

# ESSENTIALS IN OPHTHALMOLOGY

G. K. KRIEGLSTEIN · R. N. WEINREB

Series Editors



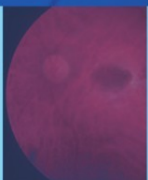
Glaucoma



Cataract  
and Refractive  
Surgery



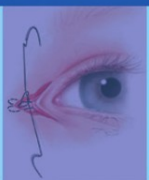
Uveitis  
and  
Immunological  
Disorders



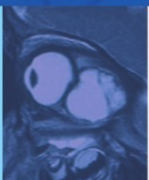
Vitreo-retinal  
Surgery



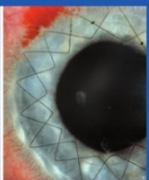
Medical  
Retina



Oculoplastics  
and Orbit



Pediatric  
Ophthalmology,  
Neuro-  
Ophthalmology,  
Genetics



Cornea  
and External  
Eye Disease

# Cornea and External Eye Disease

Edited by  
**T. REINHARD**  
**F. LARKIN**



Springer

---

**ESSENTIALS IN OPHTHALMOLOGY: Cornea and External Eye Disease**  
T. Reinhard · D. F. P. Larkin (Eds.)

---

**ESSENTIALS IN OPHTHALMOLOGY**

G. K. Krieglstein · R. N. Weinreb  
Series Editors

**Glaucoma**

**Cataract and Refractive Surgery**

**Uveitis and Immunological Disorders**

**Vitreo-retinal Surgery**

**Medical Retina**

**Oculoplastics and Orbit**

**Pediatric Ophthalmology,  
Neuro-Ophthalmology, Genetics**

**Cornea and External Eye Disease**

---

Editors T. Reinhard  
D.F.P. Larkin

# Cornea and External Eye Disease

With 138 Figures, Mostly in Color,  
and 20 Tables

 Springer

---

## Series Editors

GUENTER K. KRIEGLSTEIN, MD  
Professor and Chairman  
Department of Ophthalmology  
University of Cologne  
Joseph-Stelzmann-Strasse 9  
50931 Cologne  
Germany

ROBERT N. WEINREB, MD  
Professor and Director  
Hamilton Glaucoma Center  
Department of Ophthalmology – 0946  
University of California at San Diego  
9500 Gilman Drive  
La Jolla, CA 92093-0946  
USA

ISBN-10 3-540-22600-1  
Springer Berlin Heidelberg New York

ISBN-13 978-3-540-22600-0  
Springer Berlin Heidelberg New York

Library of Congress Control Number: 2005933478

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilm or in any other way, and storage in data banks. Duplication of this publication or parts thereof is permitted only under the provisions of the German Copyright Law of September 9, 1965, in its current version, and permission for use must always be obtained from Springer-Verlag. Violations are liable for prosecution under the German Copyright Law.

Springer is a part of Springer Science +  
Business Media

springeronline.com

© Springer-Verlag Berlin Heidelberg 2006  
Printed in Germany

Cover picture “Cataract and Refractive Surgery” from  
Kampik A, Grehn F (eds) *Augenärztliche Therapie*.  
Georg Thieme Verlag Stuttgart, with permission.

## Volume Editors

THOMAS REINHARD, MD  
Professor of Ophthalmology  
University Eye Hospital  
Killianstrasse 5  
79106 Freiburg  
Germany

D. F. P. LARKIN, MD, MRCPF, FRCS  
Cornea & External Diseases Service  
Moorfields Eye Hospital  
London, EC1V 2PD, United Kingdom

ISSN 1612-3212

The use of general descriptive names, registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

Product liability: The publishers cannot guarantee the accuracy of any information about dosage and application contained in this book. In every individual case the user must check such information by consulting the relevant literature.

Editor: Marion Philipp, Springer-Verlag Heidelberg, Germany

Desk editor: Martina Himberger,  
Springer-Verlag Heidelberg, Germany

Production: Elke Beul-Göhringer, Springer-Verlag  
Heidelberg, Germany

Cover design: Erich Kirchner, Heidelberg, Germany

Typesetting and reproduction of the figures:  
AM-productions GmbH, Wiesloch, Germany

Printed on acid-free paper  
24/3151/beau-göh 5 4 3 2 1 0

---

## Foreword

*Essentials in Ophthalmology* is a new review series covering all of ophthalmology categorized in eight subspecialties. It will be published quarterly; thus each subspecialty will be reviewed biannually.

Given the multiplicity of medical publications already available, why is a new series needed? Consider that the half-life of medical knowledge is estimated to be around 5 years. Moreover, it can be as long as 8 years between the description of a medical innovation in a peer-reviewed scientific journal and publication in a medical textbook. A series that narrows this time span between journal and textbook would provide a more rapid and efficient transfer of medical knowledge into clinical practice, and enhance care of our patients.

For the series, each subspecialty volume comprises 10–20 chapters selected by two distinguished editors and written by internationally renowned specialists. The selection of these contributions is based more on recent and note-

worthy advances in the subspecialty than on systematic completeness. Each article is structured in a standardized format and length, with citations for additional reading and an appropriate number of illustrations to enhance important points. Since every subspecialty volume is issued in a recurring sequence during the 2-year cycle, the reader has the opportunity to focus on the progress in a particular subspecialty or to be updated on the whole field. The clinical relevance of all material presented will be well established, so application to clinical practice can be made with confidence.

This new series will earn space on the bookshelves of those ophthalmologists who seek to maintain the timeliness and relevance of their clinical practice.

G. K. KRIEGLSTEIN  
R. N. WEINREB  
Series Editors

---

## Preface

This volume of the series *Essentials in Ophthalmology* aims to present recent developments regarding the cornea, with a discussion of diagnostic measures and particular emphasis being placed on treatment.

The therapeutic repertoire for surface disorders has increased considerably within the past decade. The chapter by Geerling and Hartwig reviews the application of autologous serum eye drops for this indication. Dua and coworkers in their chapters first help us to understand the limitations of amniotic membrane transplantation and then give us an overview regarding the various possibilities of limbal stem cell transplantation. Güell and coworkers provide an illustration of the potential of limbal stem cell transplantation following ex-vivo expansion.

Anterior lamellar keratoplasty is presented by Melles as a promising technique for patients with low grade keratoconus or opaque corneas with healthy endothelium. Endothelial immune reactions may be avoided using this procedure. In penetrating keratoplasty, immune reactions and astigmatism in patients still represent the major postoperative problems. Slegers and coworkers illustrate immunopathological phenomena, clinical features and risk factors of graft rejection. Antiangiogenic procedures are discussed by Cursiefen and Kruse which might contribute to minimizing the immunological problem in the future. Böhringer and coworkers

outline modern matching techniques (major – triplet, minor matching) as a prophylactic immunological measure for patients with normal-risk as well as those with high-risk keratoplasty. Modern strategies of systemic immunosuppression following high-risk penetrating keratoplasty are presented by Reis and coworkers. An overview is given by Seitz of how best to trephine the cornea in penetrating keratoplasty in order to minimize postoperative astigmatism. Watson and Daya provide an expert opinion on a serious postoperative complication following LASIK, i.e. infection.

Adenoviral corneal opacities are still a therapeutic challenge. Hillenkamp and coworkers provide an update on the different treatment regimes. Guthoff and coworkers present confocal microscopy of the cornea as a valuable tool for the in-vivo description of corneal structures on a cellular basis. Finally, a overview of ocular allergic disease is given by Manzouri and coworkers.

All the topics covered by the book have a direct clinical relevance, and we hope they will make a significant contribution to the development of optimal diagnostic and therapeutic procedures for patients with disease of the cornea.

