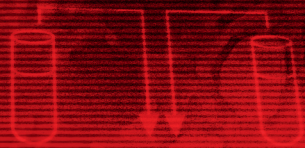
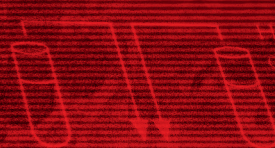


Heated patient plasma



Buffered normal pool plasma pH 7.4



Heated control plasma (Clotting factor deficient plasma)

# Quality in Laboratory Hemostasis and Thrombosis

## Second Edition

Edited by  
Steve Kitchen, John D. Olson  
and F. Eric Preston

All Factors  
Vitamin K Factors  
Factor VII  
Factor II



 WILEY-BLACKWELL



# Quality in Laboratory Hemostasis and Thrombosis



# Quality in Laboratory Hemostasis and Thrombosis

Second Edition

Edited by

**Steve Kitchen, PhD**

Clinical Scientist  
Sheffield Hemophilia and Thrombosis Centre  
Royal Hallamshire Hospital  
Sheffield; and  
Scientific Director, UK National External Quality Assessment Scheme (NEQAS)  
for Blood Coagulation  
Scientific Director, WHO and WFH International External Quality Assessment  
Programs for Blood Coagulation  
Sheffield, UK

**John D. Olson, MD, PhD**

Professor and Vice Chair for Clinical Affairs  
Department of Pathology  
University of Texas Health Science Center; and  
Director of Clinical Laboratories  
University Health System  
San Antonio, TX, USA

**F. Eric Preston, MD, FRCPath, FRCP**

Emeritus Professor of Hematology  
University of Sheffield  
Sheffield; and  
Director, WHO and WFH International External Quality Assessment Programs  
for Blood Coagulation  
Sheffield, UK

Foreword by Professor Dr Frits R. Rosendaal

 **WILEY-BLACKWELL**

A John Wiley & Sons, Ltd., Publication

This edition first published 2013 © 2009 © Blackwell Publishing Ltd; 2013 by John Wiley & Sons, Ltd  
Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific,  
Technical and Medical business with Blackwell Publishing.

*Registered office:* John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex,  
PO19 8SQ, UK

*Editorial offices:* 9600 Garsington Road, Oxford, OX4 2DQ, UK  
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK  
111 River Street, Hoboken, NJ 07030-5774, USA

For details of our global editorial offices, for customer services and for information about how to apply  
for permission to reuse the copyright material in this book please see our website at  
[www.wiley.com/wiley-blackwell](http://www.wiley.com/wiley-blackwell)

The right of the author to be identified as the author of this work has been asserted in accordance with  
the UK Copyright, Designs and Patents Act 1988.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or  
transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or  
otherwise, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior  
permission of the publisher.

Designations used by companies to distinguish their products are often claimed as trademarks. All  
brand names and product names used in this book are trade names, service marks, trademarks or  
registered trademarks of their respective owners. The publisher is not associated with any product or  
vendor mentioned in this book. This publication is designed to provide accurate and authoritative  
information in regard to the subject matter covered. It is sold on the understanding that the publisher is  
not engaged in rendering professional services. If professional advice or other expert assistance is  
required, the services of a competent professional should be sought.

The contents of this work are intended to further general scientific research, understanding, and  
discussion only and are not intended and should not be relied upon as recommending or promoting a  
specific method, diagnosis, or treatment by physicians for any particular patient. The publisher and the  
author make no representations or warranties with respect to the accuracy or completeness of the  
contents of this work and specifically disclaim all warranties, including without limitation any implied  
warranties of fitness for a particular purpose. In view of ongoing research, equipment modifications,  
changes in governmental regulations, and the constant flow of information relating to the use of  
medicines, equipment, and devices, the reader is urged to review and evaluate the information provided  
in the package insert or instructions for each medicine, equipment, or device for, among other things,  
any changes in the instructions or indication of usage and for added warnings and precautions. Readers  
should consult with a specialist where appropriate. The fact that an organization or Website is referred  
to in this work as a citation and/or a potential source of further information does not mean that the  
author or the publisher endorses the information the organization or Website may provide or  
recommendations it may make. Further, readers should be aware that Internet Websites listed in this  
work may have changed or disappeared between when this work was written and when it is read. No  
warranty may be created or extended by any promotional statements for this work. Neither the  
publisher nor the author shall be liable for any damages arising herefrom.

*Library of Congress Cataloging-in-Publication Data*

Quality in laboratory hemostasis and thrombosis / edited by Steve Kitchen, John D. Olson,  
F. Eric Preston ; foreword by Frits R. Rosendaal. – 2nd ed.  
p. ; cm.

Includes bibliographical references and index.

ISBN 978-0-470-67119-1 (hardback : alk. paper)

I. Kitchen, Steve, Dr. II. Olson, John David, 1944- III. Preston, F. E.

[DNLM: 1. Hemostatic Techniques. 2. Anticoagulants. 3. Blood Coagulation Disorders,  
Inherited—diagnosis. 4. Blood Coagulation Factors. 5. Clinical Laboratory Techniques.  
6. Thrombophilia—diagnosis. WH 310]

616.1'57075—dc23

2012044510

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print  
may not be available in electronic books.

Cover image: Internal. Top: courtesy of Bert Verbuggen; bottom: courtesy of Wayne Chandler.  
Cover design by Andrew Magee Design Ltd

Set in 9/11.5 pt Sabon by Aptara® Inc., New Delhi, India

# Contents

Contributors, vii  
Foreword, x  
Preface, xii  
*Steve Kitchen, John D. Olson, & F. Eric Preston*

## Part 1: General Quality Program

- 1 General quality planning in the hemostasis laboratory, 3  
*John D. Olson*
- 2 Hemostasis test validation, performance, and reference intervals:  
international recommendations and guidelines, 12  
*Richard A. Marlar*
- 3 Causes of errors in medical laboratories, 22  
*Giuseppe Lippi & Emmanuel J. Favaloro*
- 4 International standards in hemostasis, 32  
*Trevor W. Barrowcliffe & Anthony R. Hubbard*
- 5 Sample integrity and preanalytical variables, 45  
*Dorothy (Adcock) Funk*
- 6 Internal quality control in the hemostasis laboratory, 57  
*Steve Kitchen, F. Eric Preston, & John D. Olson*
- 7 External quality assessment in hemostasis: its importance and significance, 65  
*F. Eric Preston, Steve Kitchen, & Alok Srivastava*
- 8 The unique challenges of hemostatic testing in children, 77  
*M. Patricia Massicotte, Mary E. Bauman, Vanessa Chan, &  
Anthony K.C. Chan*

## Part 2: Quality in Coagulation Testing

- 9 Initial evaluation of hemostasis: reagent and method selection, 95  
*Wayne L. Chandler*
- 10 Assay of factor VIII and other clotting factors, 105  
*Steve Kitchen & F. Eric Preston*
- 11 Application of molecular genetics to the investigation of inherited  
bleeding disorders, 115  
*Stefan Lethagen, Morten Dunø, & Lars Bo Nielsen*
- 12 Detecting and quantifying acquired functional inhibitors in hemostasis, 124  
*Bert Verbruggen, Myriam Dardikh, & Britta Laros-van Gorkom*

- 13 Standardization of D-dimer testing, 136  
*Guido Reber & Philippe de Moerloose*
- 14 Point-of-care testing in hemostasis, 147  
*Chris Gardiner, Dianne Kitchen, Samuel Machin, & Ian Mackie*

### **Part 3: Quality in Testing for Platelet Function and von Willebrand Disease**

- 15 Diagnostic assessment of platelet function, 159  
*Paquita Nurden, Alan Nurden, & Martine Jandrot-Perrus*
- 16 Laboratory evaluation of heparin-induced thrombocytopenia, 174  
*Theodore (Ted) E. Warkentin & Jane C. Moore*
- 17 Laboratory evaluation of von Willebrand disease: phenotypic analysis, 192  
*Emmanuel J. Favaloro*
- 18 Laboratory analysis of von Willebrand disease: molecular analysis, 204  
*Anne C. Goodeve & Ian R. Peake*

### **Part 4: Quality in Thrombophilia Testing and Monitoring Anticoagulation**

- 19 Quality issues in heritable thrombophilia testing, 219  
*Isobel D. Walker & Ian Jennings*
- 20 Evaluation of antiphospholipid antibodies, 233  
*Michael Greaves*
- 21 Monitoring heparin therapy, 244  
*Marilyn Johnston*
- 22 Monitoring oral anticoagulant therapy with vitamin K antagonists, 253  
*Armando Tripodi*
- 23 Monitoring new anticoagulants, 264  
*Elaine Gray & Trevor W. Barrowcliffe*
- Index, 273



# Contributors

**Trevor W. Barrowcliffe** MA, PhD  
Formerly National Institute for Biological Standards  
and Control (NIBSC)  
Potters Bar, Hertfordshire, UK

