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# Autologous serum eye drops preparation : approach to the filtration step impact on the concentration of active molecule

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BACKGROUND

Autologous serum eye drops (ASEDs) are pharmaceutical preparations used in severe dry eye diseases. Sterility is a specification for eye drops, which can be obtained by filtration. Any molecule with a mean diameter greater than the filter porosity is then generally retained. EGF (Epidermal Growth Factor) is one of these active molecules (AMs) in ASEds. With an intermediate molecular mass (MM) (180 kDa), its investigation make possible to predict the impact of filtration on the concentration of other active molecules.

**OBJECTIVES** : To evaluate the impact of this sterilisation method on AMs by measuring EGF concentrations before/after filtration of collected sera.

MATERIAL - METHODS

**Four 4mL tubes of human serum** (numbered from 1 to 4 : P1-P4) were used, all from the hospital biological collection.

Each serum underwent the following 3 successive operations :

- **Zero filtration**
- **Clarifying filtration** (CF, at **0.45 µm** porosity)
- **Sterilising filtration** (SF, at **0.20 µm** porosity)



The assay was performed in duplicate using an ELISA technique (Quantikine® Human EGF Immunoassay kit, R&D System, USA). The impact of filtration is considered significant if the relative difference in concentrations after the process exceeds 7.5%.

**The EGF concentration (pg/mL)** in each unfiltered serum represents the **maximum concentration (100%)**, allowing the impact of filtrations to be expressed as relative percentages of this maximum.

RESULTS

	Zero filtration	Clarifying filtration	Reduction	Sterilising filtration	Reduction
P1	100%	96.2%	3.8%	94.8%	5.2%
P2	100%	97.2%	2.8%	93.4%	6.6%
P3	100%	92.8%	7.2%	91.1%	8.9%
P4	100%	97.1%	2.9%	95.9%	4.1%

CONCLUSION - RELEVANCE

As expected, EGF concentrations decrease after filtration, especially when the porosity of the filter used is low. Moreover, the significance threshold is reached for P3 under SF.

We may suppose that smaller AMs (ie IGF-1, MM 7.6 kDa; TGF-β1, MM 25 kDa) will be less retained. For larger AMs such as fibronectin (MM around 450kDa), the decrease in concentration is likely to have an impact on the ASEds efficacy, justifying a more specific study. Other methods of ensuring the microbiological safety of ASEds should probably also be considered.