Autologous Serum Eye Drops – Implementation and First Experiences at Blood Transfusion Service Zurich, Swiss Red Cross

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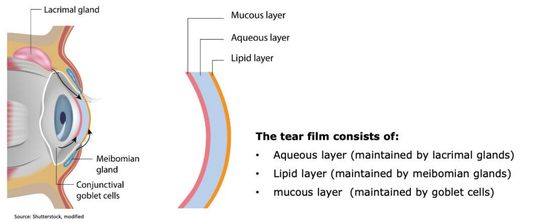
BLUTSPENDE SRK ZÜRICH

Introduction:

Autologous serum eye drops (ASED) are used in severe cases of dry eyes (Figure 1, Figure 2) and persistent corneal defects (Figure 3). ASED consist of serum prepared from patient's own blood and contain substances promoting wound healing like epidermal growth factor. About 460 mL of whole blood (WB) (Figure 4) are needed to produce 120 vials containing approx. 1.3 mL serum each (Figure 5). Here we describe the introduction of ASED manufactured with a closed system at our centre in 2020 and first experiences made in routine.

Figure 1: Schematic Representation of the Dry Eye Syndrome



Source: Shutterstock, modified

The tear film consists of:

- Aqueous layer (maintained by lacrimal glands)
- Lipid layer (maintained by meibomian glands)
- mucous layer (maintained by goblet cells)

Functions of the layers:

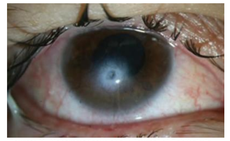
The aqueous layer nourish the corneal epithelium and is antimicrobiotic.
The lipid layer reduces evaporation and therefore stabilises the tear film.
The mucous layer links the aqueous layer to the eye surface.

Figure 2: Dry Eye Syndrome

Conjunctivitis. (2007, Sep. 11). Source: https://de.wikipedia.org/wiki/Konjunktivitis

e.g. due to Sjogren's Syndrome.

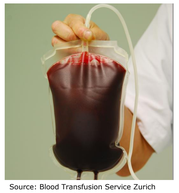
Figure 3: Corneal Ulcer



Source: M. Mohammadpour et al.; Journal of Ophthalmic and Vision Research 2016; Vol. 11, No. 1

Graft versus Host Disease.

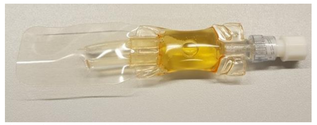
Figure 4: Whole Blood



Source: Blood Transfusion Service Zurich

Approx. 460 mL WB

Figure 5: Vial



Source: Blood Transfusion Service Zurich

One application unit ASED, containing approx. 1.3 mL Serum.

Methods:

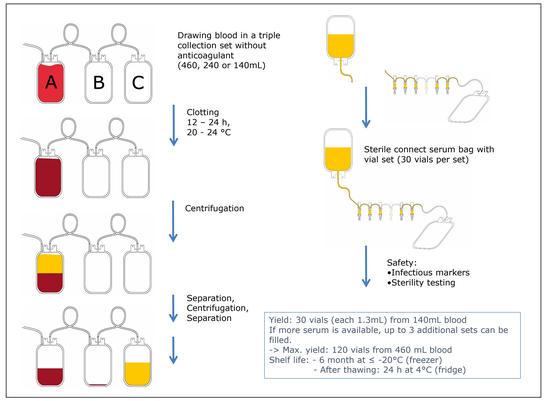
Validation lots were produced from 4x460, 4x240, and 4x140 mL WB (Table 1). 240 mL (60 vials) and 140 mL (30 vials) are low volume options. Half of donations collected in bag A of triple dry set MRV502B (Macopharma) were stored <12h and half >24h at RT to allow clotting before centrifugation (15min/4068g). Serum was separated into bag B, centrifuged, and transferred into bag C. Bag C was sterile connected to set TF30 (Meise) for final aliquotation, and stored at ≤ -20°C. (Figure 6). Release criteria were sterility acc. to Ph. Eur., appearance, and negative tests for HIV, HBV, HCV, HEV, Syphilis. Residual cells were measured for information only. In routine, donation criteria are less strict than for standard blood donors since products are autologous. ASED are delivered via physicians.