**Mary E. Bauman** RN, MN, NP  
Stollery Children's Hospital  
University of Alberta  
Edmonton, AB, Canada

**Anthony K.C. Chan** MBBS, FRCPC, FRCPath  
McMaster Children's Hospital/Hamilton Health  
Sciences Foundation  
Chair in Pediatric Thrombosis and Hemostasis  
Department of Pediatrics, McMaster University  
Hamilton, ON, Canada

**Vanessa Chan** BSc, MLT  
Department of Paediatric Laboratory Medicine  
The Hospital for Sick Children  
Toronto, ON, Canada

**Wayne L. Chandler** MD  
Coagulation Laboratory  
Department of Pathology and Genomic Medicine  
The Methodist Hospital Physician Organization  
Houston, TX, USA

**Myriam Dardikh** MSc  
Department of Laboratory Medicine  
Laboratory of Haematology  
Radboud University Nijmegen Medical Centre  
Nijmegen, The Netherlands

**Philippe de Moerloose** MD  
Haemostasis Unit  
Department of Internal Medicine  
University Hospital and Faculty of Medicine  
Geneva, Switzerland

**Morten Dunø** MD, PhD  
Department of Clinical Genetics  
Copenhagen University Hospital (Rigshospitalet)  
Copenhagen, Denmark

**Emmanuel J. Favaloro** PhD, FFSc (RCPA)  
Department of Hematology  
ICPMR, Westmead Hospital  
Westmead, NSW, Australia

**Dorothy (Adcock) Funk** MD  
Colorado Coagulation  
a business unit of Esoterix, Inc., Englewood, CO,  
USA

**Chris Gardiner** PhD  
Haematology Department  
University College London Hospitals NHS Trust; and  
Nuffield Department of Obstetrics and Gynaecology  
John Radcliffe Hospital  
Oxford, UK

**Anne C. Goodeve** PhD  
Sheffield Haemostasis Research Group  
Department of Cardiovascular Science  
University of Sheffield  
Sheffield; and  
Sheffield Diagnostic Genetics Service  
Sheffield Children's NHS Foundation Trust  
Sheffield, UK

**Elaine Gray** PhD  
National Institute for Biological Standards and  
Control  
Potters Bar, Hertfordshire, UK

**Michael Greaves** MD FRCP FRCPath  
Aberdeen Royal Infirmary  
Head of College of Life Sciences and Medicine,  
University of Aberdeen, Scotland, UK

## CONTRIBUTORS

**Anthony R. Hubbard** PhD  
National Institute for Biological Standards and  
Control  
Hertfordshire, UK

**Martine Jandrot-Perrus** MD, PhD  
INSERM U698 and Paris 7 University  
Paris, France

**Ian Jennings** PhD  
UK NEQAS for Blood Coagulation  
Sheffield, UK

**Marilyn Johnston** MLT, ART  
Hemostasis Reference Laboratory  
Hamilton, ON, Canada

**Dianne Kitchen** FIBMS  
UK NEQAS Blood Coagulation  
Sheffield, UK

**Steve Kitchen** PhD  
Sheffield Hemophilia and Thrombosis  
Centre  
Royal Hallamshire Hospital  
Sheffield; and  
UK National External Quality Assessment Scheme  
(NEQAS) for Blood Coagulation  
WHO and WFH International External  
Quality Assessment Programs for Blood  
Coagulation  
Sheffield, UK

**Britta Laros-van Gorkom** MD, PhD  
Department of Haematology  
Radboud University Nijmegen Medical  
Centre  
Nijmegen, The Netherlands

**Stefan Lethagen** MD, PhD  
Copenhagen University  
Copenhagen; and  
Medical and Science  
Haemophilia R&D  
Novo Nordisk A/S, Denmark

**Giuseppe Lippi** MD  
Clinical Chemistry and Haematology Laboratory  
Department of Pathology and Laboratory Medicine  
Academic Hospital of Parma  
Parma, Italy

**Samuel Machin** MB ChB, FRCP, FRCPath  
Haematology Department  
University College London Hospitals NHS Trust; and  
Haemostasis Research Unit, Haematology Department  
University College London  
London, UK

**Ian Mackie** PhD, FRCPath  
Haematology Department  
University College London Hospitals NHS Trust  
Haemostasis Research Unit  
Haematology Department  
University College London  
London, UK

**Richard A. Marlar** PhD  
Pathology and Laboratory Medicine  
Oklahoma City VA Medical Center; and  
Department of Pathology  
University of Oklahoma Health Sciences Center  
Oklahoma City, OK, USA

**M. Patricia Massicotte** MD, MHSc  
Stollery Children's Hospital  
University of Alberta  
Edmonton, AB, Canada

**Jane C. Moore** ART, BSc  
Departments of Pathology and Molecular Medicine,  
and Medicine  
Michael G. DeGroote School of Medicine, McMaster  
University  
Hamilton Regional Laboratory Medicine Program  
Hamilton, ON, Canada

**Lars Bo Nielsen** MD, PhD  
Department of Clinical Biochemistry  
Rigshospitalet and Department of Biomedical  
Sciences; and  
University of Copenhagen  
Copenhagen University Hospital (Rigshospitalet)  
Copenhagen, Denmark

**Alan Nurden** PhD

Centre de Référence des Pathologies Plaquettaires  
Plateforme Technologique d'Innovation Biomédicale  
Hôpital Xavier Arnoz  
Pessac, France

**Paquita Nurden** MD, PhD

Centre de Référence des Pathologies Plaquettaires  
Plateforme Technologique d'Innovation Biomédicale  
Hôpital Xavier Arnoz  
Pessac, France

**John D. Olson** MD, PhD

Department of Pathology  
University of Texas Health Science Center  
San Antonio, TX, USA

**Ian R. Peake** PhD

Sheffield Haemostasis Research Group  
Department of Cardiovascular Science  
University of Sheffield  
Sheffield, UK

**F. Eric Preston** MD, FRCPath, FRCP

University of Sheffield  
Sheffield; and  
WHO and WFH International External Quality  
Assessment Programs for Blood Coagulation  
Sheffield, UK

**Guido Reber** PhD

Haemostasis Unit  
Department of Internal Medicine  
University Hospital and Faculty of Medicine  
Geneva, Switzerland

**Alok Srivastava** MD, FRACP, FRCPA, FRCP

Department of Hematology  
Christian Medical School  
Vellore, India

**Armando Tripodi** PhD

Angelo Bianchi Bonomi Hemophilia and Thrombosis  
Center  
Department of Internal Medicine  
University School of Medicine and IRCCS Cà  
Granda Maggiore Hospital Foundation  
Milan, Italy

**Bert Verbruggen** PhD

Laboratory of Clinical Chemistry and Haematology  
Jeroen Bosch Hospital's-Hertogenbosch,  
The Netherlands

**Isobel D. Walker** MD MPhil (Medical Law),  
FRCPath

University of Glasgow, Glasgow; and  
NEQAS for Blood Coagulation  
Sheffield, UK

**Theodore (Ted) E. Warkentin** MD

Departments of Pathology and Molecular Medicine,  
and Medicine  
Michael G. DeGroot School of Medicine  
McMaster University  
Transfusion Medicine  
Hamilton Regional Laboratory Medicine Program  
Hamilton, ON, Canada

# Foreword

*Thou art always figuring diseases in me, but thou art full of error: I am sound*

*(William Shakespeare. Measure for measure (1604); Act I, Scene II)*

A correct diagnosis is the cornerstone of medicine. Without it, no remedy can be prescribed, or prognosis given. Although laboratory tests are only a part of the diagnostic arsenal, together with history taking, clinical examination, and imaging techniques, few diagnoses are arrived at without some form of laboratory test. Inadequate tests may lead to either false reassurance or false alarm. They may lead to the erroneous choice not to give treatment when treatment would be beneficial, or even to prescribe the wrong treatment, which is likely to be harmful. It is therefore of the utmost importance that whenever laboratory tests are performed, the results are reliable.

Laboratory tests in the field of thrombosis and hemostasis are notoriously difficult, which is related to the large variety in techniques that are used, and the sensitivity of many assays to small preanalytical and analytical variation. Therefore, quality assurance is crucial, and no hemostasis laboratory can afford not to invest in internal and external quality control. The book, *Quality in Laboratory Hemostasis and Thrombosis*, edited and written by authorities in the field, since its first edition in 2008, has become an indispensable help for those who wish to set up a hemostasis laboratory, as well as those who already work in such a place. For, to quote from the first chapter: “Process is never optimized; it can always be improved.”

