Investigation of the repeatability of tear osmolarity using an I-PEN osmolarity device

Raied Fagehi1*, Abdulkareem B. Al-Bishry1, Mana A. Alanazi1, Ali Abusharha1, Gamal A. El-Hiti2, Ali M. Masmali2

Abstract:
PURPOSE: To investigate the repeatability of tear osmolarity in healthy Saudi subjects using an I-PEN osmolarity device.

MATERIALS AND METHODS: Thirty typical male subjects with healthy eyes (27.4 ± 4.9 years) participated in the study. Eye abnormalities were tested with a slit lamp, and eye comfort was determined with the surface disease index. Measurements of the tear break-up time and phenol red thread tests were used for exclusion criteria. The tear osmolarity test, using an I-PEN osmolarity system, was performed three times in the right eye of each subject with a 5 min’ gap between tests.

RESULTS: The average osmolarity test score was 303.8 ± 4.8 mOsm/L. Tear osmolarity measurements showed tear osmolarity of 280–299 mOsm/L, 300–309 mOsm/L, and 310–329 mOsm/L in 14 (46.7%), three (10%), and 13 (43.3%) subjects, respectively. Correlations among the three I-PEN measurements were significant (Spearman’s correlation coefficient; r = 0.036, 0.501, and 0.603; P = 0.050, 0.006, and 0.001, respectively). The mean coefficient of variance among the three measurements was 4.4%.

CONCLUSION: The mean measurement of an I-PEN tear osmolarity was 303.8 ± 4.8 mOsm/L which is in agreement with the range of those reported for healthy subjects. The I-PEN is reliable and has the advantage of portability (hand-held) compared to the other osmolarity systems.

Keywords: Dry eye, hyperosmolarity, I-PEN osmolarity system, phenol red thread test, repeatability

Introduction

Ocular tear film stability is vital for maintaining healthy eyes and ocular surfaces. Disturbances in the tear film lead to numerous vision problems in which eye dryness is the most common. [1] Eye dryness is associated with pain, inflammation, discomfort, and redness. [2] The tear film structure is complex, but primarily contains an outer lipid layer that covers an aqueous phase representing a bi-phasic structure rather than a tri-layered structure that contains lipid, aqueous and mucin phases. [2,3]

The lipid layer plays an essential role in the stability of tear film. It spreads over the tear film during blinking, therefore preventing tear evaporation. [4,5] In addition to lipids, salts, proteins, and mucins play an essential role in maintaining the stability of tear film.

The stability of the tear film can be detected through the measurement of both the volume and quantity of tears. Various tests can be used for this purpose. However, correlations among such tests are weak. [6] Therefore, a combination of tests must be used to diagnose eye dryness. [7] The quantity of tears can be measured using phenol red

How to cite this article: Fagehi R, Al-Bishry AB, Alanazi MA, Abusharha A, El-Hiti GA, Masmali AM. Investigation of the repeatability of tear osmolarity using an I-PEN osmolarity device. Taiwan J Ophthalmol 2021;11:168-74.
The tear osmolarity test is having abnormalities, recent ocular surgery, contact examine abnormalities in the eyelids, and subjects were older than 30 years. A slit-lamp was used to complete the study. The age of all subjects ranged from 20 to 37 years; in which 33.3% of the subjects (20 to 37 years; in which 33.3% of the subjects (20 to 37 years) completed the study. The mean (± standard deviation) age of 27.4 ± 4.9 years were treated in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject before the commencement of the research.

Materials and Methods

Participants
Thirty normal male subjects with healthy eyes and a mean (± standard deviation) age of 27.4 ± 4.9 years completed the study. The age of all subjects ranged from 20 to 37 years; in which 33.3% of the subjects (n = 10) ranged from 20 to 25 years; 53.3% of the subjects (n = 16) ranged from 26 to 30 years, and 13.4% of the subjects (n = 4) were older than 30 years. A slit-lamp was used to examine abnormalities in the eyelids, and subjects having abnormalities, recent ocular surgery, contact lens wearers, smokers, and subjects with diabetes, anemia, and thyroid disorders were excluded from the study. In addition, subjects with a high body mass index (above 24.9 kg/m²), a high blood cholesterol level (above 4 mmol/L), Vitamin A and D deficiencies, and hypertension were excluded.

