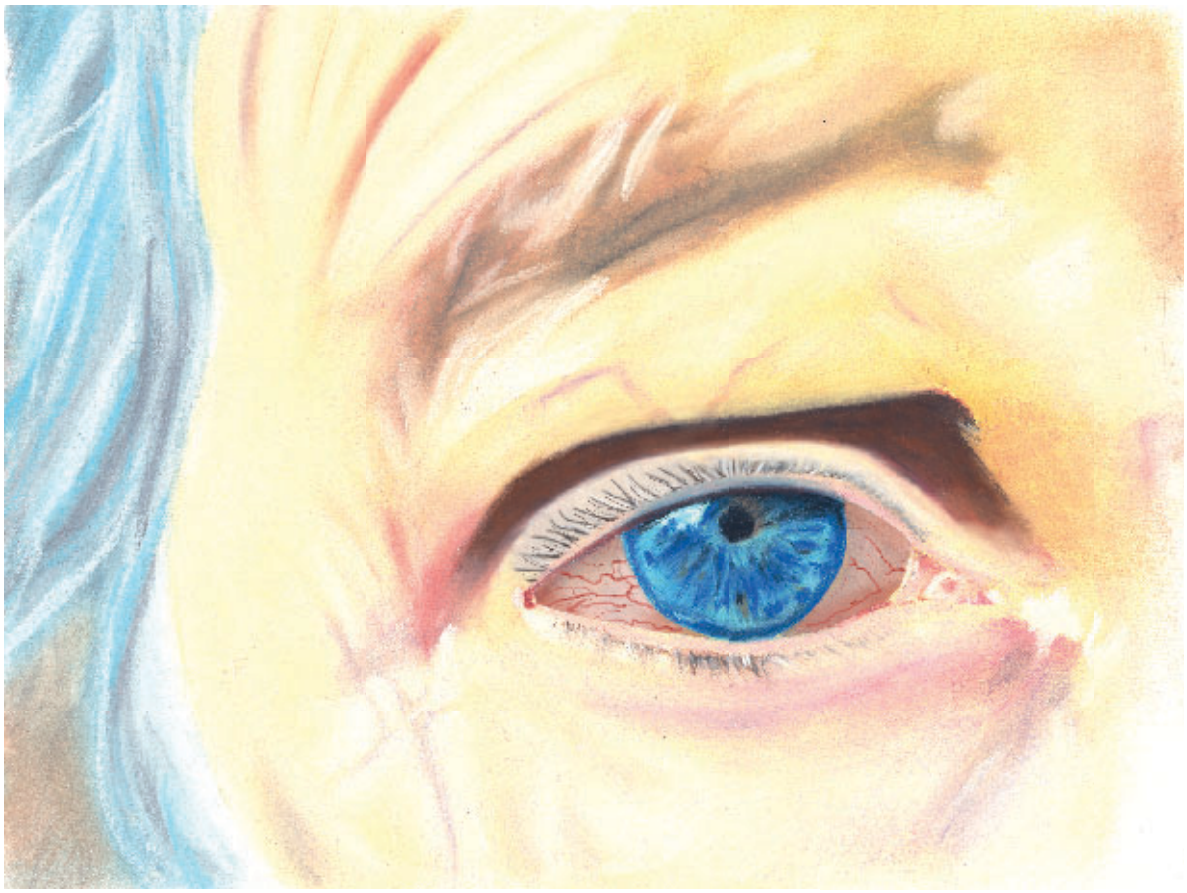

Applications and Sterility of Autologous Serum Eye Drops

Abstract

Severe dry eye syndrome can adversely affect a patient's quality of life. When preservative-free artificial tear solutions are not adequate to reduce symptoms, the patient's own serum can be compounded into eye drops that improve the ocular surface. The aim of our study was to test the sterility of autologous serum eye drops in refrigerator conditions for up to 30 days and in freezer conditions for up to 180 days. It was determined that sterility was maintained throughout this period.

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Tears, which serve the four major purposes listed below, are essential to optimal eye health:[1]

1. Smooths the cornea
2. Wets and protects the epithelium
3. Inhibits growth of microorganisms
4. Provides nutrition to the cornea

Normal tears consist of albumin, globulin, lysozymes, immunoglobulins, and other factors which are important to ocular health.[1] When the body does not produce enough tears, or tears do not have the proper components, dry eye is the result. Dry eye can produce the sensation of a foreign body in the eye, itching, photophobia, and other unpleasant symptoms. Dry eye syndrome, known as keratoconjunctivitis sicca (KCS), has many causes, including autoimmune disease, injury, and use of medications like antihistamines and beta-blockers. At first, discomfort and a slight loss of visual acuity are the main concerns of KCS. Over time, continued dryness of the ocular surface can create more severe problems, including damage to the cornea, infection, and possible loss of vision.[1]

