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"A review of the Schirmer Tear Test in Guinea Pigs"

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1. Introduction

The guinea pig is both a common family pet and lends its name as a term denoting an experimental subject. A high level summary of the available scientific literature on ocular diseases in guinea pigs is as follows:

- Information on diseases of the guinea pig eye is important given the use of this species as a laboratory animal[17].
- The Schirmer Tear Test (STT) is the simplest and most common test used in most basic ophthalmic examinations.
- A review of available literature indicates that there is no accepted standard methodology used for conducting the STT particularly for small animals.
- No assured correlation was found between corneal sensitivity and the quantity of reflex tearing. Factors other than corneal sensitivity may have a higher impact on reflex tear production[17].
- The incidence of ocular problems occurring in guinea pigs is almost fifty percent (almost in one in every two animals) in what otherwise were healthy animals[17].
- There is surprisingly very little published material available on the general health of guinea pigs despite their being so important in a laboratory sense. Limited citations were found in the literature.
- The largest study to date of ocular disease in guinea pigs was carried out on 1000 animals by David Williams and Ann Sullivan in 2010[17].
- The Schering- Plough STT strips appear to be the most commonly used product in the investigations reviewed.

The eye is probably the most important sensory faculty for all animals. The cornea is the transparent front part of the eye and must constantly be lubricated. Tears, produced by the lacrimal gland, provide this lubrication. Blinking is the process by which this lubrication is administered. When an animal blinks a thin film of lubricant is spread across the cornea. This also acts as an irrigation system to flush the eye of any foreign material. The absence of tears gives rise to the condition called “dry eye” which causes irritation of the ocular surface and can give rise to a variety of eye diseases. Measuring and understanding the blinking process, tear production and this lubrication process has occupied the minds of ophthalmologists for many years and many books have been written on the subject.

The aim of this research project was to conduct an in-depth literature review of the Schirmer Tear Test, in particular its applicability to testing guinea pigs eyes, and to conduct an *in vitro* and *in vivo* tests on a population of 20 healthy guinea pigs using both a standard (5mm wide) and modified (2.5mm wide) tear strip. In this study STT values were measured using modified test strips and compared to reference values for guinea pigs published by other researchers.

The Guinea Pig

Cavia porcellus, the domestic Guinea pig or 'cavy', is a small stocky tailless rodent of the suborder Hystricomorpha, which has become increasingly popular throughout the world as a small companion animal and as children's pets. Hobby breeders have selectively bred domestic guinea pigs for coat characteristics over the last few decades resulting in a large variety of genotypes[3]. The Guinea pig has also been used for many years as a laboratory rodent to the extent that its very name is synonymous with a term denoting an experimental subject[17]. The most common strains used in laboratories are the Dunkin-Hartley and the American Shorthair (English) guinea pig.

In carrying out ophthalmic tests in animals there are many variables which to a greater or lesser extent can influence the test results and all have been investigated to varying degree by many research studies. These parameters include species, breed, gender, left eye versus right eye, healthy versus unhealthy animals, laboratory versus pet animals and the test environment itself.

The Guinea Pig Eye

In his book *Ophthalmology of Exotic Pets*[20], David L. Williams has written extensively on guinea pigs eyes and the following is a brief summary of some aspects of the guinea pig eye.

Guinea pigs are a highly visual species using sight from the moment of birth. It remains unclear how well guinea pigs kept as domestic pets can see given the high prevalence of ocular defects found in otherwise healthy animals. It is similar in size to many rodents but its retina has no blood vessels. It is not capable of synthesising its own vitamin C so that its diet needs to be supplemented. As a result they are at a high risk of scurvy which is an early sign of conjunctivitis. Neonatal guinea pigs have very small eyes and are born with their eyes open. They are prone to many eye diseases and Keratoconjunctivitis is one such disease. Clinical signs are ocular irritation, partial closure of the palpebral aperture and a lack of clear sharp reflection of light from the ocular surface. This generally suggests "dry eye". The Schirmer Tear Test is the standard method of evaluating tear production. Dr Williams refers to data for mean STT values measured by a number of independent researchers. Schirmer Tear Test results from these studies are discussed later by this author in paragraph 2.1. Guinea pigs are also prone to ocular scarring from shards of hay or grass awns in their cages which can impair their vision and lead to keratitis.

The guinea pig is prone to a wide range of ocular diseases. The following literature review is confined to those diseases linked to tear production and how the Schirmer Tear Test is used in studying these diseases. Some other parameters reviewed including their method of measurement are summarised in Table 1 below. Guinea pigs were found to have a low CTT and virtually no reflex tearing.

The palpebral fissure length of the eye varies greatly from species to species depending on their physical size. This makes measurement a more difficult task for smaller species. An improved standardized method for recording low values (≤ strip notch) in Schirmer tear testing in smaller animals and pets who have small eye globes and palpebral fissure lengths is necessary.

Table 1: Ocular parameters reviewed and their methods of measurement.