T. REINHARD  
D. F. P. LARKIN

---

# Contents

## CHAPTER 1

### **Autologous Serum Eyedrops for Ocular Surface Disorders**

GERD GEERLING, DIRK HARTWIG

1.1	Introduction	2
1.1.1	The Rationale for Using Serum in Ocular Surface Disorders	2
1.1.2	Legal Aspects	3
1.2	Production and Application	4
1.2.1	Important Parameters of the Production Process	4
1.2.2	Current Standard Operating Procedures Used at the University of Lübeck	6
1.2.3	Quality Control	8
1.3	Clinical Results	8
1.3.1	Persistent Epithelial Defects	8
1.3.2	Dry Eye	10
1.3.3	Other Indications	13
1.4	Complications	15
1.5	Alternative Blood Products for the Treatment of Ocular Surface Disease	16
1.5.1	Umbilical Chord Serum	16
1.5.2	Albumin	17
1.5.3	Plasma and Platelets	17
	References	18

## CHAPTER 2

### **Controversies and Limitations of Amniotic Membrane in Ophthalmic Surgery**

HARMINDER S. DUA, V. SENTHIL MAHARAJAN,  
ANDY HOPKINSON

2.1	Introduction	21
2.2	Proposed Mechanisms of Action of the Amniotic Membrane	22
2.2.1	Amnion Structure	22
2.2.2	Amnion Composition	22

2.3	Intra and Inter Donor Variations of the Membrane	24
2.4	Processing and Preservation of the Membrane	25
2.5	Clinical Studies and Outcomes (Definitions of Success and Grading of Disease Severity)	27
2.6	Efficacy of Membrane in Relation to Other Established Techniques and Options	30
	References	31

## CHAPTER 3

### **Transplantation of Limbal Stem Cells**

HARMINDER S. DUA

3.1	Introduction	36
3.2	Stem Cells	36
3.2.1	Definition	36
3.2.2	Characteristics of Stem Cells	36
3.2.3	The Stem Cell 'Niche'	37
3.3	Limbal Stem Cells	37
3.3.1	The Clinical Evidence	37
3.3.2	The Scientific Evidence	39
3.4	Limbal Stem Cell Deficiency	41
3.4.1	Causes of Limbal Stem Cell Deficiency	41
3.4.2	Effects of Limbal Stem Cell Deficiency	41
3.4.3	Diagnosis of Stem Cell Deficiency	44
3.5	Limbal Transplant Surgery	45
3.5.1	Principles	45
3.5.2	Preoperative Considerations	45
3.6	Surgical Techniques	46
3.6.1	Sequential Sector Conjunctival Epitheliectomy (SSCE)	46
3.6.2	Auto-limbal Transplantation	48
3.6.3	Allo-limbal Transplantation	49
3.6.4	Adjunctive Surgery	51
3.6.5	Postoperative Treatment	52
	References	53



CHAPTER 4

**Limbal Stem Cell Culture**

JOSÉ L. GÜELL, MARTA TORRABADELLA,  
MARTA CALATAYUD, OSCAR GRIS,  
FELICIDAD MANERO, JAVIER GAYTAN

4.1	Introduction	57
4.2	Epithelial Phenotype	58
4.3	Preparation of Human Amniotic Membrane	58
4.4	Culture of Explanted Tissue	59
4.5	Tissue Procurement	59
4.6	Preliminary Clinical Experience	60
4.6.1	Principles for Taking the Biopsy	60
4.6.2	Advantages of Limbal Stem Cell Culture	61
4.7	Case Report	61
4.8	Future Standard Staging Approach for Ocular Surface Reconstruction	64
	References	64

CHAPTER 5

**Deep Anterior Lamellar Keratoplasty**

GERRIT R.J. MELLES

5.1	Introduction	65
5.2	Main Drawbacks of Conventional DALK	65
5.3	Different Concepts	65
5.4	Important Preoperative Considerations	66
5.5	Psychological Preparation of the Patient	67
5.6	Choice of DALK Surgical Technique	67
5.7	Clinical Results	68
	References	70

CHAPTER 6

**Corneal Transplant Rejection**

T.P.A.M. SLEGGERS, M.K. DALY, D.F.P. LARKIN

6.1	Introduction	73
6.2	Incidence	74
6.3	Factors Predisposing to Corneal Graft Rejection	74
6.4	Clinical Features	75
6.5	Histopathology	75

6.6	Immunopathological Mechanisms	76
6.6.1	Immune Privilege and Its Breakdown	76
6.6.2	Afferent Arm of the Allogeneic Response	76
6.6.3	Efferent Arm of the Allogeneic Response	77
6.7	Treatment of Rejection	77
6.8	Prevention of Rejection	77
6.8.1	Immunosuppression	77
6.8.2	HLA Matching	78
6.9	Future Prospects	78
	References	79

CHAPTER 7

**New Aspects of Angiogenesis in the Cornea**

CLAUS CURSIEFEN, FRIEDRICH E. KRUSE

7.1	Introduction	83
7.2	“Angiogenic Privilege of the Cornea” or “How Does the Normal Cornea Maintain Its Avascularity?”	84
7.3	Corneal (Hem)angiogenesis	85
7.3.1	General Mechanisms of Corneal (Hem)angiogenesis	85
7.3.2	Common Causes of Corneal (Hem)angiogenesis	86
7.3.3	Clinical Consequences of Corneal Hemangiogenesis	86
7.3.4	Corneal Hemangiogenesis After Keratoplasty	87
7.3.5	Corneal Angiogenesis Due to Contact Lens Wear	90
7.3.6	Angiogenesis as a Cause of Disease Progression, not a Sequel (Herpetic Keratitis)	91
7.3.7	Surgery in Vascularized Corneas	91
7.4	Corneal Lymphangiogenesis	91
7.4.1	Mechanisms of Corneal Lymphangiogenesis	91
7.4.2	Importance of Lymphangiogenesis for Induction of Alloimmunity After Keratoplasty	94
7.4.3	Non-immunological Effects of Corneal Lymphangiogenesis	94
7.5	Antiangiogenic Therapy at the Cornea	95
7.5.1	Established and Novel Antiangiogenic Therapies	95

7.5.2 Novel Antihemangiogenic and Antilymphangiogenic Therapies to Improve Graft Survival After Keratoplasty ..... 98  
 References ..... 98

CHAPTER 8

**Histocompatibility Matching in Penetrating Keratoplasty**

DANIEL BÖHRINGER, RAINER SUNDMACHER, THOMAS REINHARD

8.1 Introduction ..... 101  
 8.1.1 Immune Reactions Constantly Threaten Graft Survival ..... 101  
 8.1.2 Major Transplantation Antigens (HLA) ..... 102  
 8.1.3 Minor Transplantation Antigens .. 105  
 8.2 Time on the Waiting List Associated with Histocompatibility Matching . 106  
 8.2.1 Waiting Time Variance Has Been a Barrier to Histocompatibility Matching ..... 106  
 8.2.2 Algorithm for Predicting the Time on the Waiting List ..... 106  
 8.3 Recommended Clinical Practice ... 107  
 References ..... 108

CHAPTER 9

**Current Systemic Immunosuppressive Strategies in Penetrating Keratoplasty**

ALEXANDER REIS, THOMAS REINHARD

9.1 Introduction ..... 109  
 9.2 Immunology ..... 109  
 9.2.1 Acute Rejection ..... 110  
 9.2.2 Major Histocompatibility Complex 110  
 9.2.3 Chronic Rejection ..... 110  
 9.3 Normal-Risk Versus High-Risk Transplantation ..... 111  
 9.3.1 Normal-Risk Transplantation ..... 111  
 9.3.2 High-Risk Transplantation ..... 111  
 9.3.3 Rationale for Systemic Immunosuppression ..... 111  
 9.3.4 Why Is Immunomodulation with Topical Steroids Not Sufficient To Prevent Immunologic Graft Rejection in High-Risk Patients? .. 111