The book has two parts: the first eight chapters give a scholarly overview of the concepts that underlie quality assurance, explaining the various aspects of test validation, with its components, of which accuracy and precision are the most important: does a test measure what it is supposed to measure, and does it do so with acceptable reproducibility. Subsequent chapters in this first part explain in detail how internal quality control deals with precision and external quality control with accuracy. The development of international standards is an important and ongoing

development in improving accuracy and comparability of hemostasis laboratory tests. Here, the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, working together with the World Health Organization, has played a major role. Over the years we have witnessed the emergence of large external quality assurance programs, in which samples are sometimes sent to more than a thousand participating laboratories. Such programs not only allow laboratories to evaluate their performance, but also to group results by reagent or instrument, which leads to valuable insights, and further quality improvement. Newly added chapters to the second edition deal with the causes of laboratory error, the understanding of which is indispensable in optimizing laboratory performance, and the performance and interpretation of hemostatic tests in children.

In the second part of the book, Chapters 9 through 23, a detailed description is given of all major assays in hemostasis, grouped in a series of chapters on coagulation factor assays, on primary hemostasis (platelets and von Willebrand factor), and on thrombophilia testing and anticoagulant treatment monitoring. These chapters give the reader invaluable information on the performance and interpretation of these tests. A newly added chapter that was much missed in the first edition deals with heparin-induced thrombocytopenia.

The ultimate test for a laboratory test is whether it improves medical care, that is, reduces morbidity and mortality, which depends on the effect a negative or positive test result has on the treatment of a patient. A test that does not affect clinical management is a waste of resources. Both at the beginning and the end of laboratory tests there is usually a clinician, who first makes the decision to order a test, and subsequently has to interpret the test result. Although these clinical decisions and interpretation are not part of the content of this book, which would have made it unwieldy to say the least, these are of obvious importance, and one of the tasks of the individuals working in hemostasis laboratories is to educate clinicians

about the clinical value of the various assays. I am quite confident that in the field of hemostasis and thrombosis more useless than useful testing is done, and that in medicine as a whole the greatest waste of money is on redundant diagnostics. The practice of medicine knows a wide variety of tests, which generally serve three purposes, either to diagnose a disease, or to test for a risk factor for disease, or to screen for either of these. This distinction is rarely sharply made, while it seems that clinically one type (diagnosing a disease) is almost always indicated and useful, and another type (testing for risk factors) only rarely is. While it is logical to find out which disease a patient with complaints has, it is not so logical to try and identify the causes of that disease, or even to try and identify those risk factors in nondiseased individuals, such as relatives of individuals with thrombosis. The reason the distinction between diagnosing a disease and identifying a risk factor is not always sharply made, is possibly because in some diseases in the field, notably bleeding disorders, there is an almost one-to-one relationship between the cause of the disease and the disease itself. While excessive bleeding is the disease and the clotting factor level a cause, individuals with no factor VIII or IX will invariably have the clinical disease of hemophilia, and therefore, measuring the clotting factor level has become synonymous to diagnosing hemophilia. This is quite different for thrombosis. Thrombosis (deep vein thrombosis or pulmonary embolism) is a disease, whereas thrombophilia is not. Given the multicausal nature of the etiology of thrombosis, in which multiple risk factors need to be present to lead to disease, it is far from self-evident that testing for thrombophilic abnormalities has any clinical value. So far, there are no clinical studies that show a benefit of such testing, although it is performed on a broad scale. Whenever you order a test or are requested to perform a test, question whether the result could possibly change anything. If not, or if the only benefit is to satisfy the doctor's curiosity, the test should not be done.

The reliability of a particular assay should be viewed in the context in which the test is ordered. Suppose one would order a test for high factor VIII as a prothrombotic risk factor, the above mentioned notwithstanding, an error of five IU/dL would be irrel-

evant, since the purpose is to discriminate between levels of over 150 or 200 IU/dL versus plasma concentrations around 100 IU/dL. The same error in a factor VIII assay to diagnose hemophilia A could be disastrous.

A clinician, when ordering a test, will have to deal with so-called prior probabilities, which is of particular relevance in screening tests. A slightly prolonged aPTT has a vastly different meaning when found in a healthy woman who had four uneventful deliveries who has come to the hospital for a tubal ligation, than in an 18-month-old boy who needs to undergo a duodenoscopy with possible biopsies. She is unlikely to have a bleeding tendency, even when the aPTT is prolonged, while the young boy may suffer from hemophilia. Screening tests affect the likelihood of disease, which, according to Bayes' theorem, is also a function of the prior probability of disease. Virtually, all tests that use reference ranges based on statistical cutoff values, such as the population mean plus or minus two standard deviations, are screening tests, that do neither establish a risk factor or a disease, but only, when abnormal, affect the likelihood of that state. Nature does not use standard deviations, and using a cutoff of two standard deviations by definition finds 2.5% of the population below, or over, such a cutoff. In reality, diseases and risk factors may have prevalences that exceed, or, more usual, lie far below this figure. Tests using "normal ranges" therefore can never establish an abnormality, and should be followed by more specific tests, such as clotting factor assays or genetic tests.

Over the last decades, major progress has been made in quality assurance of hemostatic laboratory assays. In this new edition of *Quality in Laboratory Hemostasis and Thrombosis*, all chapters have been updated and several new chapters have been added. This book will remain an indispensable part of every hemostasis laboratory, where, given its hand-on nature, it will rarely sit to get dusty on the shelves.

*Frits R. Rosendaal*

Former Chairman ISTH Council, President XXIV  
ISTH Congress, Former Chairman Netherlands  
Society of Thrombosis & Haemostasis, Leiden  
University Medical Centre, The Netherlands

# Preface

In the past two to three decades, few disciplines, if any, in laboratory medicine have seen the growth in the number and complexity of testing as that experienced in the discipline of hemostasis and thrombosis. These rapid changes have presented challenges for laboratories as they develop quality programs for the oversight of this testing. The quality issues extend across all levels of testing and all sizes of laboratories. The field of laboratory medicine continues to evolve and there have been important advances since the first edition of this text was published. We, therefore, accepted the invitation to bring this text up to date in the second edition.

In our original discussions about a title for the first edition, we had an interesting discussion regarding possibilities of "... the Hemostasis and Thrombosis Laboratory" and "... Laboratory Hemostasis and Thrombosis." The distinction is subtle but relevant, the former being a place and the latter a discipline. Quality issues in this discipline extend well beyond the walls of the laboratory. In this second edition, we have retained contributions from all the original recognized experts which have been updated, and added a few new chapters based on the comments received in the intervening years since the first edition appeared. These experts have provided information on elements

of managing quality as it relates to individual tests or groups of tests extending from nuances of internal quality control to the challenges in many areas where standardization may be absent or inadequate. There is information on all aspects of testing from preanalytic to analytic and results reporting as well as external quality assurance. In addition, chapters are included regarding the development of international guidelines for methods as well as the preparation of international standard plasmas and reagents.

Quality is a changing process, continually striving to improve the product while reducing errors and improving safety. This book represents an event in this continuum, and is intended to capture the elements of quality at all levels of the practice of Laboratory Hemostasis and Thrombosis, bringing these up to date since the construction of the first edition during 2008. We believe that it will continue to provide a useful guide for those involved hemostasis and thrombosis testing, whether very simple, like the point of care, or complex, like the major reference laboratory.

*Steve Kitchen  
John D. Olson  
F. Eric Preston*

PART 1

# **General Quality Program**





# 1

## General quality planning in the hemostasis laboratory

John D. Olson<sup>1,2</sup>

<sup>1</sup>Department of Pathology, University of Texas Health Science Center, San Antonio, TX, USA

<sup>2</sup>University Health System, San Antonio, TX, USA

### Introduction

Quality:

Invisible when it is good.

Impossible to ignore when it is bad.

“So,” you might ask, “What is quality, anyway?” The word quality repeatedly infiltrates our discussions and interactions as we work to produce or choose a product. The *Oxford English Dictionary* devotes more than 3000 words in its effort to define the many variations on the use of this word [1]. We may all have difficulty with a definition, but we do know what we mean. The customer of the product or service defines many aspects of its quality while those who are producing define many others. Stated in its simplest terms, quality is the condition or state of a person, thing, or process.

### The principles

As early as the middle of the 1400s, boat makers in Venice, Italy, introduced the principle of “mass production” with the manufacture of boats in the sequential assembly of preproduced parts. This assembly line process was refined in the modern sense by Henry Ford between 1900 and 1910. The scientific elements of quality management systems began in the 1930s with the publication of Shewhart in 1931 [2],

providing a scientific and statistical basis for quality processes. He stated:

A phenomenon will be said to be controlled when, through the use of past experience, we can predict, at least within limits, how the phenomenon may be expected to vary in the future. Here it is understood that prediction means that we can state, at least approximately, the probability that the observed phenomenon will fall within given limits. [1]

The evolution of quality management systems was influenced by experiences in World War II. During the war, individuals involved in the production of reliable products for the consumer (soldier) to effectively do their job tied the entire system from raw material to the use of the finished product in a unique “team” from start to finish. Few circumstances can link the person in production so directly to the importance of the outcome. The success of the soldier was tied to the long-term well-being of the person making the tools used by that soldier. This ability to build the tight kinship and team performance on the part of people in production to the quality of the product is the goal of quality programs in all sectors of the economy today. It is, of course, very difficult to achieve this attitude in the workplace in the same way that it could be when the outcome could so directly benefit the producers.