Parameter	**Measured by**
Corneal sensitivity	CTT measured using Cocher-Bonnett in g/mm^2
Tear Production	Schirmer tear tests: STT_I, STT_{II} in mm/minute
Quantity of reflex tearing (Tear Production difference)	Schirmer tear tests: $(STT_I - STT_{II})$ mm
Palpebral Fissure length	Measured in mm with callipers

1.1 Overview of the Schirmer Tear Test

The Schirmer Tear Test I (STT_I) is the simplest and most commonly used semi-quantitative routine test used in data collection as part of most basic ophthalmic examinations. It measures the aqueous portion of the precorneal tear film production as part of the assessment of the basal level of tear secretion in a patient. Basal tear production is defined as the quantity of tears which lubricate the corneal surface, secreted under normal conditions without any stimulation[8]. Measurements are reported in millimetres /minute.

The Schirmer tear test is named after Otto Schirmer, a German ophthalmologist, who first developed the use of absorbent paper for the evaluation of the lacrimal system in humans[13]. A veterinary modification was later described in 1962. This test is recommended for all patients presenting with external ocular disease whenever any abnormal ocular discharge occurs as it aids in the diagnosis of keratoconjunctivitis sicca (KCS).

Prior to the 1980's there is no evidence of any research into the basic factors affecting the wetting rate of the paper strips. For example, did the wetting rate of the strip depend on the tear secretion rate or was it simply a function of the magnitude of the "wetting rate" (time) of the strip. Researchers rather focused their attention on the type of paper used in the test including blotting paper, litmus paper, cigarette paper, and various filter papers. Many paper types were tested *in vitro* by dipping the tip of the strip into a beaker of water (unlimited fluid supply situation) and measuring the wetting rate in millimetres per minute. The wetting rate was found to vary with the type of paper used. It was only in 1961 that Schirmer test paper strips were standardised and Black Ribbon No. 589 became the standard (which has similar characteristics to Whatman® No. 41 filter paper). Previous researchers concluded that all studies employing the Schirmer tear test should always specify the maker and the lot number of the strips used due to the propensity for variations in performance by different strips.[5]

Most test strips today are made of sterile standardised absorbent Whatman® No. 41 filter paper, with a rounded tip at one end and typically measuring 5mm wide and up to 40mm in length. Some brands have a notch 5mm from the rounded end, which demarcates the site where the strip should be folded over before insertion in the lid margin of the eye. Strip design has improved over time with newer strips incorporating a measuring rule printed on the strip marking it from 0 to 35mm in 1mm gradations, or from 2mm to 35mm. Some paper strips incorporate a dye impregnated in the strip to facilitate reading of the wetting line. The paper strips are individually wrapped and are commercially available on the market in boxes of 100 strips and are produced by a number of different manufacturers.

Schirmer tear testing should be performed as the first step in any ophthalmic examination before measurements are influenced by any further examinations. STTI is performed prior to the application of any topical solutions or medications to the eye. It should also be conducted before any treatments such as use of tonometry devices on the eye globe or any manipulative procedures such as corneal or conjunctival scrapings and flushing of the lacrimal apparatus. Such examinations may induce reflexive tear production, which is defined as the quantity of tears secreted in response to a corneal or conjunctival irritant[8]. It is arguable that the Schirmer tear test (STTI) itself may even cause a reflex tear production in some species, but this is unlikely in guinea pigs due to their very low corneal sensitivity.

If there is a notch on the strip, the strip should be folded over at this point before insertion in the lid margin of the eye. This fold should be made while the strip remains unopened in its original packet, to avoid touching the strip with gloves or fingers. Potentially this could cause contamination with foreign components or secretions which could influence or impact the absorbing surfaces of the paper.

The strip should be gently placed between the corneal surface and the lower eyelid using the fold as a hook to maintain its position over the middle to lateral third of the lower lid in the conjunctival fornix. The lids may be maintained either in an open position, or closed by gentle pressure on the upper lid if blinking and retention of the strip becomes a problem. Care should be taken not to compress the eye which may elicit reflex lacrimation and give false values[12]. The strip should remain in position for 60seconds allowing the tear fluid to migrate along the strip. The strip should then be removed from the eye and the length of strip that has now become moistened measured and recorded in millimetres. This test is then repeated with a new strip for the fellow eye. Some researchers[18] studied the kinetics of the wetting process by performing STT measurements at 5second intervals to track the wetting rate of the strip over time.

It appears from a review of available literature on this subject that even today there is no consistent or standard methodology for conducting the STT. The author considers this may be partly due to the varying designs of STT test strips that have evolved over time.

A standardized method for recording low values (≤ notch) in Schirmer tear testing, particularly in smaller companion animals or exotic pets who have smaller eye globes and smaller palpebral fissure lengths is necessary. One of the aims of this research project was to review and compare alternative methods used by researchers for measuring STT values with the objective of recommending a more appropriate standard for small animals.