9.4 Immunosuppressive Agents ..... 112  
 9.4.1 History ..... 112  
 9.4.2 Corticosteroids ..... 114  
 9.4.3 Cyclosporine A (CSA, Sandimmun, Sandimmun Optoral, Sandimmun Neoral) ..... 114  
 9.4.4 Tacrolimus (FK506, Prograf) ..... 114  
 9.4.5 Mycophenolate Mofetil (MMF, CellCept, Myfortic) ..... 115  
 9.4.6 Rapamycin (Sirolimus, Rapamune) 115  
 9.4.7 RAD (Everolimus, Certican) ..... 116  
 9.4.8 FTY 720 ..... 116  
 9.4.9 Biologic Agents ..... 117  
 9.5 Guidelines for Practitioners ..... 117  
 9.5.1 Preoperative Evaluation ..... 117  
 9.5.2 How To Use Cyclosporine in High-Risk Corneal Transplantation ..... 118  
 9.5.3 How To Use MMF in High-Risk Corneal Transplantation ..... 118  
 9.5.4 How To Use Rapamycin in High-Risk Corneal Transplantation ..... 118  
 9.5.5 How To Use Tacrolimus in High-Risk Corneal Transplantation ..... 118  
 9.5.6 Combination Therapies ..... 119  
 9.6 Conclusion ..... 119  
 References ..... 119

CHAPTER 10

**Trephination in Penetrating Keratoplasty**

BERTHOLD SEITZ, ACHIM LANGENBUCHER, GOTTFRIED O.H. NAUMANN

10.1 Introduction ..... 123  
 10.2 Astigmatism and Keratoplasty .... 124  
 10.2.1 Definition of Post-keratoplasty Astigmatism ..... 124  
 10.2.2 Reasons for Astigmatism After Keratoplasty ..... 125  
 10.2.3 Prevention/Prophylaxis of Astigmatism After Keratoplasty 129  
 10.3 Trephination Techniques ..... 130  
 10.3.1 Principal Considerations ..... 131  
 10.3.2 Conventional Mechanical Trephines 139  
 10.3.3 Nonmechanical Laser Trephination 144  
 10.4 Concluding Remarks ..... 148  
 References ..... 149

CHAPTER 11

**Infective Complications Following LASIK**

ADAM WATSON, SHERAZ DAYA

11.1 Introduction ..... 153  
 11.2 Frequency and Presentation ..... 153  
 11.3 Characteristics ..... 154  
 11.4 Differential Diagnosis ..... 154  
 11.4.1 Diffuse Lamellar Keratitis (DLK, “Sands of the Sahara”) ..... 154  
 11.4.2 Steroid-Induced Intraocular Pressure Elevation with Flap Oedema (Pseudo-DLK) . 155  
 11.5 Management ..... 155  
 11.5.1 Flap Lift ..... 155  
 11.5.2 Specimen Taking ..... 155  
 11.5.3 Treatment ..... 157  
 11.5.4 No Improvement ..... 157  
 11.6 Special Considerations ..... 158  
 11.6.2 Fungal Keratitis ..... 158  
 11.6.3 Viral Keratitis ..... 159  
 11.7 Visual Outcome ..... 159  
 11.8 Management of Sequelae ..... 159  
 11.9 Prevention ..... 160  
 References ..... 160

CHAPTER 12

**Treatment of Adenoviral Keratoconjunctivitis**

JOST HILLENKAMP, RAINER SUNDMACHER, THOMAS REINHARD

12.1 Introduction ..... 163  
 12.1.1 Etiology and Clinical Course of Ocular Adenoviral Infection .... 163  
 12.2 Socioeconomic Aspect ..... 166  
 12.3 Treatment ..... 166  
 12.3.1 Treatment of the Acute Phase .... 166  
 12.3.2 Treatment of the Chronic Phase ... 168  
 12.3.3 Prophylaxis ..... 169  
 12.4 Conclusion and Outlook ..... 170  
 12.5 Current Clinical Practice and Recommendations ..... 170  
 References ..... 170

CHAPTER 13

**In Vivo Micromorphology of the Cornea: Confocal Microscopy Principles and Clinical Applications**

RUDOLF F. GUTHOFF, JOACHIM STAVE

13.1 Introduction ..... 173  
 13.2 Principle of In Vivo Confocal Microscopy Based on the Laser-Scanning Technique . 174  
 13.2.1 Slit-Scanning Techniques ..... 175  
 13.2.2 Laser-Scanning Microscopy and Pachymetry ..... 176  
 13.2.3 Fundamentals of Image Formation in In Vivo Confocal Microscopy ... 179  
 13.3 General Anatomical Considerations ..... 180  
 13.4 In Vivo Confocal Laser-Scanning Microscopy ..... 181  
 13.4.1 Confocal Laser-Scanning Imaging of Normal Structures ..... 182  
 13.5 Clinical Findings ..... 190  
 13.5.1 Dry Eye ..... 190  
 13.5.2 Meesmann’s Dystrophy ..... 190  
 13.5.3 Epithelium in Contact Lens Wearers ..... 192  
 13.5.4 Epidemic Keratoconjunctivitis .... 195  
 13.5.5 Acanthamoeba Keratitis ..... 196  
 13.5.6 Corneal Ulcer ..... 199  
 13.5.7 Refractive Corneal Surgery ..... 200  
 13.6 Future Developments ..... 201  
 13.6.1 Three-Dimensional Confocal Laser-Scanning Microscopy ..... 201  
 13.6.2 Functional Imaging ..... 203  
 References ..... 206

**CHAPTER 14**  
**Allergic Eye Disease: Pathophysiology,**  
**Clinical Manifestations and Treatment**  
 BITA MANZOURI, THOMAS FLYNN,  
 SANTA JEREMY ONO

14.1 Introduction ..... 209  
 14.2 Pathophysiology ..... 210  
 14.2.1 Type I Hypersensitivity ..... 210  
 14.2.2 Ocular Inflammatory Reaction:  
     Late Phase ..... 211  
 14.2.3 Non-specific Conjunctival  
     Hyperreactivity ..... 211  
 14.2.4 T-Cell-Mediated Hypersensitivity  
     in Allergic Eye Disease ..... 212  
 14.3 Clinical Syndromes  
     of Allergic Eye Disease ..... 212  
 14.3.1 Seasonal Allergic Conjunctivitis ... 214

14.3.2 Perennial Allergic Conjunctivitis .. 214  
 14.3.3 Vernal Keratoconjunctivitis ..... 214  
 14.3.4 Atopic Keratoconjunctivitis ..... 215  
 14.3.5 Giant Papillary Conjunctivitis .... 217  
 14.4 Treatment of Allergic Eye Disease . 217  
 14.4.1 Antihistamines ..... 218  
 14.4.2 Mast Cell Stabilizing Agents ..... 218  
 14.4.3 Dual-Acting Agents ..... 219  
 14.4.4 Non-steroidal Anti-inflammatory  
     Drugs (NSAIDs) ..... 219  
 14.4.5 Topical Corticosteroids ..... 219  
 14.4.6 Calcineurin Inhibitors ..... 220  
 14.4.7 Future Drug Developments ..... 220  
 14.5 Conclusion ..... 221  
 References ..... 222

**Subject Index** ..... 225

---

## Contributors

DANIEL BÖHRINGER, Dr.  
University Eye Hospital  
Killianstrasse 5, 79106 Freiburg, Germany