Following World War II, the effort of reconstruction of the industry and economy of the affected countries became a major international effort and

**Table 1.1** Comparison of Deming and traditional management principles

Common company practices	“Deming” company practices
Quality is expensive.	Quality leads to lower costs.
Inspection is the key to quality.	Inspection is too late. If workers can produce defect-free goods, eliminate inspections.
Quality control experts and inspectors can ensure quality.	Quality is made in the boardroom.
Defects are caused by workers.	Most defects are caused by the system.
The manufacturing process can be optimized by outside experts with little or no change in the system afterward.	Process is never optimized; it can always be improved.
Little or no input from workers.	Elimination of all work standards and quotas is necessary. Fear leads to disaster.
Use of work standards, quotas, and goals can help productivity.	People should be made to feel secure in their jobs.
Fear and reward are proper ways to motivate.	Most variation is caused by the system.
Employees can be treated like commodities, buying more when needed and laying off when needing less.	Buy from vendors committed to quality and work with suppliers.
Rewarding the best performers and punishing the worst will lead to greater productivity and creativity.	Invest time and knowledge to help suppliers improve quality and costs. Develop long-term relationships with suppliers.
Buy one supplier off against another and switch suppliers based only on price.	Profits are generated by loyal customers.
Profits are made by keeping revenue high and costs down.	

*Source:* From Reference 6.

influenced the evolution of quality programs. The work of Deming [3] and Juran [4, 5], both associates of Shewart, extended his work. In 1951, Juran published a seminal book [4] that proposed the key elements for managing quality: quality planning, quality control (QC), and quality improvement. Following World War II, Deming presented a significant departure from the “standard” thinking about quality. He proposed a modification to the real relationships of quality, costs, productivity, and profit. The different approach to quality espoused by Deming is compared to the “standard” thinking in Table 1.1 [6]. Thus, anything that improves the product or service in the eyes of the customer defines the goals of the quality program.

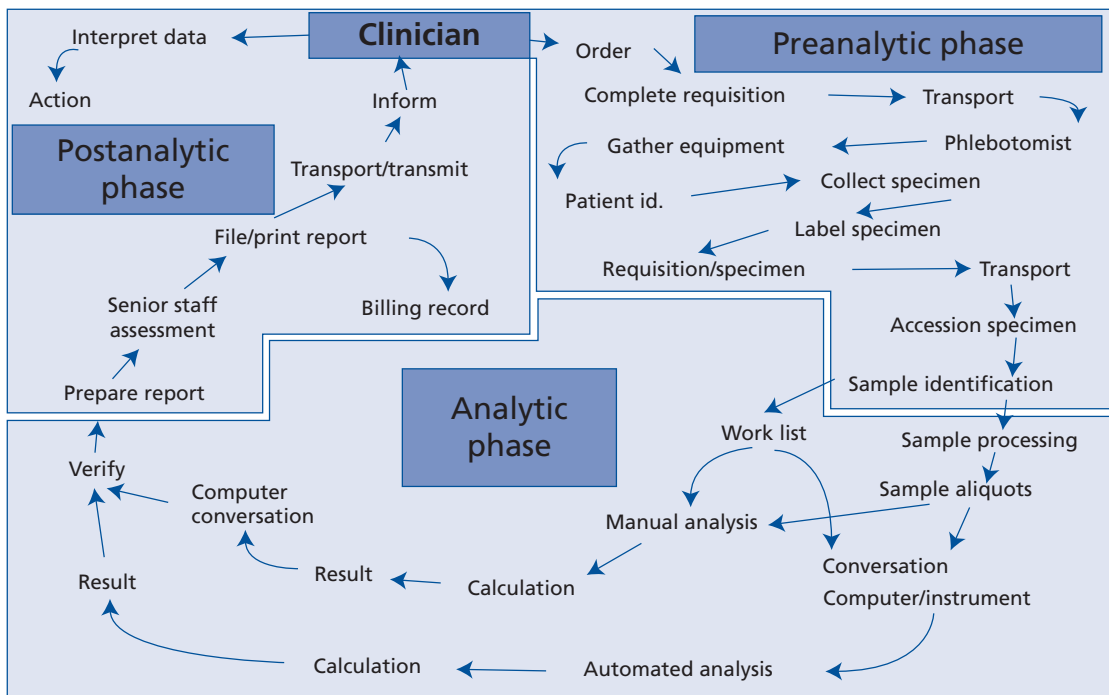
Organizations that follow Deming principles find that good quality is hard to define, but the lack of quality is easily identified. In the “standard” management of a system, the workers ultimately pay for management failure because labor costs are reduced when profits fall. In contrast, moving quality

programs as close to the worker as possible will ultimately lead to lower cost and improved consumer and worker satisfaction.

The clinical laboratory has three “consumers” of their product: (1) the patient who benefits from the best possible quality of care; (2) the ordering clinician who depends upon the right test, at the right time with an accurate result in order to make a clinical decision; (3) the hospital, clinic, or other entity that depends upon the laboratory for a positive margin when comparing cost with revenue. All three consumers benefit when the quality program drives the best possible practice.

#### Elements of quality in the hemostasis laboratory

When a clinician orders a laboratory test, he/she sets in motion a complex process that involves many individuals. More than two dozen individual actions, involvement of sophisticated instruments, and multiple interfaces of computing devices encompass the



**Figure 1.1** The laboratory cycle: depicted are the steps needed to complete a laboratory test, beginning with the ordering clinician and ending with the response of the ordering clinician to the result. Preanalytic, analytic, and

postanalytic parts of the process are indicated. More than two dozen steps (arrows) are involved, each of which may be the source of an error. Monitoring all steps by a quality program is required.

three phases: the preanalytic phase (order, collection, and transport); the analytic phase (making the correct measurement); the postanalytic phase (formulating and delivering the data and the action of the clinician in response to the result). Figure 1.1 is a graphic depiction of the laboratory cycle. Examining the figure, one might think that each arrow represents an opportunity for error that could affect the final result. A quality program must encompass all of these events including processes to prevent and detect errors, should they occur.

**The tools**

Many different quality practices/programs have evolved in the decades since the early work of Shewhart, Juran, and Deming. They all have their acronyms (i.e., TQM, CQI, ISO, IOP, ORYX, SIX SIGMA, Lean, TOC, and others) and a common goal of improving the quality of the performance (and

product) of an organization. The discussion of all these individual programs is beyond the scope of this chapter, but many of the principles are addressed below and in other chapters of this book. All programs have great strength, but they also suffer from being prescriptive, an issue that will be discussed later in this chapter.

Currently, *Six Sigma* and *Lean* are programs that are in use in laboratories and merit some description.

**Six Sigma**

Many industries and some laboratories have adopted control processes that focus on quantifying and reducing errors called *Six Sigma*<sup>®</sup> [7]. *Six Sigma* was developed by an engineer (Bill Smith) at the Motorola Company and the company began using the program in the mid-1980s. *Six Sigma* is a registered trademark of the Motorola Corporation. Application of the process has become very popular among companies internationally. *Six Sigma* processes can be applied to

**Table 1.2** Six Sigma metrics

Measure outcomes	Measure variation
Inspect outcomes and count defects	Measure variation of a process (SD)
Calculate defects per million	Calculate Sigma process capability
Convert DPM to Sigma metric	Determine QC design metric

Source: From Reference 7.

discrete events (misabeled specimens, clerical errors, etc.) and to variable events (i.e., variance of a method like the fibrinogen assay). Elements of these activities are depicted in Table 1.2. Discrete elements are expressed in defects per million events (DPM). Achieving the Six Sigma goal means that defects are less than 1:1,000,000, a level achieved in the airline industry. Errors in the healthcare industry are much more frequent with errors causing injury to hospitalized patients at 10,000 DPM ( $3.8\sigma$ ), errors in therapeutic drug monitoring 244,000 DPM ( $2.2\sigma$ ), or errors of laboratory reporting much better at 447 DPM ( $4.8\sigma$ ) [8]. Other aspects of the laboratory activity rely on analysis of the variability of data. This variability can be measured at several levels. The greatest variability is seen in External Quality Assessment (EQA) data regarding the all method variance, referred to as the National Total Quality (NTQ). EQA programs also report data for an analyte comparing many laboratories using the same method, referred to the National Method Quality (NMQ). NMQ is frequently significantly better because variability is only among laboratories using the same methods, but not among methods. The lowest variability is seen with a single method in a single laboratory, referred to as the Local Method Quality (LMQ) [9]. Greater variability occurs with method-specific interlaboratory testing with the greatest variability being observed when all methods are compared. Thus, the degree of variability is best controlled at the local level.