All measurements were performed by the same examiner at the Optometry Clinics of the College of Applied Medical Sciences between 08:00 and 11:00 a.m. under controlled conditions in terms of temperature (23°C) and relative humidity (<40%). The OSDI sheet was completed first by all subjects, followed by the NITBUT, PRT, and I-PEN tear osmolarity tests with a 5-min interval between each test.

Ethical approval was obtained from the Ethics Committee at the College of Applied Medical Sciences, King Saud University (approval number: CAMS-036-3940), the subjects were treated in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject before the commencement of the research.

The ocular surface disease index sheet
The OSDI sheet was completed first by each subject, and a score of <13 was considered a healthy eye [23].

The noninvasive tear break-up time test
The NITBUT test was performed on the right eye of each subject using EASYTEAR view+ (EasYTEAR, Rovereto, Trento, Italy) without fluorescein drops. White illumination was used to create the corneal reflection to confirm regular mires and grid on the ocular surface. Each subject was asked to blink once; then, the time between the blink and the appearance of mires and grid distortion was calculated. The test was performed three times, and the average score was calculated for each subject. The tear break-up time provides information about the status of the eye in which longer time is an indication of healthy eyes. Healthy and normal eyes have a tear break-up time of more than 10 s [23].

The phenol red thread test
The PRT test was performed on the right of each subject using PRT stripes (Zone-Quick, Showa Yakuhin Kako Co., Ltd, Tokyo, Japan). Each subject was asked to gaze at a primary position, and a 3 mm fold of the PRT thread was inserted gently into the lower lid conjunctival sac (one-third of the distance from the lateral canthus). The thread was removed after 15 s, and the length of the red portion was measured in millimeters. The length of PRT thread wetted portion is a measure of tear volume and large readings are characteristic for normal and healthy eyes. Healthy and normal eyes have a PRT reading of more than 10 mm [23].
The I-PEN tear osmolarity test

Tear osmolarity was performed using the I-PEN osmolarity system 5 min after the PRT test. The I-PEN osmolarity system was used far away from electronic devices to ensure the accuracy of the readings. Each subject was asked to close their eyelids for 30 s gently, and then, the disposable single-use sensor was softly contacted with the palpebral conjunctiva from the lower eyelid at a 30° angle. By design, after a few seconds, the I-PEN beeps and displays an osmolarity reading on the screen.[25] Tear osmolarity was measured three times in the right eye of each subject with 5-min intervals between measurements. Based on the I-PEN tear osmolarity measurements, subjects were classified as a healthy eye (<290 mOsm/L), minor dry eye (290–310 mOsm/L), mild dry eye (310–330 mOsm/L), and moderate dry eye (330–350 mOsm/L).

Statistical analyses

The data were collected using Excel (Microsoft Office 2016, Microsoft Corp., Redmond, WA, USA) and was analyzed using the SPSS statistical package for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). The data were not normally distributed (Kolmogorov–Smirnov test; P < 0.05) for the scores from the OSDI, NITBUT, and PRT measurements. For the osmolarity test, the data were normally distributed (Kolmogorov–Smirnov test; P > 0.05). Therefore, parametric tests (one-way repeated-measure analysis of variance) were used to analyze the osmolarity measurements. In addition, the intraclass correlation coefficient test among the three osmolarity readings was applied. A correlation coefficient (Spearman’s correlation coefficient; r) was used to study the relationship among parameters.[29]

Results

The median scores (median interquartile range) obtained from the OSDI, NITBUT, and PRT measurements were 8.3 (6.4), 12.3 (4.5) s, and 25.0 (7.5) mm, respectively. For the osmolarity test, the average score was 303.8 ± 4.8. The tear osmolarity readings ranged from 277 to 337 mOsm/L in which 14 subjects (46.7%) had tear osmolarity of 280–299 mOsm/L, three subjects (10%) had tear osmolarity of 300–309 mOsm/L, and 13 subjects (43.3%) had tear osmolarity of 310–329 mOsm/L. The averages obtained from the scores of OSDI sheet and tear film tests, including tear osmolarity are summarized in Table 1.