MARTA CALATAYUD, MD  
Diagonal 419 6<sup>a</sup>1a, 08008 Barcelona, Spain

CLAUS CURSIEFEN, Dr.  
Department of Ophthalmology  
Friedrich-Alexander University  
Erlangen-Nürnberg  
Schwabachanlage 6, 91054 Erlangen, Germany

M.K. DALY, MD  
Cornea & External Diseases Service  
Moorfields Eye Hospital  
London, EC1V 2PD, UK

SHERAZ DAYA, MD  
Eye Bank, Queen Victoria Hospital NHS Trust  
East Grinstead, West Sussex, RH19 3DZ, UK

HARMINDER S. DUA, MD, PhD  
Chair and Professor of Ophthalmology  
University of Nottingham, Eye ENT Centre  
Queens Medical Centre  
Derby Road, Nottingham, NG7 2UH, UK

THOMAS FLYNN, MRCOphth.  
Department of Ocular Immunology  
Institute of Ophthalmology  
University College London, Bath Street  
London, EC1V 9EL, UK

JAVIER GAYTAN, MD  
Instituto de Microcirugia, Ocular de Barcelona  
Munier 10, 08022 Barcelona, Spain

GERD GEERLING, Prof. Dr.  
Department of Ophthalmology  
University of Würzburg  
Josef-Schneider-Strasse 11  
97080 Würzburg, Germany

OSCAR GRIS, MD  
Instituto de Microcirugia, Ocular de Barcelona  
Munier 10, 08022 Barcelona, Spain

JOSÉ L. GÜELL, MD  
Associate Professor of Ophthalmology  
Instituto de Microcirugia  
Ocular de Barcelona  
C. Munier, 10, 08022 Barcelona, Spain

RUDOLF F. GUTHOFF, Prof. Dr.  
University Eye Hospital Rostock  
Doberaner Strasse 140  
18057 Rostock, Germany

DIRK HARTWIG, Dr.  
Institute of Immunology  
and Transfusion Medicine  
University of Lübeck, Ratzeburger Allee 160  
23538 Lübeck, Germany

JOST HILLENKAMP, Dr.  
Eye Hospital of the University of Regensburg  
Franz-Josef Strauss Allee 11, 93042 Regensburg  
Germany

ANDY HOPKINSON, PhD  
Division of Ophthalmology  
and Visual Sciences, Eye ENT Centre  
Queens Medical Centre, University Hospital  
Derby Road, Nottingham, NG7 2UH, UK

FRIEDRICH E. KRUSE, Dr.  
Department of Ophthalmology  
University of Erlangen-Nürnberg  
Schwabachanlage 6, 91054 Erlangen, Germany

ACHIM LANGENBUCHER, Priv.-Doz. Dr.  
Department of Ophthalmology  
University of Erlangen-Nürnberg  
Schwabachanlage 6, 91054 Erlangen, Germany

D.F.P. LARKIN, MD, MRCPE, FRCS  
Cornea & External Diseases Service  
Moorfields Eye Hospital  
London, EC1V 2PD, UK

V. SENTHIL MAHARAJAN, MS, FRCS  
Division of Ophthalmology  
and Visual Sciences Eye ENT Centre  
Queens Medical Centre, University Hospital  
Derby Road, Nottingham, NG7 2UH, UK

FELICIDAD MANERO, MD  
Instituto de Microcirugia  
Ocular de Barcelona  
Munier 10, 08022 Barcelona, Spain

BITA MANZOURI, MRCOphth.  
Department of Ocular Immunology  
Institute of Ophthalmology  
University College London  
Bath Street, London, EC1V 9EL, UK

GERRIT R.J. MELLES, MD  
Netherlands Institute  
for Innovative Ocular Surgery  
Laan Op Zuid 390  
3071 AA Rotterdam, The Netherlands

GOTTFRIED O.H. NAUMANN, Prof. Dr.  
Department of Ophthalmology  
University of Erlangen-Nürnberg  
Schwabachanlage 6, 91054 Erlangen, Germany

SANTA JEREMY ONO, PhD  
Department of Ocular Immunology  
Institute of Ophthalmology  
University College London  
11-43 Bath Street, London, EC1V 9EL, UK

THOMAS REINHARD, Prof. Dr.  
University Eye Hospital  
Killianstrasse 5, 79106 Freiburg, Germany

ALEXANDER REIS, Priv.-Doz. Dr.  
Reis Medical Institution Est.  
Landstrasse 310, 9495 Triesen  
Principality of Liechtenstein

BERTHOLD SEITZ, Prof. Dr.  
Department of Ophthalmology  
University of Erlangen-Nürnberg  
Schwabachanlage 6, 91054 Erlangen, Germany

T.P.A.M. SLEGGERS, MD  
Department of Ophthalmology  
University Medical Center Groningen  
9700 RB Groningen, The Netherlands

JOACHIM STAVE, Prof. Dr.  
University Eye Hospital Rostock  
Doberaner Strasse 140, 18057 Rostock  
Germany

RAINER SUNDMACHER, Prof. Dr.  
University Eye Hospital, Moorenstrasse 5  
40225 Düsseldorf, Germany

MARTA TORRABADELLA, MD  
Paseig Vall d'Hebron 119  
08034 Barcelona, Spain

ADAM WATSON, FRANZCO  
Corneoplastic Unit  
Queen Victoria Hospital NHS Trust  
East Grinstead, West Sussex, RH19 3DZ, UK

## Core Messages

- The viability and function of the corneal and conjunctival epithelium is supported by the antimicrobial, nourishing, mechanical and optical properties of tears, since these contain factors which promote proliferation, migration and differentiation of epithelial cells
- A lack of these epitheliotropic factors, e.g. in aqueous tear deficiency, can compromise the ocular surface and result in serious disorders such as persistent epithelial defects
- Pharmaceutical lubricants usually replace the aqueous component of tears alone and may have little efficacy in improving surface disorders
- Serum has biomechanical and biochemical properties similar to normal tears
- In vitro cell culture experiments have shown that the morphology, migration and differentiation of ocular surface epithelia are better supported by serum than by pharmaceutical lubricants
- A number of protocols for the production of serum eyedrops have been published, which vary considerably and can influence the biochemical properties of this autologous blood product
- Under EU legislation production of serum eyedrops requires a license to produce blood products by the appropriate national body, which means that an extensive evaluation and documentation process must be successfully completed
- Alternatively the use of serum eyedrops is allowed on an intention to treat basis if production and application are all performed by the physician, i.e. in ophthalmic departments that have the necessary laboratory facilities and are able to admit the patient for the duration of the treatment
- The production of serum eyedrops should be well documented and measures for appropriate quality control (i.e. serological and microbiological tests) should be established
- The results of a considerable number of clinical cohort studies have reported beneficial effects of its use for persistent epithelial defects, severe dry eyes and other indications
- The use of serum eyedrops implies the risk of transmission of infectious diseases, from the donor to other individuals involved in the production and application of the product
- In addition contamination of the dropper bottle and subsequent microbial keratitis can occur
- Such complications can be largely avoided by testing the patient for HIV, syphilis and hepatitis B and C prior to the blood donation and testing every serum eyedrop batch for bacterial contamination. In addition, the serum therapy can be combined with topical antibiotics
- Due to these risks and the lack of randomised controlled trials, the use of serum eyedrops should still be considered experimental and informed consent should be obtained from every patient treated
- Alternative blood products with epitheliotropic potential such as plasma, platelet concentrates or serum albumin should be evaluated since they are readily available as quality controlled blood derivatives from blood banks on a routine basis

## 1.1

### Introduction

In recent years the use of eyedrops produced from autologous serum has gained wide acceptance for the treatment of ocular surface disorders intractable to conventional medical therapy. Such conditions include persistent epithelial defects or severe dry eyes. Autologous serum was first evaluated in 1984 by Fox et al. [9] in search of an unpreserved lubricant, which was not available from pharmaceutical providers at that time. However, it was Tsubota who repopularised their use when he described the epitheliotropic potential of serum for the ocular surface due to its content of growth factors and vitamins [46].