2. Literature Review

Guinea pigs have increased greatly in popularity as family pets over the last three decades. On presentation to veterinarians it was observed that the incidence of ocular diseases occurring in these animals also increased greatly. There are not many studies published on ocular measurements (and hence ocular disease) in guinea pigs over the past three decades, however more published material has become available in the past decade.

The Schirmer tear test is one of the key parameters studied when performing ocular examinations on all animals. However the actual physical size of the cornea of the species being studied presents challenges when trying to accurately take measurements. Many studies were published quoting STT measurement in a variety of animal species however this literature review focuses mainly on research into guinea pigs. In this review the author also searched for new measurement methodologies in performing the STT particularly in relation to small or exotic animals. Typically STT measurements were always taken using a standard commercially available strip size (5mm wide) which is sometimes too large to place in the eye of a small animal. Some researchers performed their own modifications to the standard STT strip to reduce its size as there is no commercially available "mini strip" or modified strip available on the market for use in the eyes of very small species. The author also researched for variations in the type of tear strips used by different researchers to try to identify if STT results are a function of the type of paper used by different manufacturers of tear strips. Another factor researched in this literature review was whether any researchers adopted baseline calibration testing of STT strips, versus for example distilled water or saline, in their laboratory practice before conducting an STT on an animal.

David L. Williams (2005)[18] analysed the uptake of tears in the STT *in vitro and in vivo* for dogs. He tried to "dissect the origins of the tear wetting involved in the strip wetting for a dog". His aims were to study fluid uptake in standard Schering-Plough test strips, the actual kinetics of fluid uptake in the strips, and "to evaluate the value of determination of test strip fluid uptake over time in the diagnosis of tear film deficiencies"[18]. He stated that "fluid wetting the tear strip is comprised of tears already present in the tear lake together with those produced during the period of the test"[18]. He formed the opinion that initial rapid rise in the test strip wetting originates from tears in the tear lake, while the slower rise in the test strip wetting arises from steady state tear production. He tried to estimate the contributions of these two fluid origins. He assessed the kinetics of wetting of the STT strip both with limited and unlimited fluid availability by holding the strips in contact with distilled water and 0.9% saline and measured the length of wetting over time. He repeated this procedure for live dogs (100) before conducting the normal full STTI and STTII measurements. He found no difference in tear strip wetting with distilled water and 0.9% saline. The results from the *in vivo* study show "a marked transition between the initial rapid uptake and the steady state uptake"[18]. This suggested that the initial rapid uptake comes from the tear well and is followed by a slower uptake that approximates more closely to absolute tear production. These results were reflected in the STT measurements also, initial rapid rise in STT (uptake from the tear lake) followed by a smaller STT values (slower uptake from steady state tear production). Williams was the only researcher found to conduct this calibration check versus distilled and saline solutions. Williams also measured tear uptake over time (reading STT's every 5 seconds) rather than simply measuring a single STT after one minute as most other researchers did. In his opinion this "allows absolute measurement of tear turnover rather than merely a figure for tear uptake by the STT filter paper at one point in time".[18] His results were presented graphically and clearly demonstrate his findings.

Montiani-Ferreira et al, (2006)[9] conducted a study to determine "Reference values for selected ophthalmic diagnostic tests of the ferret" with the aim of establishing normal physiological reference values for this species. They conducted standard Schirmer tear tests on 15 healthy ferrets as part of their research. Standard Schering–Plough STT strips were used to measure STTI and STTII for each animal. "Schirmer tear test mean values were 4.80 ± 0.88mm/min for male ferrets and 5.72 ± 1.50mm/min for female ferrets, the median was 5mm/min. There was no significant difference between left and right eyes for the Schirmer results (*P < 0.15*)".

This author found their concluding comments regarding the tear strips to be of particular interest. "The commonly available 6mm-wide STT strips used in this investigation just fit the palpebral aperture of the adult ferret and require patience to be placed correctly. With such a low normal STT result (median of 5mm/min), detecting a significant reduction might be difficult as a value of 3 or 4mm/min represents a significant change (in percentage). Nevertheless, whether or not a change of this magnitude is clinically significant still needs to be investigated. A possible solution for this potential problem might be to use STT strips of half width (3mm). This would allow for a larger absorption distance in mm, making it possible to detect more subtle changes. As there are no STT strips of this size available commercially, the clinician would have to cut each individual strip in half manually. This procedure might add another variable to the test, given that small differences in the way the strip was tailored might affect the result".[9]

To test this thesis they conducted a small side experiment on 6 ferrets using manually modified STT strips cut down to 3mm wide. "A higher mean STT value was obtained (6.5mm/min) but with noticeably larger variance (standard deviation of ±2.34mm/min). Nevertheless, we believe that the mean reference STT value obtained in this investigation using commercially available standardized strips will be a useful parameter for ferrets".[9] This clearly shows that modified STT strips do give different results to standard STT strips. This author believes that measurements made using smaller modified strips may well be more accurate than the standard larger strips when working with small species.

Four studies in particular focused in some detail on the use of the STT for ocular measurement in guinea pigs in the study of ocular diseases and these studies are reviewed in some detail in the following pages.

