Received: 19 January 2024 | Revised: 14 June 2024 | Accepted: 8 July 2024

DOI: 10.1111/tme.13068

ORIGINAL ARTICLE

TRANSFUSION MEDICINE WILEY

Allogenic and autologous nondiluted serum eye drops—validation strategy compliant with good manufacturing practice

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Abstract

Background and aims: Serum eye drops alleviate ocular symptoms of diseases such as sicca syndrome, or chronic graft-versus-host disease. This study was designed for good manufacturing practice validation of our standard manufacturing, storage and transport processes for both autologous and allogenic SEDs. Specifications of quality parameters are lacking and were aimed to be defined.

Methods: Using sterile collected, coagulated whole blood, serum was separated by centrifugation and filled into single-use eye drop applicator vials. Quality control tests included visual inspection, sterility, leukocyte concentration, pH, vitamin A, TGF-ß and VEGF-A. Samples were collected after manufacture and after 24 h and 6 months of frozen storage (−20°C). Sterility testing was performed after opening the SED applicators at specified intervals. For transport validation, SEDs were packed in insulated transport bags and stored at 20–24°C and 30–32°C for 8 h.

Results: Vitamin A, TGF-ß and VEGF-A assays showed no difference in concentration between fresh and 24 h frozen serum. All specifications for pH (aim 7.4) and cellular contamination were met and microbiological contamination tests were negative. Shelf-life was defined as 6 months at −20°C. Once opened, the product must be used within 24 h to avoid bacterial outgrowth. Transporting frozen SEDs from the manufacturer via a local pharmacy to the patient within a maximum of 4 h was demonstrated.

Conclusions: The GMP compliance of our production, storage and transport processes for autologous and allogenic SEDs was successfully validated. 100% serum eye drops in single-use applicators can be safely used for up to 24 h after opening.

KEYWORDS
allogenic, autologous, serum eye drops, validation

1 | INTRODUCTION

Serum eye drops (SEDs) play an important role in the treatment of ocular manifestations of diseases such as sicca syndrome, Sjögren's syndrome and chronic graft-versus-host disease (GvHD), which can cause severe dry eye.[1–3] Up to 80% of patients undergoing allogenic stem cell transplantation develop GvHD with ocular manifestations. In the acute phase, conjunctivitis is common. Chronic GvHD often leads to severe ocular surface disease with corneal epithelial damage requiring special ophthalmologic treatment.[4] The beneficial effect of artificial tears made from autologous serum in patients with sicca syndrome was first reported by Fox et al. in 1984,[5] and has since been confirmed in numerous studies.[6–9] However, the pharmacologic mechanism of SEDs is not fully understood. Natural tears have many functions. For example, they act as a mechanical barrier, have antibacterial properties and transport vital nutrients.[10] Tears and serum have important similarities in pH (pH 7.4), osmolarity (298 osmol/L tears and 296 osmol/L serum), and biochemical composition.[9,11] Growth factors, vitamins, fibronectin and other important biochemicals normally found in tears and serum appear to be essential for healing and restoring dry eye. Therefore, we defined vitamin A and the growth factors VEGF-A and TGF-ß as functional parameters, especially regarding to shelf-life testing for our validation.[2,12,13]

Compared with serum, conventional artificial tears lack these healing properties and are insufficient in the treatment of dry eye. Providing validated SED products is therefore an important part of dry eye management. Because patients requiring SEDs generally have health problems excluding them from making autologous blood donations, allogenic blood from healthy donors is often the preferred raw material for SED production. In 2007, Chiang et al. described the successful treatment of two patients with allogenic SEDs.[14] Since then, the use of allogenic SEDs has increased worldwide. The Corona crisis also led to increased demand for allogenic products, for example at the National Health Service Blood and Transplant (NHSBT) in the UK.[15] Health services in the UK and many other countries commonly offer both autologous and allogenic SEDs.

Although specific national regulations or guidelines for the preparation and storage of SEDs are currently missing,[11,16–18] the European Pharmacopoeia (Ph. Eur. 2.6.1)[13] provides product quality specifications for ocular applications.