There was no statistically significant difference among the three I-PEN osmolarity readings (Friedman test; P = 0.786). However, the standard deviation was high for some readings in which the average coefficient of variation was 4.4%, and the cohort ranged from 1% to 9% [Figure 1]. The intraclass correlation coefficient (average measures) was 0.745. The Bland–Altman plots between three I-PEN measurements are shown in Figure 2. The difference between repeated measurements was up to 40.7 mOsm/L. The correlations among the three tear osmolarity reading were strong (r = 0.036, 0.501, and 0.603; P = 0.050, 0.006, and 0.001, respectively). However, no correlations were found among the scores from the OSDI, NITBUT, PRT, and tear osmolarity measurements, expectedly, because each test detects a different parameter. The correlations between the scores obtained from different tests including OSDI are shown in Figure 3.

Discussion

Dry eye syndrome is a common problem that requires attention and immediate management to avoid damage of the ocular tear film. The diagnosis of eye dryness is achieved by a combination of conventional tests since no single test provides high accuracy. Tear hyperosmolarity is considered the primary cause of inflammation and discomfort among dry eye patients.[30] The measurement of tear film osmolarity is considered a gold standard for the diagnosis of dry eye.[31,32] The tear osmolarity test is superior to other dry eye diagenetic tests such as NITBUT and Schirmer tests.[23] The average tear osmolarity for a healthy-eye subject is 300.8 ± 7.8 mOsm/L based on measurements of 299 subjects (218 females and 81 males) using the TearLab osmolarity system.[23] In vitro measurements of tear osmolarity using electrical impedance is affected by temperature variation.[33] While the I-PEN or TearLab osmolarity systems are used only in vivo and therefore the temperature has no significant effect on tear osmolarity readings since the palpebral conjunctiva temperature remains steady at 36.2°C ± 0.6°C.[34] No significant differences were noted among the tear osmolarity readings measured at different times of day for 30 healthy controls.[33] In contrast, the tear osmolarity recorded on the TearLab osmolarity system among a small group of dry (n = 10) and healthy eye (n = 10) subjects differed by 21.9 ± 13.5 and
The average tear osmolarity for healthy and dry eye subjects was 298.0 ± 14.2 and 304.0 ± 10.8 mOsm/L, respectively.²⁵ The reported average tear osmolarity measured using the I-PEN or TearLab osmolarity systems was 288.3–336.4 ± 7.6–22.0 mOsm/L.²³,²⁵,²⁷ In the current study, there were no significant differences among the three I-PEN readings. The average for the tear film osmolarity readings using the I-PEN osmolarity system was 303.8 ± 4.8 mOsm/L. That average was slightly higher than in some earlier studies and lower than others.²⁷ In the current study, the difference between the repeated osmolarity measurements was up to 40.7 mOsm/L, which is consistent with the literature.²⁷

Table 1: The averages (mean±standard deviation or median (interquartile range)) for the ocular surface disease index, noninvasive tear break-up time, phenol red thread, and I-PEN tear osmolarity measurements

| Test                  | Mean±SD or median (IQR) |
|-----------------------|-------------------------|
| Age (years)           | 27.4±4.9                |
| OSDI                  | 8.3 (6.4)               |
| NITBUT (s)            | 12.3 (4.5)              |
| PRT (mm)              | 25.0 (7.5)              |
| Osmolarity (mOsm/L)   | 303.8±4.8               |

SD=Standard deviation, IQR=Interquartile range, OSDI=Ocular surface disease index, NITBUT=Noninvasive tear break-up time, PRT=Phenol red thread
However, it is much higher (four-time) than that obtained using TearLab system.\(^{[10,27]}\)

Tear osmolarity of constructed tear solutions from electrolytes and proteins that have different osmolarity (297 mOsm/L for healthy eye tears and 342 mOsm/L for dry eye tears) was measured using a vapor pressure osmometer, TearLab osmolarity, and I-PEN osmolarity systems.\(^{[26]}\) The average tear osmolarity for healthy and dry eye tears using the

