Development and validation of a self-prepared phenol red thread test

Bryan E. Puentes[1], Erin A. Hisey[1], Michelle H. Ferneding[1], Vanessa N. Ureno[1], Maria A.H. Do[1], Sara M. Thomasy[1,2] Brian C. Leonard[1,2]

[1]Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California- Davis, Davis, CA, USA.
[2]Department of Ophthalmology & Vision Science, School of Medicine, University of California Davis, Davis, CA. USA.

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Background

Measurement of aqueous tear production is an incredibly important quantitative assessment in the diagnosis of dry eye disease (DED) in both animals and humans. The phenol red thread test (PRTT) is the preferred diagnostic tool to quantify aqueous tear production in small mammals, birds, and reptiles due to ease of placement under the eyelid margin and short testing duration (15 sec). Despite its benefits, commercially-available PRTTs have been discontinued, creating a void and a need for an alternative source. Therefore, we proposed to develop and validate a self-prepared PRTT to address the critical need for PRTT in clinical and research settings.

Hypothesis and Aims

Hypothesis: A self-prepared PRTT will measure aqueous tear production with high reproducibility and reliability, similar to the commercial PRTT.

Aim 1: To develop and validate a self-prepared PRTT in solutions of varying pH

Aim 2: To compare the self-prepared PRTT with the commercial PRTT in wildtype mice

Materials and Methods

Generating the Thread

- 100% white cotton thread (Connecting Threads®, item model #20869, dia: 0.25 mm, length: 110 m) dyed with 0.1% phenol red solution (Aldon Corp., 213000)- a liquid pH indicator- for 48 hours (**Fig 1A**)
- Dried for 8 hrs. at room temperature
- Cut the thread into 75 mm segments
- UV sterilized thread in sealed pouches for 1 hr.

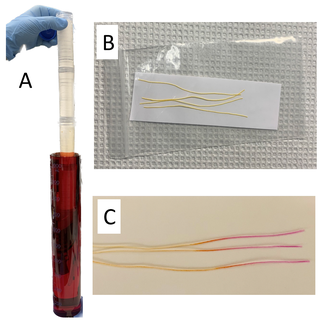

Figure 1:
A) Soaking of the thread in 0.1% phenol red solution.
B) Self-prepared PRT after drying, cutting and UV sterilization.
C) Post-PRTT color change of the self-prepared PRT

Aim 1- *In Vitro* Testing

- Inserted 3 mm tip of PRT into solutions of varying pH (range: 7.2-8.0) for 5 to 40 s
- Length of color change was measured using digital calipers
- 10 trials at each time interval for a total of 80 tests per pH

Aim 2- *In Vivo* Testing

- Performed PRTTs on 10 mice (5 females, 5 males) at 8 weeks of age in both eyes 3x/week using the commercial PRT (Zone-Quick) for one week, then using our own PRT (same lot as Aim 1) the subsequent week
- Mice were physically restrained without sedation
- Lower eyelid of each mouse was gently everted and the tip (1mm) of the PRTT was placed into the lateral ventral conjunctival fornix with forceps and allowed to wick for 15s.

Statistics

- A one phase association regression and mixed effects analysis of variance was performed on the *in vitro* data. Outliers were excluded if greater than the mean +/- 2 SD.
- ANOVA with a post-hoc Tukey multiple comparison and Bland-Altman analyses were performed on the *in-vivo* data.