The first reported comprehensive study of ocular disease in guinea pigs was carried out by Trost, Skalicky and Nell (2007)[15]. The 2007 study was performed "to establish reference values for the Schirmer tear tests STTI and STTII, the phenol red thread tear test (PRT), eye blink frequency, corneal sensitivity in the guinea pig"[15] because no information about physiologic tear production in this species existed. The precorneal tear film is essential in maintaining normal corneal health. When eyelids blink they spread the tears over the cornea. While performing their study it was observed "that the eye blink frequency in the guinea pig was very low and to quantify this impression the eye blink frequency was also determined".[15]

They reported results for the PRT, STT and CTT in 36 guinea pigs. In their study they only used the Dunkin-Hartley laboratory strain of guinea pig with similarly aged animals. Their key findings were as follows. In all tests there were no significant differences between the right and the left eye. They established reference ranges for STTI, STTII and PRT. The difference between STTI and STTII was not significant (implying no reflex tearing) but a correlation was found between the STT of the left and right eye. Corneal sensitivity was found to be higher in the central region than in the four limbal regions but they surmised that this may be attributable to the sensitivity of the instrument. Eye blink frequency in a group of ten test cases was found to be between two and five blinks in twenty minutes but increased greatly if the guinea pigs were in an excited state. This is considered to be a low blink frequency when compared with other species. Corneal sensitivity in guinea pigs is lower than in other domestic animals apart from the brachycephalic cat. For instance a dog blinks at 2 – 5 times per minute, a cat 1 – 5 times per minute, a horse 5 – 25 times per minute and cattle at 5 times per minute. Blinking maintains the physiologic thickness of the preocular surface by spreading the tears over the corneal surface. The results found for STTI and STTII are summarised in Table 3 and discussed in paragraph 2.1 below.

A year later, Coster, Stiles, Krohne and Raskin, (2008)[3] conducted a study to report values for the PRT, STT, CTT and IOP in 31 healthy guinea pigs of various breeds and ages. They focused on the evaluation methods for ophthalmic testing and ocular disease recognition. They also looked at the effect of using topical anaesthesia on the PRT and STT values. Only 24 of the test population were used for measuring STT. Like the Trost et al[15] study they found no significant correlation between PRT and STT values obtained before and after topical aesthesia in either eye but suggested that this might be due to the low sample size. One significant finding is that the PRT and STT values measured in their study were higher than those previously reported by Trost et al[15] who used a homogenous test population of 54 Dunkin-Hartley guinea pigs only. It was not clear if these were true differences and it was proposed that this could be due to natural biological variation or differences in population characteristics, as guinea pigs in their study were of various ages and breeds. They concluded that the difference in STT values between their study and the Trost et al[15] study could be related to differences in the manufacturer, design and absorptive capabilities of the tear strips used in the two studies. A further reason they concluded may be due to differences in how values were recorded for animals in which wetting did not reach to or beyond the notch in the test strip. This is discussed later in paragraph 2.1. In conclusion they proposed that a standard method for recording low STT values (< 2mm/min) is required.

The most comprehensive study of ocular disease in guinea pigs carried out to date was conducted by Williams and Sullivan, (2010)[17]. The population group tested amounted to 1000 animals. All one thousand guinea pigs tested were given a full ophthalmic examination with direct and indirect ophthalmoscopy and with slit lamp biomicroscopy. Tear production was measured using STTI. Intraocular pressure was measured using applanation tonometry after topical anaesthesia was undertaken in selected animals. This author notes that Williams and Sullivan[17] adopted a different STT measuring methodology to that of previous researchers. Approximately 45% of all the guinea pigs examined presented with some form of ocular abnormality. This statistic is surprisingly high. The majority of abnormalities found were lens lesions including 17% with cataract, and 21% with subclinical lens abnormalities such as nuclear sclerosis. Other abnormalities included conjunctivitis in 4.7% and keratitis in 3.6%. Lipid deposition in conjunctiva was observed in 2.3% of the guinea pigs and ciliary body heterotopic bone formation in 0.8% of all animals. Many of these conditions did not appear to have a significant impact on the animal's general health or behaviour and, being visible only on close ophthalmic examination, would not have been noted by owners when presented to veterinarians. They found that neither STT's nor intraocular pressures were significantly different between eyes of individual animals using a paired t-test and so an average of both eyes was used. However it is disappointing to read that in this study of 1000 guinea pigs only 50 animals were selected to have the STT measured.

This study clearly shows that a high proportion of guinea pigs eyes have some degree of abnormality in animals which otherwise were considered healthy. "Information on diseases of the guinea pig eye is important given the use of the species as a laboratory animal"[17].

Cafaro et al, (2009)[2] studied the functional & structural characteristics of the cornea of healthy guinea pigs by performing analysis including BUT and FS analysis *in vivo* and *in vitro*, but did not conduct any STT measurements. Although previous studies provide results on tear production and values for corneal sensitivity in guinea pigs of different strains and ages,[(15,3)] there appears to be little information about histological and ultrastructural anatomy of the cornea of these animals. Their objective was to provide a detailed histological description of the Guinea pig's corneal structure and the status of corneal surface. They referred to the work done by Trost et al[15] and Coster et al[3] who used the STT methodology, however no cross correlation of results was possible given the absence of STT values and the different technologies used.