---

**Figure 3:** Correlations between (a) I-PEN and ocular surface disease index scores, (b) I-PEN and noninvasive tear break-up time scores, (c) I-PEN and phenol red thread scores, (d) phenol red thread and ocular surface disease index scores, (e) phenol red thread and noninvasive tear break-up time scores, and (f) noninvasive tear break-up time and ocular surface disease index scores.
three devices was 305.6 ± 4.0 and 352.2 ± 5.5 mOsm/L, 300.6 ± 3.7 and 341.4 ± 7.9 mOsm/L, and 336.4 ± 21.5 and 342.0 ± 20.7 mOsm/L, respectively.[28] Both the vapor pressure osmometer and the TearLab osmolarity system showed exceptional consistency and accuracy. However, I-PEN was less accurate in measuring contrived tears that have a known osmolarity.[29] Another study was conducted among healthy eye subjects (n = 20) in which tear osmolarity was measured five times using TearLab and I-PEN osmolarity systems.[27] The average tear osmolarity using the I-PEN osmolarity system was higher (319.4 ± 20.3 mOsm/L) compared to the average obtained using the TearLab osmolarity system 295.4 ± 8.6 mOsm/L).[27] Again, TearLab osmolarity was accurate in identifying all the subjects having healthy eyes, while, I-PEN showed exceptionally low accuracy (15%).[27] The tear osmolarity measurements in 25 subjects using the TearLab osmolarity system was found to be higher (305.2 ± 16.1 mOsm/L) than those obtained using the Fiske 210 osmometer (293.4 ± 12.2 mOsm/L).[30]

Dry eye subjects tend to have higher tear osmolarity (312.0 ± 16.9 mOsm/L) compared to healthy eye subjects (305.6 ± 9.7 mOsm/L) when using the TearLab osmolarity system.[30] There was an association between higher tear osmolarity and discomfort, higher OSDI, and conjunctival staining scores.[19] The average tear osmolarity among those subjects with Sjögren syndrome dry eye (n = 39) using the TearLab osmolarity system was 311.1 ± 16.4 mOsm/L.[40] The tear osmolarity was collated positively with both OSDI (r = 0.405; P = 0.011) and the ocular staining score (r = 0.592; P < 0.001) and negatively with the Schirmer I test score (r = −0.625; P < 0.001).[40] The mean tear osmolarity using the TearLab osmolarity system was 296.8 ± 16.5 mOsm/L in non-Sjögren syndrome dry eye subjects, 303.4 ± 17.2 mOsm/L in Sjögren syndrome dry eye, and 303.5 ± 12.9 mOsm/L in healthy eye subjects.[41]

A study conducted among healthy (n = 14) and dry eye (n = 74) subjects using the TearLab osmolarity system showed that plasma osmolarity was higher than tear osmolarity.[42] For healthy eyes, the mean for plasma osmolarity and tear osmolarity was 288.3 ± 6.6 and 293.1 ± 2.8 mOsm/L, respectively.[42] However, for dry eye, the mean for plasma osmolarity and tear osmolarity was 288.5 ± 9.4 and 293.4 ± 5.1 mOsm/L, respectively.[42] There was no correlation between plasma osmolarity and tear osmolarity since they are independent. The tear osmolarity measured among 30 healthy eye subjects using the TearLab osmolarity system ranged from 277 to 312 mOsm/L with a mean of 299.1 ± 7.7 mOsm/L with 0.80% as a coefficient of variation.[13] The osmolarity readings have a significant (P = 0.018) positive medium correlation (r = 0.429) with the scores from McMonnies questionnaire and a significant (P = 0.001) strong negative correlation (r = −0.587) with the NITBUT scores.[12] On the other hand, there was no significant correlation (r = −0.067; P = 0.725) between the osmolarity readings and the PRT scores.[12] Various osmolarity systems can be used to detect eye dryness and have acceptable repeatability and accuracy. The current study has some limitations, such as the use of a small size sample of male-only subjects from Riyadh City.

**Conclusion**

The mean for the I-PEN tear osmolarity was 303.8 ± 4.8 mOsm/L which is in agreement with the range of those reported for healthy subjects. The I-PEN osmolarity system is reliable and had the advantage of portability (hand-held) compared to the other osmolarity system.

**Acknowledgments**

The authors extend their appreciation to the College of Applied Medical Sciences Research Center and the Deanship of Scientific Research at King Saud University, for funding this research.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

The authors declare that there are no conflicts of interests of this paper.