SEDs were prepared without preservatives, with the physiological (isohydric) pH of natural tears (pH 7.4),[12,19] with a maximum particle size of 25 μm, and supplied in single-use applicator vials to avoid microbial contamination. Since monocytes, the largest blood cells, have a particle size of 12–24 μm,[20] SEDs meet the specified particle size limit of maximal 25 μm.[13]

The aim of this study was to validate SED products for home use. It included various aspects such as validation of the manufacturing process according to GMP, testing of product quality parameters, shelf-life testing and temperature monitoring during shipment to the patient.

2 | MATERIALS AND METHODS

2.1 | Donors and patients

A total of 11 healthy donors (8 male, 3 female) and 9 patients requiring SEDs were enrolled in this study. Patients prescribed SEDs completed a medical interview and screening for blood donation eligibility. Individuals with acute infection, diarrhoea, severe heart disease or anticoagulant treatment were excluded. Eligibility for whole blood donation was based on hemogram criteria in patients (male: Hg >12.5 g/dL, Hct ≥37.5%, female: Hg >11.0 g/dL, Hct ≥33.0%) and on German national guidelines (RiLiBäk chap. 2.2.5.1) in donors (male: Hg >13.5 g/dL, Hct ≥40%, female: Hg >12.5 g/dL, Hct ≥38.0%).[21] For viral safety, serum from all donors and patients was subjected to standard blood donor safety testing (HIV, HBV, HCV, HEV, *Treponema pallidum* and West Nile virus). Data were collected from 2020 to 2022 in accordance with EU good manufacturing practice (GMP) requirements. SEDs from allogenic donors were produced for testing purposes only, whereas SEDs from patients (autologous) were produced according to an accompanying validation study and dispensed to patients by a pharmacy.

2.2 | SED preparation process

Whole blood donations (maximum: 450 mL/bag) were collected without anticoagulant using a TF304 sterile blood collection system (Meise Medizintechnik GmbH, Schalksmühle, Germany), allowed to clot overnight (19:05 h; 16:40 h-21:45 h) at room temperature and centrifuged twice at 2888 g and 20°C for 15 min (Heraeus Cryofuge 6000; Thermo Fisher Scientific Messtechnik GmbH, Darmstadt, Germany). Serum was collected as the supernatant, aliquoted into closed, sterile 1.5 mL disposable single use eye drop applicators (ATS 12 and ATS 36 eye drop systems, Meise Medizintechnik GmbH), and stored frozen at −20°C ± 2°C. Visual inspection was performed of the serum before filling the applicators. The acceptance criteria were a yellow and clear liquid with no visible agglomeration, and an intact blood collection bag without leakage.

2.3 | SED storage

After preparation, applicators were stored at −20°C ± 2°C up to 6 months in a temperature monitored freezer. The continuous monitoring was conducted with BRIEM system (BRIEM Steuerungstechnik GmbH, Nürtingen, Germany).

2.4 | Sample preparation

Samples were collected immediately after isolation of fresh serum (12.5 mL) and after 24 h of frozen storage (12 applicators). For autologous SED production, this corresponds to 6.7% of the serum volume and 8.5% of the applicators. For shelf-life testing, the applicators were thawed and evaluated after 6 months of frozen storage. Serum was visually inspected for colour and coagulation.

2.5 | Sample analysis

2.5.1 | pH

pH was determined using an ABL 90 Flex (Radiometer GmbH, Krefeld, Germany) based on the potentiometric measurement principle. The normal point-of-care temperature setting (37°C) was corrected corresponding to application temperature (20°C).

2.5.2 | Cell count

Cell concentrations were measured using an XN-550 Automated Hematology Analyzer (Sysmex Corporation, Kobe, Japan) according to the manufacturer's instructions.

2.5.3 | Sterility

Sterility testing was performed using an automated system (BD BACTEC, BD Life Sciences Diagnostic Systems, Heidelberg, Germany). Aerobic and anaerobic culture bottles (BD Bactec Standard Anaerobic/F and Aerobic/F, respectively) were incubated for 7 days at 30–32°C with a sample volume of 6 mL.