Lima et al, (2010)[7] conducted a study of chinchillas along the same lines as previous studies for guinea pigs. This paper was reviewed to see if any innovations were introduced to the Schirmer tear testing process used which might be applicable to the author's own planned work. The chinchilla is a small rodent which is closely related to the guinea pig. The objective of their study was to observe "the most important features of chinchilla ocular morphology" and "to establish normal parameters for ocular diagnostic tests to serve as reference values for future investigations”[7]. This study used a group of 57 healthy chinchillas and conducted “(i) ocular inspection including blink frequency (ii) Schirmer tear test STT (iii) palpebral fissure length measurement”.

Standard Schering-Plough tear test strips were used to perform STTI tests, only measuring basal plus a portion of the reflex tear secretion in all animals tested. However there were no innovations, such as tear strip modification, in the methodology used in conducting the STT and no details were provided on the actual measuring technique. PFL was measured using callipers which gave an average mean of 1.44cm for all animals. They found that chinchillas have a low STT and low blink frequency. An STT mean value of 1.07 ± 0.47cm was recorded for males and 1.07 ± 0.59cm for females. This STT was “higher and somewhat more measurable than the mean value of a guinea pig[15]”. The blink frequency of 2 – 4 times in 10 minutes (2.6 ± 0.84) is very low but very similar to the guinea pig. This reference data obtained from the study “will help veterinary ophthalmologists to more accurately diagnose discrete pathological changes of the chinchilla eye”.[7]

A similar study of chinchillas was undertaken in the same year by Muller, Mauler and Eule, (2010)[10] with similar overall objectives. STTI measurements were taken using standard size Essex Pharma (now NUVISAN) tear strips on 18 animals. The strip was bent at the notch as normal and inserted in the eye. Wetting measurements were taken after 1 minute. The STTI measurements recorded were zero for 13 animals and 1mm in 5 cases. As a result the STT measurement was discontinued as Muller concluded that the STT was too difficult to perform due to the small size of the palpebral fissure and they considered 1 minute as too long a test time. They concluded STT was not a suitable test for chinchillas. This result suggests that wetting below the notch was not measured (even as a negative number as demonstrated by Trost et al[15]) and further demonstrates the lack of a standard laboratory approach to measurement of STT in small animals.

The author reviewed other studies to try to identify and learn from researchers who made modifications to the Schirmer tear test procedure in their work. When working with very small animals it is recognised that it is extremely difficult to accurately perform standard tear testing such as the Schirmer test considering the very small palpebral fissures (PFL) involved.

da Silva, Sandmeyer, Gionfriddo, Montiani-Ferreira and Galera, (2013)[4] investigated tear production in canine neonates (four week old pups) using a modified Schirmer tear test. The objective of their study was to ascertain whether new-born dogs have measurable aqueous tear production at the fourth week of life. Because they were working with very small animals they developed a modified Schirmer tear test (mSTT) as an aid to conducting their study.

The modified strip was obtained by aseptically transecting a standard commercial STT strip measuring 5 x 35mm while still in its original sealed package with a ruler and scalpel. This gave two 2.5 x 35mm long strips, using only the “unnotched” half in their study. Referring to this as an mSTT test strip they carried out both STTI and mSTT measurements firstly on the adult dogs in which the length of wetting was measured and recorded in millimetres after 1 minute. The purpose was to check the performance of the modified strips versus the standard strips. This could only be done on the larger adult dogs eyes. The modified strip occupied approximately 50 – 75% of the palpebral fissure. da Silva et al[4] did "not believe that the decreased surface area of the half-width STT strip caused significant alterations in the wetting pattern, demonstrated by a difference of only 2.45 ± 0.68mm between the mean mSTTI and the mean STT values in the adult group"[4]. It was noted that "manually trimming the STT strips *per se* might directly affect the results if the strips are unevenly trimmed, increasing its variability as previously alluded to in a previous report on ferrets[9]".[4] Human error can give different mSTT strip widths if manually cut and this could affect test results. However "commercially manufactured mSTT's would help prevent potential variability in the acquisition process of mSTT".[4] The results of the STT and mSTT measurements conducted on the adult dogs were as follows. The mean STT calculated was 21.45 ± 3.42mm/min with values ranging from 15 to 29mm. The mean mSTT calculated was 23.25 ± 3.5mm/minute with values ranging from 17 to 30mm/min. "The mean mSTTI value did not differ significantly (P = 0.10) from the mean STT in these patients".[4]

In conclusion the study showed "that the mSTT, advocated in some birds due to their small palpebral fissures[6], could successfully measure total tear production in canine newborns".[4] This was a positive result overall and vindicates the concept of seeking to have standardised commercially available mSTT strips for working with small animals.

