The authors have developed an improved technique for storage of fluid microvolumes prior to osmolality measurement. To date, tear samples have been stored in a column of Cargilles B immersion oil contained within a capillary tube, sealed at one end with Critoseal. Covering the open end of this tube with Parafilm now provides reliable storage of 0.5-μl samples for up to 24 hr; using water-saturated Cargilles B immersion oil within the capillary tube, and covering the open end of the tube with Parafilm now provides reliable storage of 0.1-μl samples for up to 18 hr. Invest Ophthalmol Vis Sci 28:401–403, 1987

Nearly ten years ago, we described a new technique for the collection and storage of microvolume tear samples. This technique permits the collection of 0.1- to 0.4-μl samples from the inferior marginal tear strip without contacting the eye and inducing reflex tearing. To retard evaporation, samples were stored in a column of Cargilles B immersion oil contained within a capillary tube.

We have continued to use this technique to collect and store tear samples for osmolality measurement. We now report an improved method of tear storage that further reduces sample evaporation. We have also defined with precision the length of time that samples can be stored reliably prior to measurement.

Materials and Methods. Using a 1-μl Hamilton syringe, fixed volumes (0.5 or 0.1 μl) of milk cryoscope 621 standard (323 mOs/m/kg) (Advanced Instruments, Inc.; Needham Heights, MA) were stored in capillary tubes that had been filled with a column of Cargilles B immersion oil (Cedar Grove, NJ). In the control groups, the storage tubes were prepared as previously described: One end of the tube was sealed with Critoseal (Monoject, St. Louis, MO) to prevent migration of the oil column. In the first experimental group, the open end of the tube was covered with Parafilm (PF) (American Can Company; Greenwich, CT) immediately after sample placement. In the second experimental group, 10 ml of Cargilles B immersion oil was mixed with an equal volume of double distilled water in a beaker that was immediately covered with PF. Two days later, when the oil and water reached equilibrium in two separate layers, and air bubbles cleared, the capillary tubes used for tear sample storage were filled with the water-saturated oil. The open end of each tube was covered with PF immediately after sample placement. In the third experimental group, the Cargilles B immersion oil was saturated with double distilled water as described above, but the beaker was not covered with PF while the water and oil were equilibrating. The water-saturated oil was used to fill the capillary tubes, and the open end of each tube was covered with PF immediately after sample placement.

For each storage technique, sample osmolality was compared with that of fresh standards, alternating the measurement of stored samples with fresh standards after osmometer calibration. In each experiment, ten stored samples were measured and compared with ten fresh standards (a total of 20 measurements). Osmolality was measured with the nanoliter osmometer and method previously described. Since each measurement took approximately 7.5 min, the 20 measurements in each experiment were performed starting at storage times of 3.5, 15.5, 21.5, and 45.5 hr, and ending at storage times of 6, 18, 24, and 48 hr, respectively.

At the beginning of the series of experiments, the stability of the commercially available standard was evaluated using a new 110-ml bottle. This standard was used for the experiments described here and for routine clinical measurements performed during this period. Osmolality of the standard was checked at 1 wk and 1 month against new, unopened bottles of the same standard.

Results. At 24 hr, the osmolality of 0.5-μl samples stored without PF had increased significantly from control (mean increase 2 ± 0.8 (SEM) mOsm/kg, P < 0.05). The 0.5-μl samples stored with PF for 24 hr did not change significantly from control (mean increase 0.1 ± 0.3 [SEM] mOsm/kg; see Fig. 1). Since the 0.5-μl samples were considerably larger than the tear samples we collected from patients, we switched to storing 0.1-μl samples. We found this smaller sample typical of the size of our stored clinical tear samples. The 0.1-μl samples could be stored with or without PF for as long as 6 hr without significant osmolality increase from control (mean increases 0.2 ± 0.3 [SEM] and 0.8 ± 0.6 [SEM] mOsm/kg, respectively; see Fig. 2). At 18 hr, 0.1-μl samples stored without PF increased significantly in osmolality from control (mean increase 3.7 ± 0.7 [SEM] mOsm/kg, P < 0.01). The 0.1 μl samples stored with PF for 18 hr did not change significantly from control (mean increase 0.8 ± 0.5 [SEM] mOsm/kg). The 0.1-μl samples stored with PF for 24 hr increased significantly from control (mean increase 3.3 ± 1.6 [SEM] mOsm/kg; P < 0.005; see Fig. 2).

In the second experimental group, where the Cargilles B immersion oil had been water-saturated and covered with PF prior to placement in capillary tubes, and the capillary tubes had been covered with PF after
sample storage, osmolality decreased 14 ± 7.5 (SEM) mOsm/kg relative to control after storage for 24 hr. Osmolality of stored samples extended over a broad range (249–323 mOsm/kg), with virtually all values below fresh standard.

In the third experimental group, where the Cargilles B immersion oil had been water-saturated but exposed to air prior to placement in the capillary tubes, and the capillary tubes had been covered with PF immediately after sample placement, sample osmolality at 18 hr did not change significantly from fresh standard, and the mean increase of 0.5 ± 0.5 (SEM) mOsm/kg was the smallest seen in this study at 18 hr for 0.1-μl samples. At 24 hr, the increase in sample osmolality was significant (mean increase 1.7 ± 0.5 [SEM] mOsm/kg, P < 0.01; see Fig. 2).

The osmolality of commercially available standard, stored in and used from the manufacturer’s 110-ml bottle, was stable at the 1-wk and 1-month time points.

**Discussion.** We have been able to improve the storage of microvolume fluid samples to the point where 0.1-μl samples can now be stored for up to 18 hr without significant change in osmolality. In the second experimental group, where the water-saturated Cargilles B immersion oil was covered with PF prior to placement in the capillary tubes, fluid osmolality in the samples tended to decrease with storage. We interpret this to be the result of water moving from the oil into the stored sample and/or osmotically active particles moving out of the sample drop and into the water contained within the oil. We avoided this problem by storing the water-saturated Cargilles B immersion oil without PF prior to placement in capillary tubes.

As shown in Figure 1, 0.5-μl samples can be stored...
for longer periods of time than 0.1-μl samples. Over the years, however, we gradually shifted toward using smaller samples that enable us to collect tears even from patients with the most severe cases of keratoconjunctivitis sicca. Furthermore, the small sample size decreases the time needed for sample collection, thus minimizing the risk of inducing reflex tearing. For storage times of up to 18 hr, the new storage technique described here eliminates previous disadvantages of the 0.1-μl sample size associated with sample evaporation.

Key words: tears, tear osmolarity, keratoconjunctivitis sicca, osmolality, microvolumes