Current therapies for dry eye include preservative-free artificial tear solutions (containing saline solution or viscous molecules like hyaluronic acid and methylcellulose) or punctal plugs.[1,2] Cyclosporine emulsion (brand name Restasis) may be an option for some patients.[3] While these therapies offer relief of symptoms, artificial tears must be reapplied frequently, which can be inconvenient. Additionally, artificial tears do not contain components found in natural tears that work to improve the ocular surface, such as epidermal growth factor (EGF), transforming growth factor-beta (TGF-β), and Vitamin A.[4-6] Human serum contains these components, but at higher levels. It can be diluted with normal saline to mimic normal tears and provide relief to patients suffering from severe dry eye syndromes.[2,7] No data is available on the optimal concentration of the components of autologous serum eye drops, but it is known that TGF-β has antiproliferative effects. Since the level of TGF-β in serum are generally about 5 times that in tears, dilution to 20% serum is often used.[2]

Autologous serum eye drops have been studied since 1984 as a therapy for dry eye caused by Sjogren's syndrome, persistent epithelial defect, chronic graft-versus-host disease, and several other conditions.[2,4,5,7,8] They have been shown to improve the ocular surface (as measured by rose-bengal and fluorescein staining scores) and improve patient comfort (as measured on visual pain scale) compared to preservative-free artificial tear solutions.[7]

Autologous serum eye drops must be compounded for each patient. Actual procedures vary by country and institution, but follow a similar basic process. First, the patient must be tested for bloodborne pathogens (including HIV, syphilis, and hepatitis B and C). For patients testing positive for bloodborne pathogens, autologous serum eye drops are not a treatment option. Iron levels and measures of cardiovascular and general health are also assessed. If the patient is disease free and not in danger of becoming anemic, a predetermined amount of blood is drawn and centrifuged. The serum is then used to compound the eye drops.[6,9] The U.S. currently does not have a U.S. Food and Drug Administration (FDA)-approved protocol in place for this process.

In previous studies, vitamin A, EGF, and TGF-β in 20% serum tears were found to be stable for 1 month at 4ºC (refrigeration conditions) and 3 months at -20ºC (freezer conditions).[4,5] A 2004 study recommended storing an opened bottle of 50% serum tears for 16 hours at 4ºC and for 3 months at –20ºC to ensure stability.[6]

Materials and Methods

Clinical Testing

Serum samples need to be tested for hemolysis before they can be used to make patients' eye drops. If the patient's vein collapses as the

sample is being drawn, contamination of the sample with the components of broken cells, known as hemolysis, can occur. Instruments such as the Beckman Coulter Synchron LX-20 (Beckman Coulter Inc., Brea, California) can detect the serum condition by measuring the absorbance of the sample using wavelengths and conducting calculations to determine the index level of interfering compounds.[10] The degree of hemolysis (H), icterus (I), and lipemia (L) are given as these are the compounds that cause interference in serum samples, hence the name HIL test. The higher the index, the more interference in the sample, which can be visible to the naked eye at really high levels, seen as red tinted or cloudy.

Compounding Methods

Compounding of autologous serum eye drops was done at Reed's Compounding Pharmacy in Tucson, Arizona. Blood was drawn from the subject at a local hospital using serum-separator tubes with either red/gray ("tiger-top") or gold-colored tops and centrifuged for 20 minutes. Then, the serum was tested for hemolysis (H-index value from HIL test must be less than or equal to "1") and delivered on ice via pharmacy courier to the pharmacy. It's important to use the proper tubes because they contain an inert clotting factor and a gel-separator that divides the clotted blood cells from the serum after centrifugation, which is necessary to ensure that the serum used for compounding does not contain any blood cells. Eye drops were compounded within two days of receipt. Upon arrival, labeled serum was visually inspected to ensure that it belonged to the correct patient, and no hemolysis of red blood cells had occurred. Each label was photocopied, and the formula worksheet was affixed. Compounding was done using aseptic technique in a Class 5 horizontal airflow hood (Labconco Purifier Series; Kansas City, Missouri) within a *United States Pharmacopeia (USP)* Chapter <797> compliant cleanroom. Prior to compounding, all equipment and supplies were wiped down with nonshedding towels wetted with 2% acidified bleach. Once all materials were inside the hood and critical sites sterilized, serum was withdrawn from collecting tubes into a 60-mL sterile syringe (Becton, Dickinson and Company [BD], Franklin Lakes, New Jersey). The volume of solutions required for preparing 100 mL of 20% ophthalmic serum are provided below.