Inspired by a number of the above previous studies which showed that guinea pigs have a very low threshold of corneal sensitivity and nearly no reflex tearing compared to dogs, cats and horses Wieser, Tichy and Nell, (2013)[16] embarked on a fresh study of this topic. The aim of their study was to determine whether a correlation existed between corneal sensitivity and the quantity of reflex tearing which could be applied to a wider range of animals. They recognised that in the majority of previous studies only one parameter was investigated. Therefore no correlations were made between parameters such as corneal sensitivity and quantity of reflex tearing, nor were values for the different species assessed[16]. Their study population was 160 animals of 8 different breeds (guinea pigs, dogs, cats, rabbits, goats, sheep, cows and horses) using about 20 animals per species. They measured the corneal touch threshold (CTT) using a Cochett-Bonnet esthesiometer. They measured the length of the palpebral fissure (PFL) with a calliper ruler. An interesting innovation was in conducting the Schirmer tear test (STT) they modified the test strips by adapting the width of the STT strip to the PFL of each species. In addition they measured STTII after the application of anaesthesia. This author reviews this new STT measurement methodology in paragraph 2.1 below. The results of the Wieser et al[16] study were disappointing overall in that no assured correlation between the corneal sensitivity and the quantity of reflex tearing could be found. The high variance and low reproducibility of the results suggest that the measuring devices are inappropriate to assess the evaluated parameters. They concluded that factors other than corneal sensitivity have a higher impact on reflex tear production. Finally, by using modified STT strips to fit the PFL of each individual species, the study concluded that accurate readings could be obtained in all species, which was not possible with the standard single size STT strip for all species.

This study recommended that the modified or "small test strip should be used in all small species like chinchillas, rabbits, rats, and hedgehogs or similar-sized species. The PFL should be evaluated beforehand, and the adequate STT width calculated with the given formula".[16]

One would have expected the results of STT measurements in the above four studies on guinea pigs to show a higher degree of consistency. However this is not the case as the measurements of STT for guinea pigs reported in all four studies varied widely (see Table 3). All these studies conducted STT measurements as part of their work however all four researchers appear to have adopted slightly different measurement methodologies. As a result the reported STT values from all four studies vary appreciably (see Table 3). Table 2 summarises the type and source of paper used in these studies. A comparison of the four STT measurement methodologies and the results obtained are presented in paragraph 2.1 below.

Table 2: Type of commercially available Schirmer tear test strips used in each study.

Guinea Pig Study	Schirmer Tear Test Product Used
Trost et al, (2007)	Vetoquinol- Österreich Ges.m.b.H., Vienna, Austria
Coster et al, (2008)	Schering-Plough Animal Health Corp, Union, NJ. *lot # 6090621*
Williams & Sullivan (2010)	Schering-Plough Animal Health Corp, Union, NJ. *lot #2051527*
Wieser et al, (2013)	Intervet Inc Merck Sharp & Dohme (MSD). *lot # 9042325*
da Silva et al, (2013)	Ophthalmos®, São Paulo, SP, Brazil

2.1. Comparison of STT measurement methodologies and STT results

The following summarises the methodologies and results found for that group of four studies which conducted STT measurements specifically on guinea pigs:

(a) Trost, Skalicky and Nell, (2007)[15] used standard commercial tear test strips which measured 5mm wide (Vetoquinol- Österreich Ges.m.b.H., Vienna, Austria) for conducting the STT test. Both eyes were tested in random order. The distal end of the strip was bent and inserted into the lower conjunctival cul de sac. The strip was removed after one minute and the amount of wetting recorded. Due to the small amount of tears in the eye the notch of the tear strip was sometimes not reached. The distance from the notch back to where the strip was moistened was measured. These values were recorded as "negative values". Measurements were made for STTI and STTII after local anaesthetic was applied. The reference range measured for STTI was -1.78 to 2.5mm and the mean value calculated was 0.36 ± 1.09mm. This was considered to be a low value versus other animals (dog 18.64 ± 23.9mm, cat 16.92 ± 14.7mm). For STTII the reference range measured was -2.1 to 2.96mm and the mean value calculated was 0.43 ± 1.29mm. The difference between STTI and STTII was not significant (P=0.79) which "suggests there is no reflex secretion"[15] in guinea pigs. In addition no significant differences were found between the left and the right eye in all tests performed.

Trost et al[15] believed "it is important to measure values lower than 0mm exactly, because if all the negative values were recorded as 0mm, the mean STT I and II would be recorded as higher than their actual value. The mean STTII would be 0.67mm instead of 0.36mm, and for the STTII a mean value of 0.81mm instead of 0.43mm would be recorded."[15] In addition the minimum value of the reference range would obviously be 0mm[15]. This author agrees that the total amount of strip wetted should be measured but believes it should be recorded in total as a positive number rather than a negative value.