2.5.4 | Growth factors

VEGF was determined with Human VEGF Immunoassay (Quantikine® ELISA; R&D Systems, Inc., Minneapolis, USA). TGF-ß was either measured with 'Human/Mouse/Rat/Porcine/Canine TGF-ß1 Immunoassay' (Quantikine® ELISA, R&D Systems, Inc.) or with 'TGF-beta 1 ELISA Enzymimmunoassay zur quantitativen Bestimmung von Transforming Growth Factor beta 1 (TGF-beta 1) in Humanserum, und Zellkulturüberständen' (IBL International GmbH, Hamburg, Germany). One applicator of 1.5 mL was used for each assay. Detection was done with ELISA microplate reader Tecan Sunrise Spectrophotometer (Tecan Trading AG, Maennedorf, Switzerland).

2.5.5 | Vitamin A

Vitamin A was identified by high pressure liquid chromatography (HPLC) (VWR Hitachi High-Technologies Corporation, Tokyo, Japan) with 'one Step Vitamins A and E in serum/plasma' (Chromsystems Instruments and Chemicals, Gräfelfing, Germany) using one applicator (1.5 mL, light protected). The isocratic HPLC was conducted with a flow rate of 1.5 mL/min and UV detection at 295 nm.

2.6 | Transport validation

Transport validation was carried out using insulated PYB2 laboratory cooling bags (Versapak Europe GmbH, Haan, Germany) according to a standardised protocol. Each bag was packed with 6 boxes (3 with applicators filled with dummy material at the bottom and 3 empty boxes at the top) and 17 frozen cooling packs and stored at 20–24°C and 30–32°C, respectively, for 8 h. The internal bag temperature in each box was continuously monitored with a temperature logger (EBI 310-T, Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany). During transport to the participating patients, a temperature indicator (Timestrip Plus 0°C, Timestrip UK Ltd, Cambridge, UK) was placed in the middle upper box and activated to ensure that temperatures remained below 0°C during transport.

2.7 | Statistical analysis

Microsoft Excel 2010 and IBM SPSS Statistics 25 were used to determine median values and range, the Shapiro–Wilk test for normal distribution and significance tests (Wilcoxon test). p values below 0.05 were considered statistically significant.

3 | RESULTS

The validation process was performed in two steps, starting with the manufacturing process for allogenic SEDs followed by validation of autologous SED manufacturing with additional shelf-life testing and transport validation.

3.1 | SED manufacturing in the allogenic setting

A total of 12 whole blood donations (350 mL; 205–450 mL) were collected from 11 healthy donors, yielding 93–181 mL of serum each (152 mL), which was filled into 62 (8–83) eye drop applicators with a volume of 1.5 mL of serum per applicator. SEDs were prepared without diluting and preservatives and packaged in single use applicator vials. The serum produced was a clear liquid with no signs of coagulation. It was almost completely free of cellular components and met the European Pharmacopoeia criteria for particle-free eye drops with a maximum diameter of 25 μm. The product also met the pharmacopeia's microbiological testing requirements. Product data (Table 1) of fresh (pre) versus 24 h frozen material (post) showed no significant difference for VEGF-A ($p = 0.263$) and vitamin A ($p = 0.610$). pH increased slightly from 7.60 to 7.69 ($p = 0.017$), whereas TGF-ß receded from 30.7 ng/mL to 23.6 ng/mL ($p = 0.025$), pre to post respectively.

3.2 | SED manufacturing in the autologous setting

Using the same methods, SEDs were prepared from 11 whole blood donations from nine patients with GvHD. 190 mL serum (144–

231 mL) isolated from 450 mL whole blood (301–451 mL) was filled into 127 applicators (90–155) with a volume of 1.5 mL of serum per applicator. These autologous SEDs were visually inspected, tested for sterility and leukocyte count. Product data (Table 1) of pre- and post-frozen autologous SED showed no significant difference for pH, vitamin A, VEGF-A and TGF-ß1 ($p > 0.05$). All specifications were met.

3.3 | Six month shelf-life of the SEDs

After 6 months of storage at −20°C ± 2°C, shelf-life testing of the SEDs was conducted (Table 2). Visual inspection and sterility testing of allogenic and autologous SED led to unremarkable results. In allogenic and autologous SEDs, TGF-ß1 increased slightly from 23.6 ng/mL to 29.3 ng/mL ($p = 0.241$), and from 17.8 ng/mL to 26.8 ng/mL (patient storage, $p = 0.310$), respectively. Whereas there also was an increase of vitamin A after 6 months in allogenic SED ($p = 0.016$), VEGF-A decreased in autologous SED (patient storage, $p = 0.028$).