#### 1.1.1

#### The Rationale for Using Serum in Ocular Surface Disorders

The tear film supplies the ocular surface with many nutrient and wound healing modulating factors such as fibronectin, vitamins or growth factors, which support and modulate proliferation, migration and differentiation of the conjunctival and corneal epithelium. These epitheliotropic factors are predominantly released into the aqueous component of the tear film by the main and accessory lacrimal glands as well as conjunctival vessels, while glucose, electrolytes and amino acids are provided by the aqueous humour.

For example fibronectin, a disulphide glycoprotein that influences cell adhesion and migration of the healing epithelium, predominantly originates from plasma when conjunctival blood vessels become more permeable during an inflammatory reaction and the lacrimal gland itself secretes vitamins, neuropeptides and growth factors such as substance P and epidermal growth factor (EGF) [36]. However, proteins of the aqueous tear film not only act as essential nutrients for the ocular surface epithelia, but also determine the biomechanical properties of the tear lipid layer. Tear-lipocalin is an example for this, which by acting as a transport

protein for retinol, supports goblet cell differentiation, but also reduces the surface tension of tears. As part of inflammatory processes, additional proteins, such as lactoferrin, serum-IgA and complement factors, are released into the tears and support opsonisation and phagocytosis of microbes by macrophages and lymphocytes. Tears thus not only have a lubricating and mechanical clearance function, but also epitheliotropic and antimicrobial properties.

If the carrier, i.e. the aqueous phase, or the epitheliotropic factors of the tear film are diminished, the integrity of the surface epithelia can become disrupted and epithelial defects evolve, which may persist and progress. Surgical attempts to rehabilitate the ocular surface in severe dry eyes also fail frequently [2, 22] unless sufficient substitute lubrication is provided.

#### Summary for the Clinician

- The tear film has lubricant and nutrient properties
- In severe dry eye it is not only the increased biomechanical stress, but also the lack of epitheliotropic factors that promotes damage of the ocular surface

The ideal tear substitute would possess lubricant and nutrient properties. However – with few exceptions – currently available products are optimised for their biomechanical properties only [49, 51]. Vitamin A, EGF and fibronectin have been used in vitro and in vivo to encourage epithelial wound healing, but due to stability concerns and limited clinical success, these single compounds have not become part of clinical routine management [15, 25, 29]. Autologous blood offers a number of characteristic advantages:

1. It contains a large number of substances also present in tears, such as vitamin A, epitheliotropic and neurotrophic growth factors, immunoglobulins and fibronectin. Some of these factors are found in serum in higher concentrations than in natural tears (Table 1.1) [20, 27, 29, 45].
2. Serum can be prepared as an autologous product and thus lacks antigenicity.



**Table 1.1.** Biochemical and biophysical properties of undiluted serum and normal, unstimulated human tears (EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin like growth factor; NGF, neurotrophic growth factor; PDGF, platelet derived growth factor; SP, substance P; TGF, transforming growth factor) [11, 15, 20, 48–52]

	Tears	Serum
pH	7.4	7.4
Osmolality	298±10	296
EGF (ng/ml)	0.2–3.0	0.5
TGF-β	2–10	6–33
NGF (pg/ml)	468.3±317.4	54.0
SP (pg/ml)	157.0±73.9	70.9±34.8
IGF-1 (ng/ml)	0.031±0.015	105
PGDF	0–1.33	15.5
Vitamin A (mg/ml)	0.02	46
Albumin (mg/ml)	0.023±0.016	35–53
Fibronectin (μg/ml)	21	205
Lactoferrin (ng/ml)	1,650±150	266
Lysozyme (mg/ml)	2.07±0.24	0.001
SIgA (μg/ml)	1,190±904	2,500

- Eyedrops from serum can be produced without preservatives and hence toxicity due to additives is not an issue.

A wide range of quality controlled products can be derived from full blood. These include not only serum, but also, e.g. plasma, platelet rich suspensions or various protein fractions such as albumin. *Serum* is the fluid component of full blood that remains after clotting. It should not be confused with *plasma*, which is obtained when clotting is prevented by mixing a full blood donation with an anticoagulant and removing all corpuscular elements by centrifugation (see Sect. 1.5.3). Plasma thus does not contain the significant amount of platelet derived growth factors such as EGF, PDGF and TGF-β, which are released into serum upon activation of the platelets during clotting. *Platelet rich suspensions* (platelet concentrates) are available as a standard blood product from blood banks,

while *human serum albumin* fractions can be purchased from pharmaceutical companies. While all of these blood derived products are potentially available as growth factor containing solutions for topical application to the ocular surface, serum has been predominantly used in clinical studies.

The growth and migration promoting effect of serum on cell cultures in general and on corneal epithelial cells is well documented [13, 46]. In dose- and time-response experiments in vitro, we found that serum maintained the morphology and supported proliferation of primary human corneal epithelial cells better than unpreserved or preserved pharmaceutical tear substitutes [13]. It is also known that serum induces mucin-1 expression in immortalised, conjunctival epithelial cells as a sign of higher differentiation [45] and that it increases transcription of RNA for NGF as well as TGF-β receptors in human keratocytes [7]. The epitheliotropic properties are – again according to in vitro tests – not reduced in patients suffering from systemic autoimmune disease requiring systemic immunosuppression [17].

### Summary for the Clinician

- Plasma, platelet suspensions and albumin are available as blood derived products from blood banks
- Serum is not a standard blood product, but can easily be produced on special request and currently is by far the most often used product in clinical studies
- Serum contains many epitheliotropic factors also present in tears and can be prepared as an autologous unpreserved tear substitute that offers both lubricant and nutrient properties

### 1.1.2 Legal Aspects

Autologous serum eyedrops are a blood product. The distribution of pharmaceuticals is regulated by governmental laws in most countries. Although in the European Union the manufacturing and distribution of pharmaceuticals is regulated by the individual country, several

directives have been issued (1965/65, 1975/139, 1975/318) which have been implemented in the laws of each member state of the EU. Today every pharmaceutical product requires a marketing authorisation to be issued by a competent authority of each state. Authorisation depends on the proof of efficacy in clinical trials, implementation of quality controls, reports of adverse effects, proof of expert knowledge and other regulatory aspects. These criteria can probably only be fulfilled by professional pharmaceutical manufacturers.

An exemption from the need to obtain marketing authorisation is granted if a physician manufactures a specific medical product by himself or under his supervision with responsibility to treat his own patient on a named basis. This product has to be prepared according to the doctor's specifications and autologous serum eyedrops can therefore be produced only by the physician himself or by his staff. However, it remains the physician's responsibility that manufacturing and application are performed correctly. Since even stricter regulations may especially exist for blood products in individual states, every physician producing autologous serum eyedrops needs to inform himself about specific national regulations.

In the United States, producers of drugs and medical devices have to be registered with the Food and Drug Administration (FDA). Similar to EU regulations, registration is not necessary "for practitioners licensed by law to prescribe or administer drugs or devices and who manufacture, prepare, propagate, compound, or process drugs or devices solely for use in the course of their professional practice". Again, special regulations on testing and approval of drugs by the FDA or for using blood products need to be evaluated by the practitioner.