Volume of Solutions Required for 100-mL 20% Ophthalmic Serum Preparation

Ingredients	Volume (mL)
Autologous serum	20
0.9% Sodium chloride for injection[a]	80
Total ophthalmic serum volume[b]	100

[a]0.9% Sodium chloride for injection volume = total ophthalmic serum volume – autologous serum volume
[b]Total ophthalmic serum volume – autologous serum volume ÷ prescription strength (%)

The amount of 0.9% sodium chloride needed for the preparation of ophthalmic serum was drawn out of a Sodium chloride for injection intravenous (IV) bag (Baxter, Deerfield, Illinois) with a sterile syringe of appropriate size. Excess sodium chloride solution was emptied, and the IV bag was refilled with the required amount of sodium chloride for injection, and then the serum volume. The content in the IV bag was mixed well, and an IV administration set was attached to the bag through the IV port. A sterile 0.2 mcm low-protein binding filter (2.7 mL; Pall Corporation, Ann Arbor, Michigan) was attached to the IV tubing, and a fluid-dispensing connector was attached to the end of the filter. The roller clamp of the IV administration set was opened to let fluid flow from the bag through the tubing and filter assembly. A 3-mL slip-tip syringe (BD) used in packaging the finished preparation was attached to the end of the fluid-dispensing connector and was filled to the desired volume. The syringe containing the ophthalmic serum was removed from the dispensing assembly and capped with a self-righting luer slip cap. Upon completion of the dispensing, about 1 to 2 mL of the ophthalmic serum was injected directly into a microbial contamination tester bottle (Catalog No. GM8000, TuffTEST; Q.I. Medical, Inc., Nevada City, California) for contamination check.

Testing Methods

The compounded formulation[11] of serum tears 20% ophthalmic (Lot 11202008:71@22) was submitted to Analytical Research Laboratories, Inc. by Reed's Compounding Pharmacy for sterility testing. The samples included 10 syringes and were shipped frozen by overnight delivery. Each sample was assigned a unique number for tracking purpose, and then six syringe (Part No. 13-986-272GR; Fisher Scientific, Pittsburgh Pennsylvania) samples were stored refrigerated at 2ºC to 8ºC, while another four samples were kept frozen in a -10°C freezer (Kenmore Serial No. 363-9634716; Sears, Roebuck and Co., Oklahoma City, Oklahoma). Sterility and endotoxin tests were validated according to guidelines in *USP* chapters <71> and <85>, respectively.[12] Initial evaluation of sterility and endotoxin levels was performed using a previously thawed syringe sample labeled for time 0 (T0), then sterility was evaluated for refrigerated samples at 4, 7, 14, and 30 days from date of compounding. Frozen samples were thawed and then tested for sterility at 90, 120, and 180 days; endotoxin level was determined for the sample at the end of 180-day storage period.

Results

The serum tears 20% ophthalmic samples were found to remain sterile up to 31 days in the refrigerator and 181 days frozen at -10°C. Endotoxin level was stable and was determined to be in the range from 1 to 1.43 EU/mL through the study period.

Discussion

Earlier observations regarding the sterility of autologous serum eye drops in clinical cases were confirmed in a pharmaceutical compounding situation. When the ophthalmic serum was compounded using aseptic technique, retention of sterility was demonstrated for samples in frozen state after 6 months storage at -10°C. This is encouraging news for patients for whom autologous serum eye drops may be an appropriate therapeutic option. Previous studies demonstrated stability and sterility for periods of 3 months. If the preparation remains stable and sterile for 6 months, this could reduce the time burden of the blood donation process. For donors who are able to donate a sufficient quantity of blood, perhaps a 6-month supply of eye drops could be compounded and dispensed with confidence throughout the time frame. In a previous study, increased endotoxin level in transplanted corneas was not associated with a higher risk of adverse outcome.[13] More research is needed to determine the significance of this parameter for diluted autologous serum in ophthalmic preparations.

Proper aseptic technique is essential to producing a quality preparation and maximizing patient care. It is understood that validated sterility

testing of the preparation is to be performed if beyond-use dating exceeds the duration allowed for sterile compounded preparations.[14] For a preparation such as this, sterility is especially important because preservatives cannot be used due to their irritant effects on the eye, especially with prolonged usage.[1] Demonstration of the sterility of this preparation has the potential to encourage greater acceptance and use. In order to assure sterility and stability, analytical testing should be preformed on a sample of preparation compounded by the pharmacy. Once sterility can be assured, compounded autologous serum eye drops provide a novel treatment approach that greatly benefits qualifying patients with dry eye syndrome.

At this time, the eye drops carry a beyond-use date of 7 days if kept refrigerated. Sterility has been confirmed for 31 days in refrigerated storage, but the possibility of contamination of drops during patient use was not addressed in this study. Therefore, it seems prudent to use caution when determining a beyond-use date.

Conclusion

Autologous serum eye drops are useful for dry eye treatment. If compounded using aseptic technique, they may remain sterile through 180 days. Guidelines for use currently recommend keeping the preparation frozen until ready to use. Once thawed, drops should be refrigerated and discarded if not used within 7 days. Our data suggest that the preparation may have a longer shelf life than previously thought, allowing greater access to this therapy and better flexibility in practice.

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