(b) Coster, Stiles, Krohne and Raskin, (2008)[3] used tear test strips (notched) manufactured by Schering-Plough to conduct the STTI. These were 5 x 35mm strips and had a scale imprinted on them. Both eyes were tested for every guinea pig. The test strip was folded at the notch and placed in the inferior conjunctival fornix. The eyelids were held shut for one minute and the length of the wetted portion of the strip was determined by comparison with the scale imprinted on the strip. A value of 0mm/min was recorded for eyes in which wetting did not reach the notch on the strip. A value of 1mm/min was recorded for eyes in which wetting stopped at the notch on the strip. In the authors opinion recording measurements in this manner is not an accurate methodology. The range for STTI measured in the right eye was 0 to 11mm/minute and 0 to 12mm/min for the left eye. The Median STTI value reported was 3mm/min. This author suggests that greater measurement accuracy would have been achieved by using a modified 2mm/ 2.5mm wide strip.

There is a marked contrast in the STT values recorded by Coster et al, (2008)[3] and Trost et al, (2007)[15] (see Table 3). This they attributed potentially to inconsistencies or “to differences in the manufacturer, design and absorptive capacities of the tear strips used in the two studies[5]”[3]. The Hawkins and Murphy (1986)[5] *in vitro* study demonstrated these manufacturing inconsistencies clearly when they conducted tests on a range of strips. However with modern production techniques this author believes that standardization of strip manufacture is more achievable today and manufacturing inconsistencies probably plays less of a role now than the differences in how the actual tear wetting measurement values themselves were recorded in each study (particularly when wetting did not reach the notch or beyond the notch).

(c) Williams and Sullivan, (2010)[17] also used STT strips manufactured by Schering-Plough (Batch No. 2051527). These were normal 5mm wide standard unmodified strips as per Coster et al, (2008)[3]. Both eyes were tested but only the average resulting measurement was recorded for each animal. This study does not mention the measurement technique used or if negative values were used for below the notch wettings. This makes a comparison with data reported in other studies difficult. The only useful STTI measurement reported was as follows. For the test a sample of 50 randomly selected guinea pigs with normal healthy eyes were chosen from the 1000 population in the study. The mean STTI calculated was 3.8 ± 1.3mm/min. Williams noted the substantial difference in the mean values for STTs determined in his study from those reported by Trost et al[15] and Coster et al[3] and was closer aligned to Coster’s results than those of Trost. He attributed this difference to the breed specificity in the Trost study. This author is of the opinion that these differences are more likely attributable to the difference in the measurement technique of the wetted strips i.e. the use of negative values by Trost et al[15]. This author recognises that breed specificity may play some role as was seen in previous studies on tear production in rabbits[1], which demonstrated “that different breeds can have substantially varying STT values and the same may be the case in guinea pigs; here is an opportunity for further research.”[17].

(d) Wieser, Tichy and Nell (2013)[16] used standard STT strips 5mm wide by 40mm, manufactured by Intervet (single batch). An interesting innovation is they modified the size of the test strip to suit the species. The width of the strip was adapted to the size of the palpebral fissure length (PFL) for each of the 8 species measured. They used a mathematical formula to calculate the ideal strip width per species. The palpebral fissure length is the latitudinal measure across the eye and is measured in mm with callipers. The average PFL was measured for 8 species (using 5 animals per species). This data was used as an aid to calculating the optimum size STT strip width for each species. The PFL data was inserted into a formula which they developed to arrive at the strip width. This process optimised the strip width per species which helped to improve the accuracy of STT measures for each species.

For dogs and cats they used standard Intervet strips (5mm wide by 40mm long). However for cows, horses, sheep and goats they manually constructed their own strips from Whatman® No.41 filter paper. They used 10mm wide by 100mm long strips for cows and horses. They used 6.5mm wide by 70mm long strips for sheep and goats. For guinea pigs they used the original Intervet strips but cut them manually to strips 3mm wide. In the author's opinion Wieser et al[16] may have introduced a compatibility question mark into their results because Intervet strips and Whatman® No. 41 paper do not necessarily have the same absorptive and other characteristics. The study did not report whether any calibration tests were conducted to validate this.

In evaluating tear production (STTI) the strip was bent at the notch and placed in the middle third of the lower conjunctival sac for 1 minute. "The length of the wetted paper was measured including the rounded end beyond the notch"[16] i.e. the total length was measured. A second strip was used to measure the STTII after application of anaesthesia. The quantity of reflex tearing, defined as the difference between STTI and STTII, was calculated in percentage terms for each species.

Test results reported for guinea pigs was a mean STTI of 9.65 ± 3.48mm and a mean STTII of 10.45 ± 3.27mm using the 3mm wide modified strips. Rabbits displayed the biggest decrease in reflex tear production (STTI – STTII) followed by sheep and dogs. The guinea pig showed no decline but a small gain (-16%).

Of the 8 species tested sheep were found to have the most sensitive cornea followed closely by cows, horses, goats, dogs and cats. There were only small differences between the 8 species. However guinea pigs and rabbits were found to have a significantly higher CTT, meaning they have low corneal sensitivity, than the other 6 species. In the final analysis "a small but significant correlation" was found between the corneal touch threshold (CTT) and reflex tearing (STTI – STTII). They concluded that factors other than corneal sensitivity have a higher impact on the reflex tear production.