### Summary for the Clinician

- Serum eyedrops are a blood product that can be produced on a named patient basis according to the doctor's specifications
- It remains the physician's responsibility that manufacturing and application are performed correctly

- Every patient should give their informed consent before the production of autologous serum eyedrops is initiated
- Quality control measures must be implemented for the production as well as the application
- Serum should only be applied under the supervision of the prescribing doctor

## 1.2 Production and Application

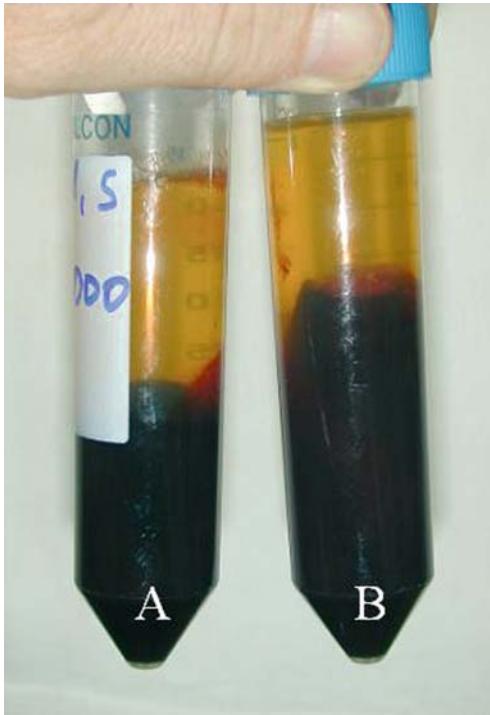
### 1.2.1 Important Parameters of the Production Process

Although the complex composition of full blood will certainly vary between individuals, it is also known that a number of production parameters can significantly influence the biochemical composition of blood derived products. These critical steps in the production of serum eyedrops should therefore be standardised.

These include:

- Clotting phase: duration and temperature
- Centrifugation: centrifugal force and duration
- Dilution: dilution factor and diluent
- Storage: container, temperature, duration

In the absence of any controlled clinical trial evaluating the impact of such differences in the production, in vitro models have been helpful in assessing a large number of protocol variations. The published clinical studies often fail to mention important parameters such as for how long the blood was allowed to clot before centrifugation. If the full blood sample is centrifuged before the clotting process is completed, the release of platelet derived factors is reduced. We have established that the concentration of EGF, HGF, and TGF- $\beta$  are higher after a 2-h clotting time when compared with paired full blood samples that were allowed to clot only for 15 min and this was associated with a trend towards better corneal epithelial cell proliferation [24]. Thus we allow the full blood donation to clot for at least 2 h at room temperature. However, a



**Fig. 1.1.** Demonstration of the influence of the  $g$ -force on the volume of serum obtained from 50 ml of full blood centrifuged 2 h after donation: **A** at 3,000  $g$  for 15 min; **B** at 500  $g$  for 5 min

48-h period of storage at 4 °C, to allow transportation of full blood donations from peripheral ophthalmic departments to a centralised production unit, seems an equally acceptable procedure [53].

The volume retrieved from a given blood donation as well as its biochemical composition are also influenced by the centrifugation, which is determined by the centrifugal  $g$ -force and the time used to spin a sample. The  $g$ -force itself depends upon the revolutions of the rotor per minute (rpm) as well as on the diameter of the rotor. Thus  $g$ -force and not rpm is the parameter that should be stated in a protocol. The  $g$ -force in the studies published so far – if mentioned at all – probably varies by at least 1 log. A higher  $g$ -force not only helps to yield a larger volume of serum from a full blood sample (Fig. 1.1), but also reduces membranous platelet remnants in the supernatant, which in high con-

centrations have been shown to induce apoptosis [5]. Also a higher  $g$ -force can result in a lower concentration of TGF- $\beta_1$ . As TGF- $\beta$  is able to slow down epithelial wound healing, Tsubota suggested diluting the serum 1:5 with saline, which, however, reduces the concentration of other growth factors, such as EGF, that are proven to support proliferation of corneal epithelial cells. Combinations of 1,500 rpm – in an average size centrifuge equal to about 300  $g$  – to 4,000  $g$  (ca. 5,000 rpm) for 5–20 min have been used. A 15-min centrifugation at 3,000  $g$  results in good separation of serum and blood clot, without inducing haemolysis [41].

It is obvious that the dilution of the obtained serum sample to the final concentration in the eyedrops determines the concentration of epitheliotropic factors. In clinical studies, 20%, 33%, 50% or 100% have been used. Since the protocols for the production of serum eyedrops also varied in other parameters, there is no clear clinical evidence to favour any specific concentration. However, *in vitro* experiments show that cell proliferation is best supported at a 20% concentration of serum. It was also shown that the type of diluent has an impact and that BSS rather than saline should be used [24].

In some indications, e.g. dry eye, autologous serum eyedrops are applied for many months. They are also usually produced without preservatives or stabilising additives to minimise the risk of drug induced toxicity. Since the production is labour intensive, a large number of aliquot samples is prepared from a single blood donation to keep the number of donation and processing efforts limited. To preserve the activity of the biological substances thought to be beneficial for the ocular surface, the drops can be refrigerated or stored frozen. The concentration of growth factors, vitamin A and fibronectin in pure and diluted serum was found to remain stable for at least 3 months if stored at –20 °C and for 1 month if stored at 4 °C. However, it is known that many protein concentrations in tears are reduced if stored for several weeks at 4 °C. While in developed countries access to freezers is rarely a problem, it seems preferable to store unused daily dosage vials of serum frozen [45, 38]. From this stock one container is then removed every morning and kept

refrigerated at 4 °C until it is discarded at the end of the day. Alternatively dilution of the serum with chloramphenicol 0.5%, which has few toxic side effects, has also been advocated to allow the use of dropper bottles for up to 1 week.

### Summary for the Clinician

- The list of protocol variations for the production of autologous serum eyedrops is long
- The biochemical composition of serum depends on the time and conditions of clotting, the centrifugation, dilution and storage
- No controlled clinical trial has determined so far which of the protocols published offers the best epitheliotropic support
- In the absence of such trials, the production protocol has been optimised in vitro
- The stock of serum eyedrops can be stored frozen for several months to preserve the biologically active components of the product