Examples of this degree of variability are shown for prothrombin time, international normalized ratio (INR), and fibrinogen assay in Table 1.3 [9]. The data in Table 1.3 are very specifically based on the data from the 2004 EQA data of the College of American Pathologists, as reported by Westgard [9]. Should a

**Table 1.3** Sigma metrics for common coagulation tests

Test	Sigma metric			
	TEA (%)	NTQ	NMQ	LMQ
Prothrombin time	15	na	1.77	5.35
INR	20	na	2.39	3.52
Fibrinogen assay	20	1.78	2.01	3.24

TEA, total acceptable error; NTQ, national total quality (all methods); NMQ, national method quality (within method); LMQ, local method quality (single laboratory); na, not available.

Source: From Reference 9.

number of different EQA data sets be analyzed, there would be a range of sigma statistics of a similar magnitude. The low sigma values shown mean that adequate control will demand more rigorous attention to control procedures, often necessitating multiple control rules. Common goals in industry are to strive for  $6\sigma$  processes and to accept  $3\sigma$ . At  $3\sigma$  or below, effective error detection could not be achieved, even with as many as six QC rules. There is much progress yet to be made in the quality of many coagulation procedures.

## Lean

Concepts of *Lean* appear to have originated with Henry Ford and his assembly line production. He actually sent engineers to the automobile junkyard to examine automobiles that could no longer function. Two types of information were gathered: first, to determine which parts failed, leading to the failure of the automobile, information used to develop improved parts in order to increase the usable life of the automobile; second, to determine those parts that were not worn out at all (or minimally), information used to examine whether alternative parts of lower cost could suffice. In the latter case, the motive is to provide sufficient performance of the part at the lowest cost to the customer. Representatives of the Toyota Motor Company visited Ford in the early 1930s. They applied and refined the principles, developing the Toyota Production System, later to be known as *Lean* [10]. *Lean* is a business management system designed to improve productivity and quality by elimination of

waste. Goals are customer satisfaction; employee satisfaction; increased workplace safety; long-term working relationships with suppliers; improved quality; reduced cost; elimination of waste. Any activity, no matter how trivial, that does not offer benefit to the product (and the customer) is a candidate for elimination. Companies involved with *Lean* are continually examining every process for opportunities to save time and improve quality. Several common activities used in other business models rarely add value. Examples include approval (delegate as much as possible); batching (delay results as little as possible, balance this with cost); searching and walking (keep all supplies immediately at hand, locate tasks as few steps as possible from each other); waiting (work with suppliers for delivery “just in time”). Thus, *Lean* aims to make processes simple enough to understand, do, and manage by the worker.

Organizations using Six Sigma and Lean rely on the common structured problem solving strategy used in business called DMAIC (Define the problem; Measure events; Analyze and understand the data; Improve the process; set up Controls that maintain the improvements). The strategy can be applied to all problem solving; however, more complex issues, such as restructuring a process, require the assembly of a team, the setting of clear goals, and a planned timeline for completion. Further details regarding application of Lean and Six Sigma can be found in George et al. [11].

### Error detection and correction

McGregor contrasted two theories of company management that he referred to as X and Y [12]. A company following theory X assumes that the worker prefers to be directed and wants to avoid responsibility. In contrast, a company that is following theory Y assumes the workers enjoy what they do and, in the right conditions, will strive to do their very best. In general, the company that follows theory X manages from the “top down” with dependence of the worker upon management as he/she performs tasks. A hallmark of theory X is toughness, the rules are laid out, and every employee must “obey.” The workplace has an element of fear that an error might occur and a reprimand will result. The style of the company that follows theory Y is different. Management works from the “bottom up.” The workplace is configured to satisfy the worker and to encourage commitment

to the organization. Workers are encouraged to be self-directed and the management/supervisory style is supportive. Theory Y has been described as operating with a “velvet glove.” Stated in another way, management under theory X strives to “drive” the organization and the workers to success, while the management under theory Y strives to “lead” the organization and the workers to success. The goal in both cases is essentially the same, but the means to the goal are very different. This brief description of diverging management styles can impact process improvement within the laboratory.

A later chapter in this book (Chapter 3) addresses the causes of medical errors and reemphasizes the need for a system in the quality program for capturing and categorizing errors. In order for any method, process, or laboratory to improve, it is paramount to correct and understand the cause of the errors that interfere with performance. The laboratory needs a system for capturing and categorizing errors. Such a system becomes the infrastructure for improvement in a quality program. It is obvious that for a system to be successful, there needs to be an aggressive program to identify all errors, optimally at the time of the occurrence. The ideal process is one that looks prospectively at activities seeking to prevent errors. Deming [6] pointed out that inspection is too late. Once again the airline industry provides an example. Considerable effort is applied to understanding what causes the big error, an airplane crash. However, major efforts are now actually directed at the near misses both in the air and on the ground, a proactive effort to understand the “close call” to help prevent the major event. The laboratory needs a similar aggressive approach that must begin with each individual owning their part of an activity and identifying the problems as they occur, or seeing ways to prevent problems by changing procedures. In order for such a process to be most efficient, the worker should not be threatened by the mechanism to report errors. The following examples regarding the differing approaches may be useful.

First, a technologist has just completed a run on an automated instrument using expensive reagents and producing many patient results. He/she notices that two required reagents were placed in the wrong position, causing them to be added in the wrong order. The error caused erroneous patient results, but not to the degree that it would be easily detected. The consequence of repeating the run is twofold: the cost of

the reagents and time of the technologist are expensive and the delay in completing the testing results in complaints from clinicians. In this scenario, management under theory X results in a reprimand from the supervisor and a letter being placed in the technologist's personnel file for negative consideration at the next performance evaluation. The consequences may be severe enough for the technologist to consider not reporting the error. In contrast, management under theory Y would result in the supervisor complimenting the technologist for detecting the problem and engaging the technologist in an investigation of the reason that the error occurred. The supervisor and the technologist understand that the goal is to prevent this from happening in the future, whether this person or another performs the procedure. The assumption is that the process contributed to the error.

Second is a case in which the error that occurred above was not detected by the technologist performing the test, but at a later time during the supervisor's inspection of reported results. Managing under theory X, the supervisor will confront the technologist with the data and, just as in the prior example, will issue a reprimand and a letter. Managing under theory Y, the supervisor will present the information to the technologist and ask the technologist to assist in understanding how the problem occurred and how it might be avoided in the future.

Errors like those described that are detected and investigated are most frequently found to be problems in the process, not exclusively with the individual doing the procedure at the time. Improving the process to help workers prevent errors is the goal and can only succeed if errors are detected and investigated. Contrasting the approaches, one can see that punishing the worker and failing to examine process will not improve the quality and the worker will not be enthused about reporting future errors. The second approach engages the workers and rewards activities that improve quality in the laboratory.

### Internal quality control

The control of the testing procedure (QC) evolved with the transition of research testing into the clinical arena. In general, internal QC provides a method to verify the imprecision of a test. To be confident that the method returns the correct result requires that steps be taken to ensure all elements are within

the control of the operator. Technologists are taught that instruments/methods are designed to fail and that they can rely upon results only if the entire method performs within defined limits with specimens of known value. The frequency of these control events are method specific and a function of the stability of all of the elements (reagent, specimen, instrument) and must be driven by historical data from the method itself. Internal QC is the grandfather of quality programs in the laboratory and is detailed elsewhere in this book (Chapter 6).

### Quality assurance

During the 1980s, laboratories began looking beyond the analytic procedure with quality programs called Quality Assurance. QC remained a part of the Quality Assurance program, but the program expanded to consider such items as laboratory orders, requisitions, collection techniques, and other issues directly impacting the result of the test but not always directly in the control of the laboratory. Preanalytic issues are detailed elsewhere in this book (Chapter 5). Postanalytic issues also became a part of quality initiatives this same era: such issues as reporting formats, verification of calculated results, timely reporting, and even action taken as a result of the data reported. It was during this period that computer applications in both the laboratory and the clinical environments began to grow, requiring the validation and continued verification of computer function and interfaces for electronic result reporting between computers as well as between instruments and computers. Encouraged (or demanded) by accreditation and/or regulatory agencies, laboratory professionals also began asking questions of and listening to clinicians regarding the quality of service and needs to provide new tests shown to have clinical value and to remove antiquated tests that no longer offer added clinical information. These activities started the interaction of the quality programs in the laboratory with similar programs in the rest of the healthcare institutions.

### External quality assessment

In the 1930s [13], the need for interlaboratory standardization for public health programs (a method to verify accuracy) led to early efforts at External Quality Assurance. The concept of an unknown specimen

being sent from a central EQA agency to the laboratory for testing with the results sent back to the agency for evaluation added an important new level of assurance for the quality of analysis. In addition, results were reported in a way that allowed a laboratory to compare their performance to other laboratories using the same or similar methods. Laboratory participation in EQA programs grew rapidly in the 1950s and 1960s. In large part this growth was due to the development accreditation and regulatory programs requiring EQA; however, the recognition by unregulated laboratories that EQA was vital to the quality of their own programs has also led to widening acceptance.