However, the pH of both allogenic and autologous SEDs increased significantly ($p < 0.05$) after 6 months of frozen storage (Table 2). As the range for the pH meter is pH 6.3–8.0, measurements were possible up to pH 8.2; for higher results, the pH meter showed error messages. This led to 5 instead of 8 valid results of the autologous SEDs.

Autologous SEDs were shipped to patients after product inspection and release. The question aroused whether storage in conventional home freezers without temperature monitoring is suitable for long-term storage of SEDs. Patients received separate boxes of validation-only and normal-use applicators and stored them in their home freezers. After 6 months, the validation applicators were returned to laboratory for testing. In parallel, additional validation applicators were stored at the hospital. There were no discrepancies in sterility and visual quality ($n = 8$) as well as in quality parameters between products stored for 6 months in home freezers and those stored at −20 ± 2°C in temperature monitored hospital freezers ($p > 0.05$).

3.4 | Stability of SEDs after opening

In order to detect possible microbiological contamination during use, allogenic SEDs were tested in repeat determination to validate in-use stability 1 day after opening. Samples were refrigerated (2–8°C) during thawing and intermediate storage, except during opening. Five thawed applicators from each of five different batches were opened every hour for the next 8 h and then stored for up to 24 h after first opening. The contents of the applicators from each batch were then inoculated into blood culture bottles and tested for microbiological purity. All samples tested negative for microbiological contamination.

TABLE 1 Product data of allogenic and autologous SED.

	Allogenic serum		Autologous serum	
Parameter	Fresh	24 h frozen	Fresh	24 h frozen
pH	7.60 (7.51–7.62)	7.69 (7.59–7.76)	7.63 (7.52–7.90)	7.64 (7.55–7.76)
Vitamin A [µg/ml]	0.63 (0.50–0.76)	0.61 (0.51–1.03)	0.58 (0.38–1.08)	0.56 (0.43–1.03)
VEGF-A [pg/ml]	240 (92–545)	336 (87–530)	683 (100–993)	608 (209–991)
TGF-ß1 [ng/ml]	30.7 (22.4–52.3)	23.6 (13.4–31.5)	20.1 (7.7–28.0)	21.3 (14.9–29.0)
Leukocytes [$*10^3/\mu l$]	0.01 (0.00–0.07)	0.02 (0.00–0.08)	0.01 (0.00–0.03)	0.00 (0.00–0.01)

Note: Data of pH, vitamin A, VEGF-A, TGF-ß1 and leukocytes from allogenic ($n = 8$) and autologous serum ($n = 9–11$) are given before freezing and 24 h post-freeze as median and range.
Abbreviation: SED, serum eye drop.

TABLE 2 Storage data of allogenic and autologous SED.

	Allogenic		Autologous		
Parameter	24 h frozen	6 month frozen	24 h frozen	6 month frozen patient	6 month frozen hospital
pH	7.75 (7.61–8.02)	8.03 (7.91–8.16)	7.61 (7.59–7.66)	8.08 (7.90–8.19)	8.04 (7.91–8.10)
Vitamin A [µg/mL]	0.58 (0.38–0.68)	0.60 (0.42–0.79)	0.60 (0.43–1.03)	0.61 (0.32–1.13)	0.67 (0.35–0.99)
VEGF [pg/mL]	359 (97–870)	313 (81–661)	641 (209–991)	611 (105–843)	575 (159–990)
TGF-ß1 [ng/mL]	23.6 (13.3–44.5)	29.3 (16.9–57.9)	17.8 (15.8–29.0)	26.8 (7.6–38.3)	24.0 (7.6–39.5)
Leucocytes [$*10^3/\mu L$]	0.02 (0.00–0.08)	0.01 (0.00–0.02)	0.00 (0.00–0.01)	0.01 (0.00–0.01)	0.02 (0.00–0.03)

Note: Data of pH, vitamin A, VEGF-A, TGF-ß1 and leukocytes from allogenic ($n = 10$) and autologous serum ($n = 5–8$) are given 24 h post-freeze and after 6 months storage at −20°C ± 2°C. Median and range are shown.
Abbreviation: SED, serum eye drop.

TABLE 3 Temperature monitoring.