**References**

1. Stapleton F, Alves M, Bunya YV, Jalbert I, Lekhanont K, Malet F, et al. TFOS DEWS II Epidemiology Report. Ocul Surf 2017;15:334-65.
2. Wilcox MD, Argüeso P, Georgiev GA, Holopainen JM, Laurie GW, Millar TJ, et al. TFOS DEWS II Tear Film Report. Ocul Surf 2017;15:366-403.
3. Cher I. A new look at lubrication of the ocular surface: Fluid mechanics behind the blinking eyelids. Ocul Surf 2008;6:79-86.
4. Kulovesi P, Rantamäki AH, Holopainen JM. Surface properties of artificial tear film lipid layers: Effects of wax esters. Invest Ophthalmol Vis Sci 2014;55:4448-54.
5. Rantamäki AH, Wiedmer SK, Holopainen JM. Melting points. The key to the anti-evaporative effect of the tear film wax esters. Invest Ophthalmol Vis Sci 2013;54:5211-7.
6. Sullivan BD, Crews LA, Messmer EM, Fouls GK, Nichols KK, Baenninger P, et al. Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: Clinical implications. Acta Ophthalmol 2014;92:161-6.
7. de Monchy I, Gendron G, Miceli C, Pogorzalek N, Mariette X, Labetoulle M. Combination of the Schirmer I and phenol red thread tests as a rescue strategy for diagnosis of ocular dryness associated with Sjögren’s syndrome. Invest Ophthalmol Vis Sci 2011;52:5167-73.
8. Masmali A, Alqahtani TA, Alharbi A, El-Hiti GA. Comparative study of repeatability of phenol red thread test versus Schirmer’s test in normal adults in Saudi Arabia. Eye Contact Lens 2014;40:127-31.
9. Cho P, Ho KY, Huang YC, Chui HY, Kwan MC. Comparison of...
non-invasive tear break-up time measurements from black and white background instruments. Optom Vis Sci 2004;81:436-41.
10. Masmali A, Alrabiah S, Alharbi A, El-Hiti GA, Almubrad T. Investigation of tear osmolarity using the TearLab osmolarity system in normal adults in Saudi Arabia. Eye Contact Lens 2014;40:74-8.
11. Masmali AM, Al-Anazi SA, Almagren B, El-Hiti GA. Assessment of the tear film in normal eye subjects after consumption of a single dose of hot peppermint drink. Clin Optom (Auckl) 2019;11:39-45.
12. Masmali AM, Murphy PJ, Purslow C. Development of a new grading scale for tear ferning. Cont Lens Anterior Eye 2014;37:178-84.
13. Schüllman RM, Christiansson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease index. Arch Ophthalmol 2000;118:615-21.
14. Nichols KK, Nichols JJ, Mitchell GL. The reliability and validity of McMonnies dry eye index. Cornea 2002;21:365-71.
15. Tomlinson A, Khanal S. Assessment of tear film dynamics: Quantification approach. Ocul Surf 2005;3:81-95.
16. Tomlinson A, Bron AJ, Korb DR, Amano S, Paugh JR, Pearce EI, et al. The international workshop on meibomian gland dysfunction: Report of the diagnosis subcommittee. Invest Ophthalmol Vis Sci 2011;52:2006-49.
17. Srinivasan S, Nichols KK. Collecting tear osmolarity measurements in the diagnosis of dry eye. Expert Rev Ophthalmol 2009;4:451-3.
18. Sweeney TE, Beuchat CA. Limitations of methods of osmometry: Measuring the osmolarity of biological fluids. Am J Physiol 1993;264:R469-80.
19. Nelson JD, Wright JC. Tear film osmolality determination: An evaluation of potential errors in measurement. Curr Eye Res 1986;5:677-81.
20. Bron AJ. Diagnosis of dry eye. Surv Ophthalmol 2001;45 Suppl 2:S221-6.
21. Benelli U, Nardi M, Posarelli C, Albert TG. Tear osmolarity measurement using the TearLab Osmolarity System in the assessment of dry eye treatment effectiveness. Cont Lens Anterior Eye 2010;33:61-7.
22. Khanal S, Tomlinson A, McFadyen A, Diaper C, Ramaesh K. Dry eye diagnosis. Invest Ophthalmol Vis Sci 2008;49:1407-14.