EQA is generally viewed as a process to examine the analytic phase of testing, offering little or no information regarding the pre- and postanalytic phases. Described below is a method to examine a portion of the preanalytic process and all of the postanalytic process if the laboratory uses a laboratory information system (LIS) with electronic reporting to an electronic medical record (EMR).

Within the LIS and the EMR, one can create an additional floor on the hospital, or clinic in the outpatient department. Doing so allows for development of as many “beds” or clinic visits as necessary to handle all EQA challenges. Next, the Medical Records and/or billing departments assign a block of medical record numbers for laboratory use only. The laboratory then assigns a medical record number and name to each of the EQA challenges to which it subscribes (coagulation limited, coagulation special, etc.). Each challenge may have several analytes.

Having created this for each challenge, when the specimen arrives, the specimen is accessioned into the computer with the same method as a patient, the testing is performed in the same manner as a patient, and the reporting into the LIS and the EMR will occur in the same manner as a patient. The data reported to the EQA provider can be that reported to the EMR.

The advantage of such an approach is that all instrument/computer interfaces are validated and the evaluating, accessioning, and reporting process becomes a part of the EQA program. In addition, with time, the laboratory can query the EMR by the name and medical record number of the EQA challenge to see the longitudinal data reported by analyte.

Detailed discussion of EQA programs is addressed elsewhere in this book (Chapter 7).

## The application of the tools in the laboratory

### Quality system essentials

Development and maintenance of a quality program in a laboratory requires that there be an infrastructure of support in order for internal and external QC and quality assurance to be successful. The field of hemostasis provides an excellent example of this issue. The hemostasis laboratory has the entire spectrum of testing from the highly automated to the complex manual tests that are time-consuming and demand a different skill set. Thus, in addition to a good QC program, there is need for an effective program for development and continuing education of the staff. The same can be said of a host of essential activities in the laboratory including such things as acquisition and maintenance of capital equipment; supply inventory; safety of staff and patients; and others. In the late 1990s and early 2000s, recommendations began to appear for the comprehensive management of the quality of all aspects of the laboratory operations. International Standards Organization (ISO) developed the ISO 17025 (primarily a laboratory management program) [14] and ISO 15189 (a program specifically for clinical laboratories) [15]. The Clinical Laboratory Standards Institute (CLSI), at the time named NCCLS, published the Quality System Essentials (QSE) [16]. The ISO programs have achieved acceptance in Europe and internationally, while the QSE programs are more commonly in use in North America. Both approach the issues of quality with a very broad perspective, covering all elements of laboratory operations.

The list presented in Table 1.4 is an example of the QSE for a given laboratory. The list is not intended to be the list for use in every laboratory. Each laboratory needs to develop its own essentials, formulated to help manage issues within their own laboratory. The list is ordinarily 9–12 items in length and the types of issues to be addressed are encompassed in Table 1.4. Each of the items on this list will be controlled by a set of three levels of documents:

*Policies:* Statement of intent with regard to rules and requirements of regulations, accreditation, and standards. Each QSE will have one or a small number of policies that will provide the framework for all activities within the QSE. In the case of test

**Table 1.4** Quality system essentials (CLSI—1999)

Purchasing and inventory	Information management
Organization	Deviations, nonconformance
Personnel	Assessments: internal and external
Equipment	Process improvement
Document and records	Customer service
Process control	Facilities and safety

Source: From Reference 14.

development, policies may address such things as validation, QC, EQA, and others.

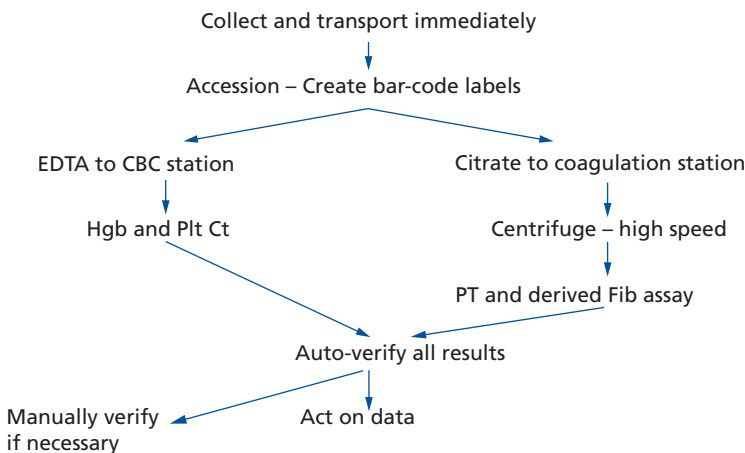
*Process descriptions:* This is a description of how the policies are implemented. Process descriptions will often cross more than one department, section of departments, and procedures within a section. Flowcharts and tables are often used to describe processes. An example of a process requiring control is given below.

*Procedures and related forms:* The standard operating procedure (SOP) is a step-by-step description of how to perform a method or task.

The Policy and the SOP are documents commonly used in all laboratories; however, the process description may not be as familiar. An example is shown in Figure 1.2. The purpose of this process is to provide the surgeon and anesthesiologist with information needed to manage blood transfusion therapy in the

rapidly bleeding patient. The data needed are the Prothrombin Time, Fibrinogen, Hemoglobin and Platelet count. The process needs an order, specimen collection, transport, laboratory receipt/accession, testing in two separate sections of the laboratory, reporting, and delivery of the data to the clinician. Ownership of the various steps in this process is in the control of the physician, nurse, and three different sections of the laboratory. In order for this to occur in a meaningful time frame in the clinical setting (less than 15 minutes), there must be well-understood coordination among all of those involved. Each step in the process described has its own SOP for the action taken. In this case, there are at least ten SOPs supporting a single process.

Implementation of a program can be challenging. Most laboratories have a quality program that can provide the beginning for the development of QSE. Most laboratories also have most of the essentials that they will define in their QSE; they are just not under the umbrella of the program and not easily identified. Thus, an initial step in changing the program will be gathering key individuals with knowledge and energy for the process to identify the QSE for the organization. Technologists should also be represented in this process. Once the QSE are identified, teams can be formed to begin drafting of policies. Leadership from the highest levels, supporting the changes that need to be made, and leading the infrastructure of a management structure base upon McGregor’s theory Y are crucial elements.



**Figure 1.2** Process for the Bleeding Profile: This process for reporting the results of the Prothrombin Time (PT), Fibrinogen Assay (Fib Assay), Platelet Count (Plt Ct), and Hemoglobin (Hgb) involves the activity of at least four different units in the health system and execution of as many as ten SOPs. As a part of the QSE, a process description would be needed to ensure return of results rapidly enough for clinician action when managing an actively bleeding patient.



Possibly the most important issue is putting reality into fault-free reporting of errors, followed by an investigation to improve process to prevent future occurrences.

For many laboratories, instituting the concepts that are described in this chapter would necessitate significant change in the quality program, the perspective of the manager, and the attitude of the employee. Such a change in the culture is difficult. It is tempting to try to “buy, install and run” a program from a quality vendor. Such an approach is likely to meet with resistance from workers who view it as “just another of those quality things that the administration is going to force on us.” In the past two decades (or more) most laboratories have instituted more that one new quality program in an effort to find a solution that works well in their setting. One possible difficulty in such an approach is the proscriptive nature of the process. They provide everything that is needed, policies, forms, SOPs, and so on. What they do not provide is the personal ownership that can come from the internal development of the quality process. Managers may find a smoother and more lasting solution in providing policies that allow for each unit to develop their own approach to the gathering of data, the identification of errors, and the many other elements of the quality program.

## Summary

Over the course of the past 70 or more years, elements of the quality program have evolved in a somewhat stepwise fashion, beginning with internal QC and progressing to more comprehensive programs that encompass all activities in the workplace. In the remainder of this book you will find information regarding quality in all aspects of the hemostasis laboratory. Experts provide information regarding the highest level of development of standards (both methods and materials) to the finest details of the nuances of selected methods. Integrated into a comprehensive quality program, similar to that described above, the information should help in the development of a “QUALITY HEMOSTASIS LABORATORY.”