### 1.2.2

#### Current Standard Operating Procedures Used at the University of Lübeck

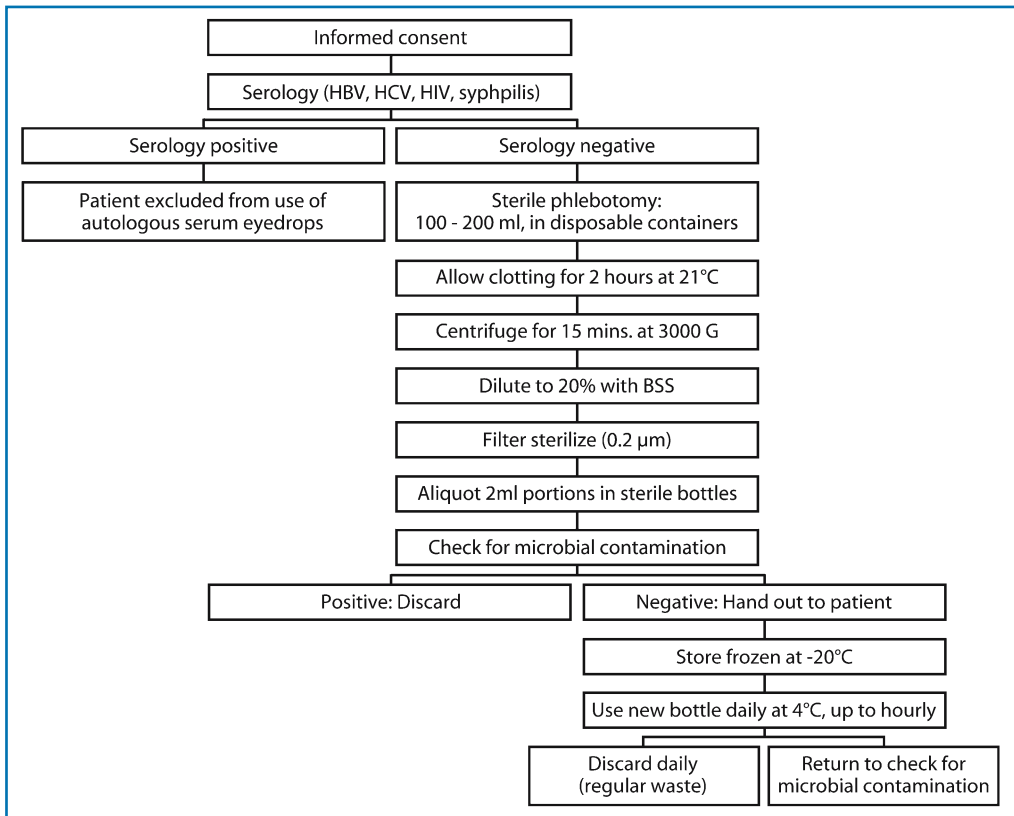
Following the principles of Good Manufacturing Practice and based on extensive evaluation in vitro, the following standard operating procedures are currently used at the University of Lübeck (Fig. 1.2) [24, 19].

Patients are assessed for their suitability to donate according to the guidelines of the Bundesärztekammer and Paul-Ehrlich Institute for blood donation and use of blood products. This requires them to be in reasonably good health, with no significant cardiovascular or cerebrovascular disease, and free of bacterial infection. Anaemia (Hb < 11 g/dl) is a relative contraindication. If only a small amount of blood (50–100 ml) is taken, mild anaemia or circulatory disorders need not be considered contraindications. To minimise the danger of bacterial contamination, no blood should be taken from patients suffering from suspected septicaemia. To exclude transmission of infection, patients must be tested for hepatitis-B/-C, syphilis and

HIV serology (HbsAg; antibodies to HCV, HIV-I/-II, HIV-NAT, syphilis; HCV-NAT) before blood is donated for the production of eyedrops. A positive serology excludes the patient from the donation of autologous blood for serum eyedrop production. Prior to venisection, the patient must be informed in writing about the planned therapy, its experimental nature, the risks involved (e.g. bacterial contamination) and alternative methods of treatment. The patient's consent should be obtained and kept with the notes.

Venipuncture is performed at the antecubital fossa under aseptic conditions. Depending on the expected duration of treatment, 100–200 ml of whole blood is collected into sterile containers. For larger volumes a sterile blood pack without anticoagulant can be used to collect up to 470 ml. A 100-ml donation of whole blood will yield 30–35 ml of serum, which diluted to 20% is sufficient for at least 3 months of serum eyedrops 8 times daily. Larger volumes are recommended in patients who require long-term treatment, in order to minimise labour intensive production. The containers are left standing upright for 2 h at room temperature to ensure complete clotting before they are centrifuged at 3,000 g for 15 min. The supernatant serum is removed under sterile conditions in a laminar air flow hood with sterile 50-ml disposable syringes. The volume retrieved is determined and diluted 1:5 with sterile BSS. Gentle shaking ensures homogenisation before portions of 2 ml are aliquoted through a 0.2-µm filter into sterile dropper bottles. The effect of filtration has not been evaluated, but Fox recommends filtration to remove fibrin strands, suspected to reduce the effect of serum eyedrops. The bottles are sealed and labelled with the name, date of birth of the patient, the date of production and the instruction "Autologous blood serum for topical use in the eye. To be stored frozen and used within 3 months after date of production. To be discarded 24 hours after opening." Two millilitres of the solution is – as required by the European Pharmacopoeia Addendum 2000 – sent for microbiological evaluation.

The product is available approximately 6 h after venesection, but is only dispatched once negative serology and microbiology of donor



**Fig. 1.2.** Standard manufacturing protocol for the preparation, storage and use of serum eyedrops. Parameters which influence the biochemical character of the product are also shown

and product are confirmed. Usually the drops are applied 8 times daily. A new bottle is opened everyday. It is recommended to be stored at +4 °C and to be discarded after 16 h of use with regular household waste. The remaining bottles are stored frozen (ideally at -20 °C) for up to 3 months. If the domestic freezer has no thermometer, it is recommended to place one inside and control the temperature when taking a new vial out every day. If the temperature cannot be adjusted to about -20 °C, the dispensing doctor may consider recommendation of shorter storage episodes.

The costs of production – i.e. labour and consumables alone – for a day’s dosage of serum eyedrops following this protocol are well below 5 €. This is the approximate equivalent of one bottle of preserved pharmaceutical lubricant

and thus should be acceptable for the rare occasion where the ocular surface disease is so severe that the use of topical autologous serum is justified [14].

### Summary for the Clinician

- Every physician producing or prescribing autologous serum eyedrops should inform himself about specific national regulations
- Any production protocol should follow the principles of Good Manufacturing Practice
- The dropper bottles must be carefully labelled with the patient’s details and instructions for storage and use
- If produced without preservatives the drops should be kept frozen at -20 °C until the day of use

### 1.2.3

#### Quality Control

In order to guarantee quality, control measures should be initiated and if strictly interpreted serum eyedrops should be produced only by personnel supervised by the doctor directly in charge of the patient treated. Bacterial contamination of the product during the production as well as from the application of serum eyedrops is a potential risk. Sterile manufacturing conditions, beginning with thorough skin disinfection, are of the utmost importance. It is preferable that further processing is performed in a closed system. To minimise the risk of infection to third parties (e.g. production or nursing staff), it is strongly recommended that the donor is tested for HIV, HBV, HCV and syphilis before donation of larger amounts of blood are collected for the production process itself. For quality control purposes bacterial contamination resulting from the production process needs to be ruled out by a microbiological examination of the product prior to initiation of the clinical application and a control system must be implemented to ensure that the product is only used when the microbiological and serological tests are clear.

Prior to venipuncture and application, the identity of the patient must be confirmed. The packaging and dropper bottles must be clearly labelled with:

- The patient's name and date of birth
- The name and address of the manufacturer
- The date of manufacture and the date of expiry
- The instructions on how to store and use the drops
- A comment that the material is an autologous blood product, which is solely for application with the named patient

To minimise the variability of the product and to maximise the safety of its use, a written version of the standard operating procedures (SOP) should be established. Conscientious documentation is indispensable for good medical and manufacturing practice. Each step relevant to manufacture as well as application (in-

cluding the dates of application and any unwanted effect) should be recorded. From this it becomes obvious that strict guidelines for good manufacturing, quality control and documentation must be established and maintained prior and throughout the therapeutic use of autologous serum eyedrops. All steps of the production should be documented on a form which – together with the patient's consent – is kept with the patient's notes.

#### Summary for the Clinician

- A written protocol of the standard operating procedures should be established
- All production steps must be documented on a SOP form
- Serum eyedrops should only be produced and released once the negative serology of the donor for hepatitis, syphilis and HIV and the negative microbiology of the product have been confirmed

## 1.3 Clinical Results

Serum eyedrops have predominantly been used for persistent epithelial defects and severe dry eye, but also as a supportive measure in ocular surface reconstruction and for a number of other indications.