23. Lemp MA, Bron AJ, Baudouin C, Benitez Del Castillo JM, Geffen D, Tauber J, et al. Tear osmolarity in the diagnosis and management of dry eye disease. Am J Ophthalmol 2011;151:792-8.
24. Tomlinson A, Khanal S, Ramaesh K, Diaper C, McFadyen A. Tear film osmolarity: Determination of a referent for dry eye diagnosis. Invest Ophthalmol Vis Sci 2006;47:4309-15.
25. Chan CC, Borovik A, Hofmann I, Gulliver E, Rocha G. Validity and Reliability of a Novel Handheld Osmolarity System for Measurement of a National Institute of Standards Traceable Solution. Cornea 2018;37:1169-74.
26. Rocha G, Gulliver E, Borovik A, Chan CC. Randomized, masked, in vitro comparison of three commercially available tear film osmometers. Clin Ophthalmol 2017;11:243-8.
27. Nolfi J, Caffery B. Randomized comparison of in vitro performance of two point-of-care tear film osmometers. Clin Ophthalmol 2017;11:945-50.
28. Masmali AM, Maeni YA, El-Hiti GA, Murphy PJ, Almubrad T. Investigation of ocular tear ferning in controlled and uncontrolled diabetic subjects. Eye Contact Lerts 2018;44 Suppl 2:S70-5.
29. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd ed.. Hillsdale: Lawrence Erlbaum Associates; 1988.
30. Lemp MA. Report of the national eye institute/industry workshop on clinical trials in dry eyes. CLAO J 1995;21:221-8.
31. The definition and classification of dry eye disease: Report of the Definition and Classification Subcommittee of the International Dry Eye Workshop (2007). Ocul Surf 2007;5:75-92. Available from: https://pubmed.ncbi.nlm.nih.gov/17508116/.
32. Sullivan BD, Whitmer D, Nichols KK, Foulks GN, Geerling G, et al. An objective approach to dry eye disease severity. Invest Ophthalmol Vis Sci 2010;51:6125-30.
33. Malmberg CG. Electrical conductivity of dilute solutions of “sea water” from 5 to 120°C. J Res Natl Stand Sec A 1965;69A: 39-43.
34. Holdon BA, Sweeney DF. The oxygen tension and temperature of the superior palpebral conjunctiva. Acta Ophthalmol (Copenh) 1985;63:100-3.
35. Oncel BA, Pinarci E, Akova YA. Diurnal variation of the tear osmolarity in normal subjects measured by a new microchip system. Eur J Ophthalmol 2012;22 Suppl 7:S1-4.
36. Li M, Du C, Zhu D, Shen M, Cui L, Wang J. Daytime variations of tear osmolarity and tear meniscus volume. Eye Contact Lens 2012;38:282-7.
37. Baenninger PB, Voegeli S, Bachmann LM, Faes L, Iselin K, Kaufmann C, et al. Variability of tear osmolarity measurements with a point-of-care system in healthy subjects-systematic review. Cornea 2018;37:938-45.
38. García N, Melvi G, Pinto-Fraga J, Calonge M, Maldonado MJ, González-García MJ. Lack of agreement among electrical impedance and freezing-point osmometers. Optom Vis Sci 2016;93:482-7.
39. Mathews PM, Karakus S, Agrawal D, Hindman HB, Ramulu PY, Akpek EK. Tear osmolarity and correlation with ocular surface parameters in patients with dry eye. Cornea 2017;36:1352-7.
40. Kim M, Kim HS, Na KS. Correlation between tear osmolarity and other ocular surface parameters in primary sjögren’s syndrome. Korean J Ophthalmol 2017;31:25-31.
41. Szalai E, Berta A, Szekanecz Z, Sütös G, Módös I Jr. Evaluation of tear osmolarity in non-Sjögren and Sjögren syndrome dry eye patients with the TearLab system. Cornea 2012;31:867-71.
42. Kobayashi M, Igarashi T, Takahashi H, Fujimoto C, Suzuki H, Takahashi H. The correlation between plasma osmolarity and tear osmolarity. Int Ophthalmol 2018;38:493-501.