## References

1. Oxford English Dictionary.
2. Shewhart WA. *Economic Control of Quality of Manufactured Product*. New York: D. Van Nostrand; 1931.
3. DeAguayo R. *Deming: The American Who Taught the Japanese about Quality*. New York: Simon & Schuster; 1990:1–66.
4. Juran JM. *Juran's Quality Control Handbook*. 4th ed. New York: McGraw-Hill; 1988.
5. Juran JM. *Juran on Leadership for Quality: An Executive Handbook*. New York: Collier Macmillan; 1989: 1–80.
6. Travers EM, McClatchy KM. Basic laboratory management. In: McClatchy KM, ed. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002:3–31.
7. Westgard JO. *Six Sigmas Quality Design and Control*. Madison, WI: Westgard QC; 2001:11–22.
8. Nevalainen D, Berte L, Kraft C, Leigh E, Morgan T. Evaluating laboratory performance on quality indicators with the Six Sigma scale. *Arch Pathol Lab Med*. 2000;124:516–519.
9. Westgard JO, Westgard SA. The quality of laboratory testing today: an assessment of  $\sigma$  metrics for analytic quality using performance data from proficiency testing surveys and the clia criteria for acceptable performance. *Am J Clin Pathol*. 2006;125:343–354.
10. Womack JD. The Lean Enterprise Institute. <http://www.lean.org>; 2009. Accessed 14 Jan 2013.
11. George ML, Rowlands D, Price M, Maxey J. *The Lean Six Sigma Pocket Toolbox*. New York: McGraw-Hill; 2005.
12. McGregor D. *The Human Side of Enterprise*. New York: McGraw-Hill; 1960:1–58.
13. Cumming HS, Hazen HH, Sanford FE, et al. Evaluation of serodiagnostic tests for syphilis in the United States: report of results. *Vener Dis Informat*. 1935;16:189.
14. International Organization for Standardization. ISO/IEC 17025:2005: General requirements for the competence of testing and calibration laboratories. [http://www.iso.org/iso/catalogue\\_detail?csnumber=39883](http://www.iso.org/iso/catalogue_detail?csnumber=39883). Accessed 14 Jan 2013.
15. International Organization for Standardization. ISO 15189:2007: Medical laboratories: particular requirements for quality and competence. [http://www.iso.org/iso/iso\\_catalogue/catalogue\\_tc/catalogue\\_detail.htm?csnumber=42641](http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=42641). Accessed 14 Jan 2013.
16. National Committee for Clinical Laboratory Standards. *A Quality System Model for Health Care: Approved Guidelines*, Wayne, PA: NCCLS; NCCLS document GP26-A; 1999.



# Hemostasis test validation, performance, and reference intervals: international recommendations and guidelines

*Richard A. Marlar*<sup>1,2</sup>

<sup>1</sup>Pathology and Laboratory Medicine, Oklahoma City VA Medical Center, Oklahoma City, OK, USA

<sup>2</sup>Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

The clinical hemostasis or coagulation laboratory is a complex testing arena that does not fit well into the mold of hematology (“counting” of particles—red blood cells or platelets) or chemistry with known concentrations of analytes (sodium charge and albumin mass). The hemostasis or coagulation assay inventory spans multiple test types (from clotting tests to chromogenic and immunologic assays to specialized tests such as electrophoresis, aggregation, and radioactive-based tests) and results are expressed in a wide variety of units: time, percentage, units, mass, optical density units, and even visual interpretation. International standards are available for some analytes (see Chapter 4); however, many still await the development of such standards. As a result, values are based on local or manufacturer’s units. These parameters complicate the development, validation, performance of methods in the routine coagulation laboratory, and the more complex methods of the “special” coagulation laboratory.

Modifications of assay methods such as using one manufacturer’s kit on another manufacturer’s instrument or in-house (“home brew”) tests or components lead to many challenges of method standardization and validation to produce accurate diagnostic, monitoring, or therapeutic information. Before a new method can be introduced into clinical use, both analytical and clinical performances must be verified under standard operating parameters of the laboratory. This chapter is intended to review the

validation procedure and outline a systematic approach for hemostatic assay validation, helping laboratories meet the daily needs of internal quality standards and external certification requirements.

The general and continuing assessment of clinical coagulation testing falls to accrediting agencies sanctioned by each country. The accreditation requirements of “good laboratory practices” vary for each oversight agency. For many hemostasis tests, significant problems are encountered: differences in reagents generating results in different arbitrary units (prothrombin time (PT) and activated partial thromboplastin time (aPTT)); tests with multiple protocols (Bethesda vs. Nijmegen inhibitor assays); and test results based on experience or visual interpretation (platelet aggregation or von Willebrand factor (vWF) multimers).

The processes of validation and performance evaluation are presented followed by a discussion of the reference interval, a difficult concept in hemostasis and coagulation. The use of a standardized validation protocol will help to objectively evaluate method performance. The parameters of this validation protocol must be established prior to any studies better defining the limits of the method, reference interval, and certainly its use in the clinical laboratory (Table 2.1). Validation is the process of proving that a procedure, process, system, equipment, reagents, and methods work singly and together as expected to achieve the intended result. Method validation assesses not only

**Table 2.1** The basic components and responsibilities of a validation study for new or modified coagulation assay

Supervisor responsibilities
Confirmation of no existing patents (if applicable)
Written laboratory procedure (CLSI GP2-A5 format)
Validation study: accuracy, precision, analytical sensitivity and specificity
Interferences, LOD, limit of quantification
RR
Quality control procedure
MSDS update
Training plan: training method, list of staff requiring training, competency
Assessment, new-employee training
Prepare memo to announce availability of test
Cost analysis of test and recommended test charge
Laboratory information manager
Establish “test definition”
Add test to LIS and HIS files
Verify report format is acceptable in all systems
QA supervisor
Add document-to-document inventory log and assign number
Add test to specimen collection manual
Add test to activity menu
Order proficiency testing materials
Laboratory director
Written approval of test establishment
Summary statement with director signature and date of implementation

Not all the listed components may be needed for every assay. The laboratory director must decide which aspects are relevant.

the major characteristics of the method but also continues assay performance over time assuring the same characteristics as initially assigned. After the assay has been deemed valid and the performance characteristics established, the final aspect of method characterization is to determine the value range(s) present in the populations in which the assay will be used. This concept of reference range evaluation and population

sampling in conjunction with the reference interval establishment methods will be discussed.

## Hemostatic test validation concepts

The purpose of test method validation is to ensure high-quality data for the accurate diagnosis of disease. The time invested in the initial validation of an analytical method will ultimately provide the necessary diagnostic advantages in the long run. Procedural, methodological, or instrumentation validation will demonstrate that the procedure, method, or instrument, respectively, is acceptable for the overall intended use. The validation steps must be thorough for each aspect of the process. The validation components should include (but not limited to) specificity, accuracy, precision, limits, linearity, and robustness (Table 2.1). Validation of coagulation methods, whether assays, instruments, or reagents, is the cornerstone of coagulation laboratory diagnostics and is the process to determine acceptability of the analytical method.

The validation protocol is necessary for the determination of the performance characteristics. A written procedure (protocol) detailing the validation process should include (1) procedural steps necessary to perform the test; (2) necessary instrumentation, reagents, and samples; (3) method for calibration; (4) formulae for generating results; and (5) source of reference standards and controls (Table 2.1). In addition, the common statistics (see Appendix) used in assay validation must also be incorporated. The typical validation parameters are discussed below using descriptions from formal definitions but slanted toward hemostatic testing [1, 2]. These include specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), and robustness.

A validation process for hemostasis/coagulation methods must be designed to ensure that the result of the method will accurately support the diagnosis of patients with coagulation defects. The samples, reagents, controls, calibrators, and instruments to be used for validation purposes should be carefully selected. Samples and specimens for validation must be collected, processed, and stored by established guidelines and identical to routine collection and storage methods used in the laboratory [3]. In the validation process for diagnostic and/or therapeutic control methods, the reagent lots and instruments

must be those that will be used in the laboratory when the methods are put in place [2, 3].

Specificity is the ability to unequivocally assess the analyte in a standard specimen in the presence of components that may be expected to be present [2, 4]. Typically this includes such components like the plasma (matrix) and degraded or inactive components. The method should be capable of the differentiation of similar analytes or interfering substances that could have a significant effect on the value. In commercially available methods (in the United States, especially FDA-approved methods), these evaluations should have been performed by the manufacturer. In “home-brew” assays, the user must demonstrate specificity, a task that may be a very difficult.

Accuracy is the closeness of agreement between the test value and the true value [5]. In hemostasis testing, this can be one of the most difficult or even impossible parameters to determine; in fact the concept of “true value” may not even apply to many coagulation tests especially those that report results as time values (PT, aPTT, and thrombin time) [2, 6]. In addition, the majority of hemostasis/coagulation tests has no “gold” standards or even established true values. This concept is changing as international standards are being developed and accepted (fibrinogen, factor VIII, protein C, antithrombin, and vWF) [7, 8]. For some standards (fibrinogen, protein C, protein S, antithrombin, and factor VIII), accuracy issues still arise due to differences in the methods used (clotting vs. chromogenic assays). The laboratory must make certain that their standards are linked, if available, to the international standard through a secondary standard of the manufacturer [2, 6]. Preparation of international standards is addressed elsewhere in this book (Chapter 4).

Precision is defined as the closeness of agreement (degree of variability) among a series of measurements obtained from multiple sampling from a single sample or reference material [2, 9]. Imprecision is measured using within-assay variability (intra-assay) and day-to-day variability (inter-assay). Intra-assay variability is the imprecision determined under the same operating conditions. Inter-assay reproducibility is the imprecision of the method when the assay components may be slightly different (different days, different operators, and different reagent vials). Precision is established irrespective of accuracy since it is the closeness of the reproducibility of the result data that

is important. The imprecision is usually expressed as coefficient of variation (CV).