### 1.3.1 Persistent Epithelial Defects

A persistent epithelial defect (PED) is defined as a defect of the corneal epithelium that – in the absence of microbial keratitis – fails to heal within the expected time course (e.g. 2 weeks) despite topical lubricants [26, 50]. A PED can occur as a result of many different pathologies, including rheumatoid arthritis, neurotrophic keratopathy or dry eye [8]. “Success” of treatment is best defined as percentage of defects healed in a given time or as total time to complete epithelial wound closure.

**Table 1.2.** Clinical studies using serum eyedrops to treat persistent epithelial defects. The production parameters and results are given. Success is defined as percentage of eyes/patients with complete epithelialisation. Note that the scale used to measure these changes as well as the baseline level varied between the studies (NA, not applicable; NR, not reported; rpm, revolutions per minute)

Author	Concentration	Diluent	Centrifugation (g force)	Duration	Clotting time	Frequency of application	Eyes (patients)	Success objective
Alvarado	20%	0.9% NaCl	5000 RPM	10 min.	NR	NR	17 (14)	83%
De Souza	100%	NA	NR	NR	NR	Hourly	70 (63)	81%
Garcia	20%	0.9% NaCl	5000 RPM	10 min.	NR	10×	11 (11)	55%
Matsumoto	20%	0.9% NaCl	3000 RPM	10 min.	NR	5–10×	14 (11)	100%
Poon	50–100%	0.5% chloramphenicol	4000 RPM (2200 G)	10 min.	2 h	8×	15 (13)	60%
Tsubota	20%	0.9% NaCl	1500 RPM	5 min.	NR	6–10×	16 (15)	63%
Young	20%	0.9% NaCl	1500 RPM	5 min.	NR	6–14×	10 (10)	75%

### 1.3.1.1 Currently Available Published Data

In five prospective case series, 20% serum diluted in 0.9% saline has been used 5–14 times daily for this indication (Table 1.2). In 1999 Tsubota was the first to report a series of 16 eyes (15 patients) in whom the PEDs had persisted despite medical treatment with lubricants or bandage contact lenses for a mean of  $7.2 \pm 9.4$  months. Ten out of these 16 defects healed completely within 4 weeks after initiation of therapy [11, 46]. Garcia-Jimenez reported complete epithelial wound healing in 6 of 11 eyes with persistent epithelial defects with healing beginning within 3–4 weeks of treatment with serum eyedrops [1, 52]. In a group of 9 patients with predominantly diabetic or postherpetic neurotrophic keratitis, all 12 epithelial defects healed within  $15.8 \pm 7.9$  days and this was associated in 9 eyes with an improvement of corneal sensitivity [27] (Fig. 1.3).

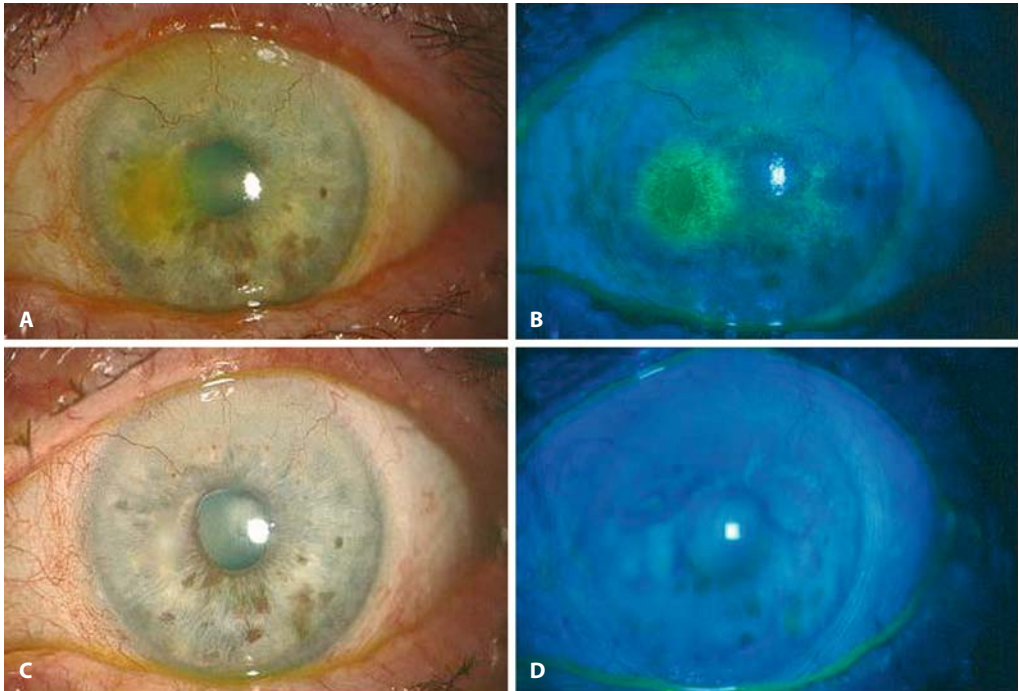
A different concentration of serum was used in two other studies. Poon et al. substituted unpreserved pharmaceutical lubricants with 50–100% serum eyedrops and observed closure of a PED with a mean duration of  $7.5 \pm 5.8$  (1–24) weeks in 9 of 15 eyes after  $3.6 \pm 2.5$  weeks (3 days–8 weeks) [33]. De Souza et al. treated 70

epithelial defects with undiluted serum hourly in addition to routine medication, 45 of which had occurred early after penetrating keratoplasty [8] and had persisted for a mean of  $15 \pm 17$  days. Eighty-one percent of these defects with a relative short history healed within  $14 \pm 12$  days.

Healing generally starts within 2 weeks after initiation of the serum therapy [33]. So far no study has been able to show a correlation between size or localisation of the defect with success or failure, but the older and deeper stromal defects tended to heal less successfully. Also, when serum eyedrops are changed back to pharmaceutical lubricants the epithelial defects may recur, as happened in 6 out of the 9 eyes in Poon's and 9 out of 70 eyes in De Souza's group. These figures are difficult to compare since none of the studies was placebo controlled and the study population seems to differ significantly in terms of underlying pathogenesis and duration of the PED.

#### Summary for the Clinician

- Pathogenic factors that can be avoided or treated, such as toxicity due to preserved eyedrops, steroids or active herpetic keratitis, should be ruled out before a PED is treated with serum eyedrops



**Fig. 1.3.** Epithelial defect persisting unaltered for 2 weeks in the left eye of a female patient with severe aqueous tear deficiency due to secondary Sjögren's syndrome before (A, B) and 1 week after (C, D) treatment with 20% autologous serum eyedrops 8 times daily. The defect started to heal within 2 days

- No signs of microbial keratitis should be present if serum application is considered for an epithelial defect, since the effect of serum in this situation is unknown and may support bacterial growth
- Twenty percent serum eyedrops are applied approximately every 2 h until the defect is healed
- PEDs begin to heal generally within 2 weeks after initiation of serum eyedrops
- Older and deeper stromal defects tend to heal less successfully
- If 20% serum fails, a higher concentration of serum may help to achieve epithelialisation
- When serum eyedrops are changed back to pharmaceutical lubricants the epithelial defect may recur

### 1.3.2 Dry Eye

Dry eye is a group of disorders, of diverse pathogenesis, that share as common manifestations signs and symptoms due to the interaction of both an abnormal tear film and an abnormal ocular surface. It is subdivided into aqueous deficient and evaporative, i.e. lipid or mucin deficient dry eyes. Although it is believed to be one of the most common ocular problems in the Western world with an incidence of symptoms of dry eye in up to 14.6%, ocular surface changes are observed clinically in only 0.5%. Severe aqueous tear deficiency, however, can lead to blindness and serum eyedrops have been used in this situation.