Results

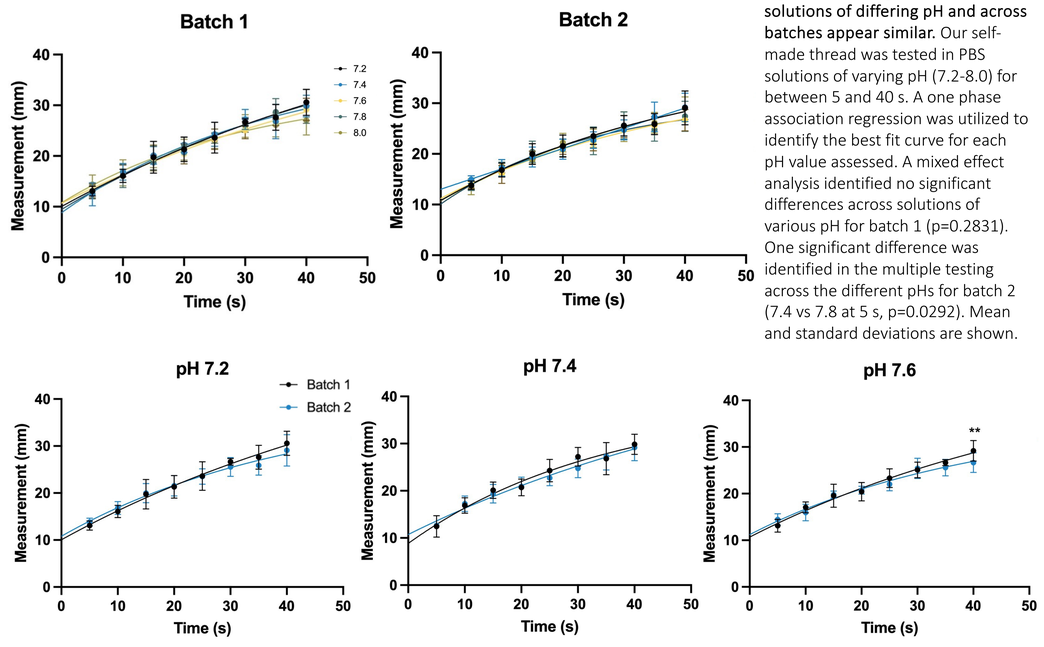

Figure 2: Wicking kinetics across solutions of differing pH and across batches appear similar. Our self-made thread was tested in PBS solutions of varying pH (7.2-8.0) for between 5 and 40 s. A one phase association regression was utilized to identify the best fit curve for each pH value assessed. A mixed effect analysis identified no significant differences across solutions of various pH for batch 1 (p=0.2831). One significant difference was identified in the multiple testing across the different pHs for batch 2 (7.4 vs 7.8 at 5 s, p=0.0292). Mean and standard deviations are shown.

Figure 3: There were negligible differences in the wicking kinetics of the two batches of our self-prepared PRT. The same one phase association regression was utilized to visually compare the wicking kinetics of the first and second batches at each pH. A mixed effects analysis was used to compare the measurements at each timepoint, which identified two significant differences between the two batches (pH of 7.6 at 40 s and pH of 7.8 at 35 s). Mean and standard deviations are shown.

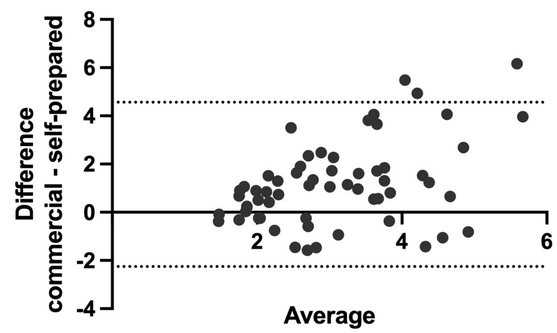

Figure 5: Commercial PRTT tended to have higher measurements compared with the self-prepared PRTT. A Bland-Altman analysis was utilized to compare the PRTT measurements from the same eye on the same relative day between the two different thread types. The bias (average difference in the values) was 1.155 with a standard deviation of 1.739. The 95% limits of agreement were between -2.253 to 4.564.

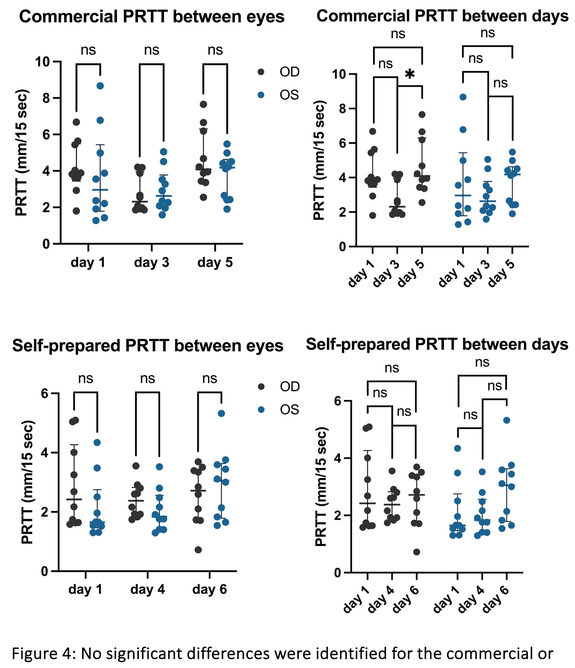

Figure 4: No significant differences were identified for the commercial or self-prepared PRTT between left and right eyes across testing sessions. PRTTs were performed on wildtype mice (n=10) at three different testing sessions using the commercial PRT then at three separate testing sessions using the self-prepared PRT. A post-hoc Tukey test performed on the commercial PRTTs identified no significant differences between the right and left eyes at each testing session. However, a significant difference between day 3 and 5 for the right eye was identified (p=0.0144). The same analysis was performed on the self-prepared PRTT, which identified no significant differences between eyes or between testing sessions for the same eye. Median and interquartile ranges are shown.

Conclusion

Our self-prepared PRTT demonstrated consistent wicking characteristics across solutions and between batches, showing reproducible generation of the PRTT. Additionally, the self-prepared PRTT provides statistically similar measurements compared to the commercial test indicating its applicability to serve as a valid and reliable replacement for the discontinued commercial PRTT.

Future Directions

Determine whether our self-prepared PRTT can accurately discriminate between wildtype and mutant mice with known differences in aqueous tear production.

Acknowledgments

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References

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Questions? Contact Bryan at bepuentes@ucdavis.edu or Brian Leonard at bcleonard@ucdavis.edu