Table 1: Organisation of Validation Runs

12 donations:	6 donations:	Target range clotting: 12–24 h	6 donations:
4 x 460 mL	2 x 460 mL		2 x 460 mL
4 x 240 mL	2 x 240 mL		2 x 240 mL
4 x 140 mL	2 x 140 mL		2 x 140 mL
Donation	Incubated for 9 – 12 h at RT (clotting)		Incubated for 24 – 27 h at RT (clotting)

Half of donations were stored <12h and half >24h at RT to allow clotting before centrifugation.

Figure 6: Manufacturing of ASED from Whole Blood



Produced with triple dry set MRV502B (Macopharma). For final aliquotation sterile connected to set TF30 (Meise).

Results:

Sterility testing for serum was successfully validated on the BACT/ALERT system (bioMérieux) acc. to Ph Eur (aerob bottle) (Figure 7). All validation lots passed release criteria (Table 2). However, 1 failed the initial sterility test, but retesting strongly suggested sample contamination during handling. Hence, to reduce contamination risk, a sterile docking device was developed to link sampling bag and syringe (Figure 8). Residual cells ranged 0.2–50.7 leukocytes/µL, 0.2E6–1.8E6 erythrocytes/mL, 0.9E9–9.0E9 platelets/L (Figure 9).

In routine, 11 lots were produced from 7 patients (25–89y). 2 patients had to be deferred and 3 qualified for 240mL only. All lots passed release criteria and residual cells didn't exceed validation results (Figure 9). The transport box kept temp. <-10°C for >10h.

Figure 7: Validation Sterility Testing (aerobic) acc. to Ph. Eur.

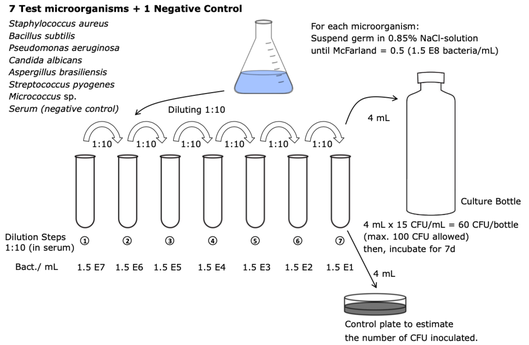


Table 2: Validation Results

Yield: passed

Results:
- 460 mL WB -> yield ≥120 units
- 240 mL WB -> yield ≥ 60 units
- 140 mL WB -> yield ≥ 30 units

Appearance: passed

Results:
- Clear
- No smears
- Not cloudy

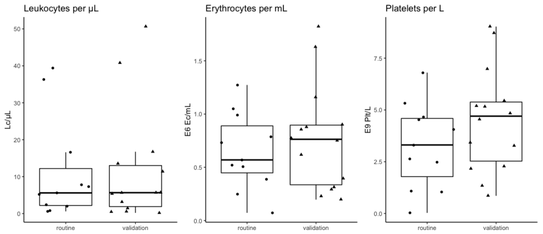
Residual Cells: for information only

Results:
Lc ranged 0.2 – 50.7 Lc/µL
Ec ranged 0.2 E6 – 1.8 E6 Ec/mL
Tc ranged 0.9 E9 – 9.0 E9 Tc/L

Sterility: passed

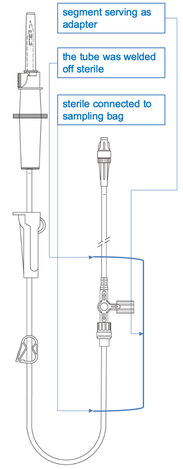
Results:
- 11/12 passed without complications
- 1 showed microbiological growth but 2 re-tests were negative
  (i.e. contamination happened most likely at the testing lab during handling of culture bottle)

Figure 9: Residual Cells



Residual cells during validation and routine.

Figure 8: Sterile Docking Device



Docking Device. Image (modified) with courtesy of Arcomed AG.

Source: Blood Transfusion Service Zurich

Sterile docking device developed to link sampling bag to syringe.

Conclusion:

Validation and implementation of ASED was successful and positive feedback was given by physicians. Unfortunately, not all patients qualified for ASED. Clotting conditions chosen (12–24h, RT) avoided night shifts and post manufacturing coagulation even if anticoagulant medication was taken. The BACT/ALERT system can be used for sterility testing of ASED if validated appropriately and the use of an in-house developed docking device facilitates sample handling and reduces contamination risk.

Acknowledgement: We would like to thank Prof. Dr. Hitzler, Dr. Conradi, and all other team members of the Transfusion Center at the University Medical Center of the Johannes Gutenberg University Mainz for the introduction into manufacturing autologous serum eyedrops and the sharing of their experiences.