	Storage at 20–24°C			Storage at 32°C		
Logger position	4 h [°C]	6 h [°C]	8 h [°C]	4 h [°C]	6 h [°C]	8 h [°C]
Box 1	−6.5	−4.4	−2.8	−2.7	−0.7	−0.2
Box 2	−8.0	−5.7	−4.3	−6.8	−4.2	−2.9
Box 3	−5.0	−3.0	−2.0	−3.8	−2.0	−1.4
Box 4	−8.0	−5.8	−4.1	−4.8	−2.8	−1.9
Box 5	−8.7	−6.0	−4.4	−6.2	−3.5	−2.9
Box 6	−7.0	−5.0	−4.2	−5.3	−2.8	−1.8

Note: Temperatures were measured in the six boxes inside the transport boxes, stored at ambient temperatures of 20–24°C and 32°C for 4, 6 and 8 h, respectively.

3.5 | Transport (shipping)

The manufactured SEDs were shipped from the hospital to the user via a local pharmacy in insulated, temperature-controlled cooler bags. Each transport bag was packed with six boxes stacked in two rows of three boxes (duplex stacking) and 17 ice packs. We then tested whether the internal temperature remained below 0°C for 8 h ($n = 12$, median 8:05 h) in bags stored at room temperature ($n = 6$; median internal −4.2°C) and at 32°C ($n = 6$, median internal −1.9°C). As expected, the temperature in the three boxes at the bottom of the bag (numbers 4–6) was lower than in the three boxes at the top of the bag (numbers 1–3) after 8 h of storage, regardless of the ambient temperature. Therefore, the applicators were transported in the boxes at the bottom of the bags with empty boxes on top for temperature buffering and robust packaging. After 4 h, the maximum temperature measured inside the bag was −2.7°C (Table 3). During transport temperature monitoring, additional temperature indicators placed inside the bags did not detect internal temperatures exceeding 0°C ($n = 7$). The median duration of the entire process, from SED manufacturing to quality control to delivery to the pharmacy, was 15 days.

4 | DISCUSSION

SEDs contain several biological substances, including growth factors and cytokines that promote epithelial proliferation and differentiation. The healing properties of SEDs have been used effectively in the treatment of various ocular diseases for decades.[4,6,7]

The manufacture of SEDs is not complicated, but production methods may vary between manufacturers and countries. SEDs may be supplied in single versus multiple use applicators, with or without physiological saline for serum dilution, produced with allogenic or autologous serum, and subject to different quality or safety standards. In addition, the regulatory status of SEDs is still unclear: blood product or drug?

The unclear regulatory status and the missing guidelines result in a various number of protocols, diluted or undiluted, different times for clotting, varying centrifugation steps and a differing storage (temperature and time).[9] Literature described many parameters for quality testing of SEDs like vitamin A, fibronectin, albumin and number of growth factors as TGF-ß, VEGF, EGF and PDGF.[22] Therefore, it was decided to validate our entire process chain for the manufacture, storage and transport of SEDs to ensure GMP compliance and to define quality specifications for process standardisation. The two-stage validation process started with the production of allogenic SEDs from whole blood donations, followed by the analogous manufacturing of autologous SEDs. This two-stage approach had the advantage of validating first the manufacturing and storage processes for SEDs derived from allogenic blood donors. This enabled us in the second stage to provide the patients with SEDs from their own blood during the validation process.

No relevant variation of the tested parameters was observed when the SEDs were stored at −20°C ± 2°C for 6 months. In addition, no significant differences were observed between SEDs stored in our hospital freezers and those stored in patients' home freezers. Based on these results, the shelf-life of frozen and thawed SEDs after opening was verified and defined as 6 months when stored at −20°C ± 2°C and 24 h after opening and storage at 2–8°C. This is consistent with other SED manufacturers whose product shelf-life recommendations range from 3 to 12 months when frozen at ≤ − 20°C.[2,6,16,23] However, it has been shown that freezing and frozen storage of SED partially had an effect on growth factors or vitamin A, but these variations should not have any clinical relevance.

