before the beginning of the experiments (pre-wound) was assessed using the vital dyes trypan blue (TB, TB-S 0.25%, AL.CHI.MI.A. srl) and sodium fluorescein (Fluo). 1-heptanol soaked paper disks (6 mm) were applied in the centre of the corneas for 1' to trigger a chemical damage at the epithelial layer. Afterwards, sodium fluorescein and TB stainings were repeated to quantify the damaged area and to monitor healing progression. The damaged area (mm2) was calculated for each time point with Fiji software. Wound healing rate (HR, mm2/die) was calculated for both Fluo (HRF) and TB (HRTB) measurements using the previously described formula:

Arithmetical averages (HRFAVG and HRTBAVG) of HRs were calculated and correlated by Pearson correlation coefficient with the following donor's parameters: age, sex, post-mortem time (PMT, time between death and tissue procurement), stromal defects, septicaemia, body temperature, diabetes.

**Results** The execution of the heptanol wounding is highly reproducible, as highlighted by Fluo and TB staining. The average time for full recovery from wounding was 3,8 ± 0,41 days for Fluo and 3,5 ± 0,63 days for TB. Fluo and TB stainings are interchangeable as they significantly correlate (Pearson correlation coefficient = 0.630; $p>0.05$). A negative linear correlation was observed between HR and PMT (HRFAVG: corrected R2: 0.243, $p = 0.003$; HRTBAVG: corrected R2: 0,132, $p = 0.028$), but not with the other donors' parameters.

**Conclusion** Our wound/healing model might be of great interest for studies of epithelial regeneration kinetics and validation of drugs for the treatment of ocular defects. The inverse correlation between PMT and HR provides valuable insights for scientists investigating the regenerative properties of the corneal epithelium, as well as for eye bank personnel aiming to preserve the regenerative potential of corneal epithelium.

## P21-A141 QUANTIFICATION OF BIOACTIVE FACTORS IN HUMAN SERUM EYEDROPS

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**Purpose** NHS Blood and Transplant supply serum eye drops (SED) for the treatment of severe dry eye syndrome, however, understanding of what components of SED contribute to their activity is limited. SEDs are produced from a patient's own blood or from an allogeneic donor source. The serum component is separated from the whole blood which is then diluted 50/50 with sterile saline, and contains bioactive molecules that are believed to help heal and maintain the ocular surface. The objective of this study is to quantify the amount of bioactive molecules in donor serum, and to understand how processing variables effects these factors.

**Methods** Samples of SEDs from 28 male allogenic donors were taken from ultra-low temperature storage and thawed. They were then centrifuged at 13,000 rpm at 4oC to remove potential contaminants such as residual red blood cells. Duplicate test samples were analysed for epidermal growth factor (EGF) and fibroblast growth factor (FGF) using ELISA kits. Analysis was carried out using Excel.

**Results** The age range of the donors was 17 to 79 years (mean 47.9).

Mean time from venepuncture to refrigerated storage was 6 hours 12 minutes with time ranging from 2 hours 40 minutes to 9 hours 35 minutes.

The concentration of EGF found in the diluted serum ranged from 0.048 to 1.90 ng/ml (mean 0.87 ng/ml), and FGF concentration ranged from 4.88 to 39.50 pg/ml (mean 12.37 pg/ml).

Analysis showed that there was no correlation between either age of the donor, or sample transfer time and growth factor concentration.

**Conclusion** Our study demonstrated that with both types of growth factors measured in the SED, a wide range of concentrations were found in the donor samples. Compared to published data EGF was at higher range while FGF was lower. Further analysis of other factors present in the donor serum is being undertaken to determine if any pattern can be found.

## P22-A142 VALIDATION OF AN IMPROVED CLOSED SYSTEM FOR DISPENSING SERUM EYE DROPS

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**Purpose** NHS Blood and Transplant Tissue and Eye Services provide a serum eye drop (SED) service to patients suffering from severe dry eye syndrome. Currently SED are dispensed using an automatic closed filling system (TF) manufactured by Meise Medizintechnik (Germany). An improved version (ATS) has recently been introduced by Meise, based on patient feedback on the TF system. ATS vials are easier to open, with a more secure, tamper evident closure and a better quality nozzle.

To evaluate the suitability of ATS vials, a validation protocol, previously developed for TF vials, was repeated. It comprised assessment of their integrity following simulated storage and transport, and the stability and sterility of SED stored in them.

**Method** Firstly, a process simulation assessment was performed using bovine serum. Vials were filled, and frozen to -80oC. They were then removed from frozen storage and checked for damage, before being put into transport containers and shipped on a round-trip journey to simulate delivery to patients. On return the vials were thawed and the integrity of each vial checked visually and by application of a standard force.

Subsequently a shelf-life study was carried out using three batches of human SED. The vials were initially frozen to -80oC, then stored for set time points of 1, 3, 6 and 12 months in a standard domestic freezer set at 20oC (to mimic a home freezer). At each time point, 10 vials were thawed and examined for integrity, and the sterility and stability of the contents. Stability was assessed by measuring serum albumin concentrations and sterility by testing for presence of microbial contamination, under aerobic and anaerobic conditions.

**Results** No vial damage or leakage was found at any time point in the ATS vials. No microbial contamination was