Imprecision evaluation consists of a two-prong assessment: within-run variation and between-run or day-to-day variation. Variation for the within-run assessment is determined by performing the assay on the same specimen or control sample within a single run using the same reagent batch for a minimum of 20 measurements. The CV should usually be 3–6% for clotting, chromogenic, and most immunologic analytes but never more than 10%. However, for the more complex assays (platelet aggregation, vWF, and lupus anticoagulant), the imprecision in terms of CV may be 10–20%.

Between-run precision is evaluated by repeating the same specimen (usually controls) on the same instrument but with other variables (such as new reagent vials, different operators, and different environmental conditions) for a minimum of 10 runs. In general, the precision for between-run studies is greater than that observed for within-run precision studies. Usually, the CV for between-run studies is 4–8% but never more than 12%. Again for the more complex assays, the precision can increase to a significant 20–40%. The acceptable limits of precision during the validation phase is difficult to define and will vary among laboratories. No hard and fast rules apply for acceptability of coagulation testing precision; however, the laboratory must decide on the acceptable limits of precision based on publications, manufacturer’s data, or published guidelines. At least three samples that span the reportable range ((RR) including normal and abnormal values) must be used as part of the precision study. The acceptable levels of precision may be different between normal and abnormal samples, the type of assay, and the reagent–instrument combinations. The precision results should mirror the values reported by the reagent and/or instrument manufacturer. Precision within the manufacturer’s reported limits are acceptable. If the precision value obtained is greater than the manufacturer’s reported values, then the laboratory may still accept the results if their method parameters justify the increased imprecision.

### Limits

In the validation of an assay’s performance, two types of “limits” must be evaluated: LOD and LOQ [2, 6, 10]. The LOD of a method is the level at which

the assay can distinguish a sample without analyte present (blank) from the sample with analyte present; however, the assay may not accurately quantify the amount [10]. The LOD is usually defined as 3 standard deviation (SD) above the mean of the blank, making the limit above the “noise” of the method, thus the probability of a false positive is minimal (<1%). The accuracy and precision of the method (including all components and reagents) and pre-analytical variables play an important role in determining the LOD. Although these components are important for a coagulation assay, an added layer of assay complexity occurs with the time-based result assays (PT and aPTT) as these methods have no specific analyte to determine. Some coagulation methods have poor LOD due to imprecision including poor differentiation at levels that are clinically relevant. The standard protocol for determining the lower LOD is to measure a zero standard (no analyte present) multiple times (20 replicates) and calculate the standard deviation. The 3 SD range is considered “noise” and the value at the upper end of the 3 SD is the lower LOD. In coagulation, this lower limit is sometimes difficult to ascertain since finding a true “zero” standard that is plasma-based is not available. Usually, the “zero” standard plasma is an artificially created sample since clinically relevant “zero” samples are not available. It is important to understand the lower LOD of the assay in relation to the clinical use of the assay. A good example of this relationship is found in hemophilia testing in which it is important to clinically distinguish between a level of <1% and 3%. If the lower LOD is only 3%, then patients with severe hemophilia (major bleeding symptoms) cannot be differentiated from moderate hemophilia (milder bleeding symptoms). The laboratory must decide what analyte level is necessary for clinical utility for each method and then make sure the assay meets those criteria.

There are a number of different “detection limits” that must be taken into account in the overall evaluation of the coagulation assay method (instrument LOD, method LOD, reagent LOD, and plasma substrate LOD). Both the instrument detection limit and the method detection limit are the main parameters for the evaluation of a new method or new reagent-instrument system. This information is usually supplied by the manufacturer but should be verified by the laboratory before using the assay. Confirmation studies must be performed.

LOQs define the lowest amount of analyte that is quantifiable in the assay, and in addition, the LOQ defines the level at which two values can be distinguished with acceptable precision and accuracy [10]. In standard practice, the lower LOQ is statistically defined as 5–10 SD from the “zero” standard control value; however, each method must be evaluated independently to determine the lowest level of the LOQ.

The laboratory in consultation with the clinical staff must determine the clinically relevant lower LOQ for each assay. For clinical purposes, the assay must be able to accurately differentiate the medical decision points. However, the LOQ can be drastically different among methods, types of methods, types of results reported, and among laboratories. Coagulation assays such as the PT, aPTT, and some lupus anticoagulant tests have a large difference in LOQ since they are global assays measuring multiple factors.

The analytical measurement range (AMR) of an analytical method is the interval between the upper and lower analyte concentrations for which the analytical method has demonstrated a suitable level of precision, accuracy, and linearity without pretreatment (dilutions) [2, 10]. Whereas the RR is the range of analyte concentration in which the analytical method demonstrates suitable precision, accuracy, and linearity with pretreatment (dilutions or concentration). For RR, recovery studies are required to verify that pretreatment (dilution or concentration) does not affect the reported value. The precision and accuracy of a method at the lower and upper limits of the RR and AMR are important, but even more important are the clinical needs (medical decision points) for the specific analyte. The lower end of the linear range is usually the most important in coagulation, but may be the most difficult to establish at a clinically relevant level. The laboratory cannot report values lower than the lowest standard of the calibration curve. If the test method cannot be reported to the level necessary for clinical utility, then modifications of the method or alternative methods must be used to achieve the desired level including curves established for lower ranges, different dilutions of the standards or other methods [2, 11].

The linearity is the ability to obtain results that are directly proportional between the instrument response and the concentration of analyte within a given range [2, 12]. Linearity acceptance criteria are usually based on the statistical correlation coefficient of linear

regression. Mathematical transformations of data can help to promote linearity if there is scientific evidence that transformation is appropriate for the method. A good example is the factor assay that may use semi-log or log-log transformations. It is important not to force the origin to zero in the calculation as this may skew the actual best-fit slope through the clinically relevant range of use. For quantitative coagulation methods used for diagnosis and monitoring, the analytical method must have a good proportional relationship between analyte concentration and instrument response. The limit for the upper range must not exceed the level of the highest linear standard.

Methods for linearity determination of a coagulation quantitative method have changed over the last decade [2]. Linearity computations have evolved from visual assessment of the line to statistical analysis via linear regression [2]. However, linear regression will not readily define acceptable limits because many of the quantitative coagulation assays may be imprecise and have a poor linear fit. In the future, refined statistical methods including polynomial analysis will be a standard linearity assessment tool.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small to moderate variations in method parameters and pre-analytical variables [13, 14]. It provides an indication of the assay's reliability during normal usage [13, 14]. The important analytical parameters include different machines, operators, reagent lots, and sample preparation, among a host of others.

Robustness is difficult to truly assess and is generally associated with more problems with increasing complexity of the analytical system. The majority of the analysis for robustness is determined by the manufacturer and usually approved by a regulatory agency such as the FDA. However, if the reagents are not designed and evaluated by the manufacturer or used differently than intended, then stated claims of the method are not validated. In this case, the coagulation laboratory must assume responsibility for determining robustness. The pre-analytical variables that the laboratory must evaluate are those encountered in the clinical setting, whereas the analytical parameters are evaluated based on the assay's method, equipment, and reagents.

Ideally, robustness should be explored during the development of the assay method through the use of a

protocol. For such a protocol, one must first identify variables in the method that may influence the results. One might expect storage conditions and processing of the sample, minimal and maximal dilution, and the type of dilutant; level of interference (hemolysis, lipemia, etc.) to have an effect on the assay. The pre-analytic issues are addressed elsewhere in this book (Chapter 4). This type of method manipulation will ensure that the system components are robust.

### Protocol

Before initiating any validation study, whether establishing a new test, changing reagents or instruments, or changing methodology, a well-planned validation protocol must be developed using sound scientific and clinical criteria [2, 15]. Commercial companies that supply the reagents and/or instruments typically provide protocol outlines. The major components of a validation protocol are presented in Table 2.1. The protocol must describe in detail the planned studies including the statistics and defined acceptance criteria [15]. The protocol must be performed in a timely manner with adequate samples, standards, and calibrators. Much of the general information on validation protocols is described in documents provided by accrediting and standards organizations [1, 2, 6, 15].

After performing the validation protocol, the data must be analyzed with predetermined statistical methods such that the results and conclusions are presented in a validation summary report. If the defined criteria established in the protocol are met or any variations that might affect the overall conclusions are justified, then the method can be considered valid. The final validation report, along with all the data, statistical analyses, and the signatures and titles of all participants, including supervisors, laboratory director, section director, administrators, consultants, and reviewers are placed in the official standard operating procedure manual or kept readily available for inspection.

### Continued performance of coagulation/hemostasis assays

To ensure the continued accurate diagnosis and treatment of hemostatic disorders, long-term consistent assay precision and accuracy is necessary. For each