Interestingly, the pH of the SEDs increased after freezing and continued to increase during storage. This effect may be due to gas exchange during the freezing and thawing processes. In terms of logistics, the transport conditions from the manufacturer to the patient via a pharmacy were also evaluated. We found that the internal temperature of the transport bags, which stayed closed throughout the entire transport period, remained below 0°C for up to 8 h at ambient temperatures of up to 32°C. Considering that the temperature of dissolved substances in serum decreases the freezing point due to lower vapour pressure osmolality, we expected the freezing point of serum to be <0°C.[24] Using the physical equation for calculating osmolality and freezing point depression based on the statement that an aqueous solution with an osmolality of 1 Osm/kg H_2O melts at −1.858°C, we estimated the freezing point to be −0.56°C for human serum with an osmolality of 275–300 mOsm/kg.[25] Therefore, SEDs shipped in the investigated transport bags must be delivered within 4 h to avoid thawing of the product. Four h are considered sufficient to cover the transport to the patient via a pharmacy in the circumference of 2–3 h concerning also transport delays.

In conclusion, the validation of our 100% SEDs, both allogenic and autologous, has been successfully completed. Due to the often problematic nature of their health conditions, it is important, as an alternative, to provide these patients with safe and effective allogenic SEDs. In future studies, diluted SEDs should also be tested for a number of reasons. Dilution may, for example, increase the yield of SEDs and provide cost savings. In addition, there is reason to believe that high concentrations of TGF-ß1 may interfere with dry eye healing.[11] Therefore, an important goal will be to identify the concentration that optimally balances the positive and negative effects of dilution on the ocular healing process.

AUTHOR CONTRIBUTIONS

Project administration was the responsibility of R.Burkhardt and R. Offner. The conceptualization was done by R. Offner, V. Hähneland F. Dormann. Investigation and data collection was performed by V. Müller, F. Dormann and V. Hähnel. F. Dormann and V. Hähnel analyzed the data and wrote thepaper. All authors reviewed and edited the paper.

ACKNOWLEDGEMENTS

We would like to thank the apheresis operators, physicians, and laboratory assistants who collected the blood samples, prepared the SEDs and assisted in data collection.

FUNDING INFORMATION

The authors did not receive any third-party funding for this research.

CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

PATIENT CONSENT STATEMENT

Written informed consent was obtained from all patients and donors.

