines Fluoreszenz-Detektors" at the German Diabetes Research Institute. This analytical principle of measurement was used for routine measurement in the clinical laboratory for many years. Moreover, the method could be adapted to determine glibenclamide for preclinical research. In a research study the rat brain tissue concentrations of glibenclamide after intravenous or local application have been determined, to investigate the effect of the drug on the ischemia-induced $K^+$ efflux changes.

Since the strategy of continuous improvement in analytical quality is still in process, in the meantime an LC-MS method for the determination of glibenclamide in serum has been developed on the basis of this diploma thesis in the German Diabetes Research Institute. As will be shown with digoxin, digitoxin (see 4.4) and immunosuppressive drugs (see 4.5) this method is on the way to be further improved by using the new analytical principle in LC-MS/MS (measurement of $Cs^+$ adducts) to a reference measurement procedure at INSTAND e.V. and will be used for setting target values in a new EQAS, thus promoting the comparability of results in the drug monitoring of antidiabetic drugs.

### 4.2 Determination of theophylline by GC-IDMS and HPLC diode array detection

The presented reference measurement procedure for determination of theophylline is a gas chromatography isotope-dilution mass spectrometry method using the stable isotope –labelled compound 2-$^{13}$C ,1,3-$^{15}N_2$-theophylline as internal standard. The extraction and derivatization procedures were examined in detail and optimized stepwise during the development of the method. Since no certified reference material was available the evaluation of the trueness of the established GC-IDMS method was performed by recovery studies. In serum samples

spiked with theophylline in therapeutic ranges the mean recovery was 99.78 % with a bias 0.22 %. The imprecision in commercial control material was between 0.527 % and 1.14 %, respectively. To verify the analytical specificity and accuracy of the GC-IDMS method a HPLC-diode array method was developed for comparison and confirmation of results. The HPLC-diode array method allows the separation of all dimethylxanthines and related compounds, which may cause interferences: theophylline, theobromine, paraxanthine, paracetamol, caffeine, and uric acid. The good correlation between the results obtained from GC-IDMS and HPLC-diode array measurements confirms the specificity of GC-IDMS measurement procedures.

The use of this GC-IDMS reference measurement procedure for theophylline was demonstrated for setting target values in external quality assessment schemes run by INSTAND e.V..

### 4.3 Determination of $HbA_{1c}$ by LC-MS

The IFCC reference measurement procedure for $HbA_{1c}$ is a LC-MS method with quantification by external standards. This method has been optimized and evaluated. The long-term stability of several LC-MS measuring instruments and their effect on the quality of the results has been evaluated. It could be shown that the technical design of the electrospray ionisation source of the mass spectrometer is the critical element for the stability of the ratios of the ß-N-terminal hexapeptides of $HbA_{1c}$ and $HbA_0$. Stable ratios and congruent calibration curves within a measuring sequence of 24 h could be obtained only by the use of the API 4000.The HPLC conditions were optimized by changing the composition of the buffer, the gradient elution profile and introduction of a splitting system. Using the optimized method excellent peak symmetry, stable retention times, a good column batch –to-batch reproducibility, stable and linear calibration curves were obtained. By the use of the appropriate LC-MS equipment and the optimized HPLC conditions successful measurements were made in several international intercomparison studies of the IFCC Working Group on $HbA_{1c}$ Standardisation. The method had a bias between 0.1 % and 0.65 %.The imprecision of the procedure was below 2.0 %, which was well within the acceptability criteria for the reference measurement laboratories of the IFCC network for $HbA_{1c}$. The reference laboratory of INSTAND e.V. is accepted as an IFCC-Reference Laboratory for $HbA_{1c}$ (one of less than 20 in the world). The use of the improved reference measurement procedure for $HbA_{1c}$ was demonstrated for setting target values in external quality assessment schemes run by INSTAND e.V..

### 4.4 Determination of digoxin and digitoxin by LC-MS and LC-MS/MS

An important progress in the development of LC-MS methods as reference measurement procedures for the determination of digoxin, digitoxin and the immunosuppressive drugs sirolimus, everolimus, tacrolimus and cyclosporin A was the discovery, that these compounds are forming adducts with $Cs^+$ ions, which are detectable both in SIM and in MRM mode. By adding an excess of $Cs^+$ ions to the elution buffer $Cs^+$ adducts of the drugs were obtained. In the MRM mode $Cs^+$ is measurable as product ion of the $Cs^+$ adducts of the drugs with high accuracy and precision. This new principle in HPLC-mass spectrometry analysis results in a higher analytical specificity and better signal-to-noise ratio, by

- replacement of undesirable adduct formations with $Na^+$ and $K^+$
- a stabilizing effect of the $Cs^+$ adduct on undesirable in source fragmentation
- a clearly defined fragmentation pattern in MRM transmission

Our reference measurement procedure for the determination of digoxin and digitoxin is an isotope dilution LC-MS analysis with electrospray ionisation after a liquid-liquid extraction. The isotope-labelling of the pure analytes was performed in our laboratory.

In the SIM mode the $Cs^+$ adduct of deuterated and native digoxin and digitoxin has been measured. The LC-MS measurement procedure was further modified to a LC-MS/MS method.

Since no certified reference material is available the trueness of the newly established method was investigated by recovery studies. The mean recovery in spiked serum samples in the therapeutic range for digoxin was 99.95 % and for digitoxin 100.2 %, the bias was 0.05 % and 0.2 % respectively. In the SIM mode the imprecision was between 1.26 % and 2.91 % for digoxin and 0.79 % and 2.00 % for digitoxin. In EQAS samples the corresponding coefficients of variation were between 1.26 % and 3.18 % and 1.21 % and 1.46 %, respectively. In the MRM mode the coefficients of variation were between 1.65 % and 2.28 % for digoxin and 1.13 % and 1.44 % for digitoxin. The use of the newly developed reference measurement procedure for digoxin and digitoxin was demonstrated for setting target values in external quality assessment schemes run by INSTAND e.V..

### 4.5 Determination of sirolimus, everolimus, tacrolimus, cyclosporin A by LC-MS/MS

A new basis was developed for a reference measurement procedure for the determination of immunosuppressive drugs. The immunosuppressive drugs sirolimus, everolimus, tacrolimus and cyclosporin A were measured as $Cs^+$ adducts in the MRM mode. Analogues of the drugs were used as internal standards. In contrast to existing routine methods the immunosuppressive drugs could be chromatographically separated using a ternary gradient profile. In standard solutions the coefficient of variation was between 2.11 % and 2.57 %. On the basis of this principle of measurement the further development of the method to a reference measurement procedure is on the way. For a better quantification we are producing stable isotope-labelled internal standards in our laboratory.

## 5. SUMMARY

Reference measurement procedures in laboratory medicine are necessary to get reliable and comparable results in laboratory analyses and thus improve patient care.

This study demonstrates that the development of reference measurement procedures is a highly dynamic process. Maintenance, evaluation, and progressive improvements to the highest technical level are a permanent objective of reference measurement laboratories.

Three reference measurement procedures and the basis for the development of two additional reference measurement procedures are presented in this study:

- reference measurement procedure for determination of theophylline
- reference measurement procedure for determination of $HbA_{1c}$
- reference measurement procedure for determination of digoxin and digitoxin
- basis for a reference measurement procedure for determination of sirolimus, everolimus, tacrolimus, cyclosporin A, and glibenclamide

The study demonstrates how reference measurement procedures are developed, improved and evaluated. Reference measurement procedures are thoroughly investigated measurement procedures enclosed in a dynamic process of optimization, leading to traceable and reliable results on highest metrological order. Providing and using reference measurement procedures improves the quality of the medicine laboratory performance.