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REFERENCES

1. Ogawa Y, Okamoto S, Mori T, et al. Autologous serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. *Bone Marrow Transplant*. 2003;31(7):579-583. doi:10.1038/sj.bmt.1703862
2. Tsubota K, Goto E, Fujita H, et al. Treatment of dry eye by autologous serum application in Sjögren's syndrome. *Br J Ophthalmol*. 1999;83(4):390-395. doi:10.1136/bjo.83.4.390
3. Deutsche Ophthalmologische Gesellschaft. Leitlinie Nr. 11, Trockenes Auge. https://augeninfo.de/leit/leit11.pdf
4. Dietrich-Ntoukas T, Steven P. Okuläre Graft-versus-Host-Disease. *Ophthalmologe*. 2015;112(12):1027-1038; quiz 1039-40. doi:10.1007/s00347-015-0149-9
5. Fox RI, Chan R, Michelson JB, Belmont JB, Michelson PE. Beneficial effect of artificial tears made with autologous serum in patients with keratoconjunctivitis sicca. *Arthritis Rheum*. 1984;27(4):459-461. doi:10.1002/art.1780270415
6. Weischnur L, Xanthopoulou K, Munteanu C, Leonhard M, Daas L, Seitz B. Autologe Serumaugentropfen bei therapieresistenten Epitheldefekten der Kornea: Einfluss der Grunderkrankung und einer simultanen Amnionmembrantransplantation bei 990 Anwendungen. *Fortschr Ophthalmol*. 2023;120(1):43-51. doi:10.1007/s00347-022-01677-5
7. Tahmaz V, Gehlsen U, Sauerbier L, et al. Treatment of severe chronic ocular graft-versus-host disease using 100% autologous serum eye drops from a sealed manufacturing system: a retrospective cohort study. *Br J Ophthalmol*. 2017;101(3):322-326. doi:10.1136/bjophthalmol-2015-307666
8. Noble BA, Loh RSK, Maclennan S, et al. Comparison of autologous serum eye drops with conventional therapy in a randomised controlled crossover trial for ocular surface disease. *Br J Ophthalmol*. 2004;88(5):647-652. doi:10.1136/bjo.2003.026211
9. de Pascale MR, Lanza M, Sommese L, Napoli C. Human serum eye drops in eye alterations: an insight and a critical analysis. *J Ophthalmol*. 2015;2015:396410. doi:10.1155/2015/396410
10. Geerling G, Hartwig D. Autologe Serum-Augentropfen zur Therapie der Augenoberfläche Eine Ubersicht zur Wirksamkeit und Empfehlungen zur Anwendung. *Ophthalmologe*. 2002;99(12):949-959. doi:10.1007/s00347-002-0661-6
11. Partal A, Scott E. Low-cost protocol for the production of autologous serum eye drops by blood collection and processing centres for the treatment of ocular surface diseases. *Transfus Med*. 2011;21(4):271-277. doi:10.1111/j.1365-3148.2011.01072.x
12. Wittmann S. Herstellung von Augentropfen Praxistipps für die Apothekenrezeptur. *Deutsche Apotheker Zeitung*. 2015;44:86. https://www.deutsche-apotheker-zeitung.de/daz-az/2015/daz-44-2015/herstellung-von-augentropfen
13. Europäisches Arzneibuch: 9. Ausgabe: Deutscher Apotheker Verlag.
14. Chiang C-C, Lin J-M, Chen W-L, Tsai Y-Y. Allogeneic serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. *Cornea*. 2007;26(7):861-863. doi:10.1097/ICO.0b013e3180645cd7
15. Roberts LLR. 2 impact of COVID-19 on a national serum eyedrops programme in the UK. *BMJ Open Ophthalmol*. 2022;7(Suppl 2):A. doi:10.1136/bmjophth-2022-EEBA.2
16. van der Meer PF, Seghatchian J, Korte D d. Autologous and allogeneic serum eye drops. The Dutch perspective. *Transfus Apher Sci*. 2015;53(1):99-100. doi:10.1016/j.transci.2015.05.017
17. Dietrich T, Weisbach V, Seitz B, et al. Herstellung von Eigenserumaugentropfen zur ambulanten Therapie: Kooperation von Augenklinik und transfusionsmedizinischer Abteilung. *Ophthalmologe*. 2008;105(11):1036-1042. doi:10.1007/s00347-008-1692-4
18. Offner R. Uncertainty: are serum eye-drops blood Productes? Patient care suffers from regulatory stumbling blocks, deficiency of transparency and acceptance. *Transfus Med Hemother*. 2022;49(Suppl. 1):55. doi:10.1159/000525893
19. Geerling G, Maclennan S, Hartwig D. Autologous serum eye drops for ocular surface disorders. *Br J Ophthalmol*. 2004;88(11):1467-1474. doi:10.1136/bjo.2004.044347
20. *Reallexikon der Medizin und ihrer Grenzgebiete*. Urban & Schwarzenberg; 1977.
21. Bundesärztekammer. Richtlinie zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Richtlinie Hämotherapie). Fortschreibung 2019 und 2021. Updated 2017.

22. Anitua E, Muruzabal F, Tayebba A, et al. Autologous serum and plasma rich in growth factors in ophthalmology: preclinical and clinical studies. *Acta Ophthalmol*. 2015;93(8):e605-e614. doi:10.1111/aos.12710
23. Harritshøj LH, Nielsen C, Ullum H, Hansen MB, Julian HO. Ready-made allogeneic ABO-specific serum eye drops: production from regular male blood donors, clinical routine, safety and efficacy. *Acta Ophthalmol*. 2014;92(8):783-786. doi:10.1111/aos.12386
24. Ebel S, Roth HJ, eds. *Lexikon der Pharmazie*. Thieme; 1987.
25. Hooper L, Abdelhamid A, Ali A, et al. Diagnostic accuracy of calculated serum osmolarity to predict dehydration in older people: adding value to pathology laboratory reports. *BMJ Open*. 2015;5(10):e008846. doi:10.1136/bmjopen-2015-008846

How to cite this article: Dormann F, Hähnel V, Müller V, Burkhardt R, Offner R. Allogenic and autologous nondiluted serum eye drops—validation strategy compliant with good manufacturing practice. *Transfusion Medicine*. 2024;34(4):319-325. doi:10.1111/tme.13068