**LIST OF PUBLICATIONS RELATED TO THE THESIS**

**[I]** Xie, Y., Zacharias, E., **Hoff, P.**, Tegtmeier, F. Ion channel involvement in anoxic depolarisation induced by cardiac arrest in rat brain. J. Cereb. Blood Flow Metab.,15, 587-594 (1995)

**[II]** Kress, M., Meißner, D., **Kaiser, P.**, Hanke, R. Wood, W.G. Determination of theophylline by HPLC and GC-IDMS, the effect of chemically similar xanthine derivatives on the specificity of the method and the possibility of paracetamol as interfering substance. Clin. Lab. 48, 541-551 (2002)

**[III]** Kress, M., Meißner, D., **Kaiser, P.**, Hanke, R., Wood, W.G. The measurement of theophylline in human serum or plasma using gas chromatography and isotope dilution-mass-spectrometry (GC-IDMS) taking other substituted xanthines into consideration. Clin. Lab. 48, 535-540 (2002)

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**[V]** **Kaiser, P.**, Kramer, U., Meißner, D., Kress, M., Wood, W.G., Reinauer, H. Determination of the cardiac glycosides digoxin and digitoxin by liquid chromatography combined with isotope-dilution mass spectrometry (LC-IDMS) - a candidate reference measurement procedure. Clin. Lab. 49, 329-343 (2003)

**[VI]** **Kaiser, P.**, Akerboom, T., Wood, W.G., Reinauer, H. A novel LC-IDMS/MS method for the determination of the cardiac glycosides digoxin and digitoxin using caesium adducts. Clin. Lab. 52, 37-42 (2006)

**[VII]** **Kaiser, P.**, Akerboom, T., Wood, W.G., Reinauer, H. A new approach for the determination of immunosuppressive drugs using HPLC-MS/MS and $Cs^+$ adducts. Ger.Med.Sci.4: Doc01 (2006)

**Scientific lectures**

1. **Kaiser, P.** Reference method for the determination of $HbA_{1c}$ in human haemolysates by HPLC-ESI-MS. WHO post graduate training course in medical laboratories, INSTAND e.V. , Düsseldorf, Germany 2003

2. **Kaiser, P.** Principle of HPLC-ESI-MS by example of the $HbA_{1c}$ reference method. Training course for technical assistants in medical laboratories, INSTAND e.V. , Düsseldorf, Germany 2003

3. **Kaiser, P.** Principle of HPLC-ESI-MS by example of the digoxin, digitoxin reference method. Training course for technical assistants in medical laboratories, INSTAND e.V., Düsseldorf , Germany 2004

4. **Kaiser, P.** Reference method for the determination of digoxin and digitoxin by HPLC combined with isotope dilution ESI-MS. WHO post graduate training course in medical laboratories, INSTAND e.V., Düsseldorf, Germany 2005

5. **Kaiser, P.** Referenzmethode HbA1c. Symposium Qualitätssicherung in der klinischen Chemie, Berlin, Germany 2005

6. **Kaiser, P.** Reference measurement procedures and quality assessment in laboratory medicine- Reference measurement procedure for determination of digoxin and digitoxin in serum. 30$^{th}$ FEBS Congress and 9$^{th}$ IUBMB Conference, Budapest, Hungary 2005

7. **Kaiser, P.** IFCC reference measurement procedure for determination of HbA1c in blood. External and internal quality assurance in medical laboratories. 5$^{th}$ INSTAND/WHO seminar (IWS), Cairo, Egypt 2005

8. **Kaiser, P.** Reference measurement procedures for determination of target values for EQAS samples. European Model of External Quality Control in Medical Diagnostic Laboratories Seminar NGO "Association of Promoting Medical Diagnostic Activity & Quality Defending of Georgia", Tbilisi, Georgia, 2005

**ACKNOWLEDGEMENTS**

I would like to thank my supervisors Prof. Dr. Dux and Prof. Dr. Reinauer for giving me the topic and opportunity to perform this study and for all their help during these years.

I am also grateful to Prof. Dr. Wood for all his useful advice and support during the preparation of this thesis.

My thanks are due to my colleagues in the reference laboratory at INSTAND e.V. Udo Kramer, Michael Kress, Dieane Meißner, Ulrike Speith, and Dr. Akerboom for their valuable suggestions and support, and I express my thanks to Karin Schumann for help in preparation of documents and presentation for publications and lectures.

Finally I like to thank my husband Dipl.-Ing. Christian Kaiser for all his support and understanding patience.