Table 3: Results of key guinea pig studies.

Study	**Mean STTI mm/min**	**Reference range STTI mm**	**Mean STTII mm/min**	**Reference range STTII mm**	**Measurement method**
Trost et al, (2007)	0.36 ± 1.09	-1.78 to 2.5	0.43 ± 1.29	-2.1 to 2.96	Std 5mm strip Negative if < 0
Coster et al, (2008)	3 (Median)	0 to 12	N/A	N/A	Std 5mm strip Value = 0 if < 0 Value = 1 if = 0
Williams & Sullivan, (2010)	3.8 ± 1.3	N/A	N/A	N/A	Std 5mm strips Normal wetting (>0)
Wieser et al, (2013)	9.65 ± 3.48	N/A	10.4 ± 3.27	N/A	Mod 3mm strip Total wetting (< notch)

2.2. Formula for calculating optimal STT with per species

Wieser, Tichy and Nell (2013)[16], developed a mathematical formula for use in formulating the optimal size Schirmer tear strip for each individual species. This was a very innovative approach to resolving the question of what is the ideal STT paper width to suit very small animals which have very small eyes. It is therefore worthy of separate note in this review.

The only variable in this formula is PFL which varies by species. The study hypothesized that "as dogs and humans have a similar PFL and STTI and STTII work well in these species, the width of the original STT strips (5mm) was taken as appropriate in relation to the size of the canine PFL"[16] (mean 21mm). In other words they selected the dog as the base case for the standard STT strip width and the strip width for all other species was calculated using their formula. Based on this PFL and STT ratio in the dog, they used the following formula to calculate the ideal STT width:

$$\frac{\boldsymbol{X}\ (\boldsymbol{mm})}{\boldsymbol{PLF}\ (\boldsymbol{mm})} = \frac{\mathbf{5}\ (\boldsymbol{mm})}{\mathbf{21}\ (\boldsymbol{mm})} \qquad \boldsymbol{X}\ (\boldsymbol{mm}) = \left(\frac{\mathbf{5}(\boldsymbol{mm})}{\mathbf{21}\ (\boldsymbol{mm})}\right) \times \boldsymbol{PLF}\ (\boldsymbol{mm})$$

Where,

X is the width of the STT strip for new species (mm);
PFL of the new species (mm);
5(mm) is constant width of original STT; and
21(mm) is constant mean PFL of dog.

In this way they arrived at an optimal strip width for each species and manually cut them from Whatman® No. 41 paper.

2.3. Corneal Touch Threshold Test and relationship with STT values

Corneal sensitivity is defined as the minimum pressure that may be exerted against the cornea which can just be felt and is measured in mg/mm^2.[8]

The capability of the cornea to respond to stimulation or touch is assessed by an aesthesiometer. It measures the corneal touch threshold (CTT), which is the reciprocal of corneal sensitivity. Sensitivity varies across the cornea, with the centre being the most sensitive[8]. A low value in g/mm^2 indicating a very sensitive cornea is defined as a low CTT value. A high value in g/mm^2 indicating a less sensitive cornea is defined as a high CTT.[11]

The most likely explanation for the lack of difference between STTI and STTII values measured for guinea pigs is attributed to their lower corneal sensitivity (and hence low reflex tearing) than that seen in other species. This is quite plausible as in a study by Strughold, (1930)[14] a direct comparison of corneal sensitivity in various laboratory animal species found that the CCT of guinea pigs was less than ranges found for other species (cats, dogs, pigeons, rats and rhesus macaques). This implies that one needs to apply a higher pressure to the cornea to elicit a blink reflex. Trost et al, (2007)[15] found that corneal sensitivity, although quite low in relation to other species, was significantly higher in the centre than in the four limbal regions. Their results also raised a question about corneal sensitivity in guinea pigs. This relatively low corneal sensitivity in guinea pigs may account for the lack of effect of topical anaesthesia on their tear test values. "If reflex tearing contributed substantially to tear test values in guinea pigs, corneal anaesthesia should result in a lower STT value".[3] The corneal sensitivity in guinea pigs is lower than in dogs and other domestic animals but the values are fairly similar to brachycephalic cats.[15] The question which arises is whether this low CTT value "is the only explanation for the low reflex secretion?".[15]

A dog blinks at a rate of 3 – 5times/min. By comparison a guinea pig's eye which blinks at a frequency of 2 to 5 times in 20 min is very low. If excited the guinea pigs eyes will have an increased blink frequency as is the case with other animals (e.g. a restrained dog blinks at a rate of 10-20 times/min.[15] "Blinking maintains the physiologic thickness of the preocular surface by spreading the tears over the corneal surface. As regards the small STT values, the low eye blink frequency in rest, and missing signs of evaporation or corneal dryness, the question arises about quantity and quality of the lipid and the mucin layer of the guinea pig tears, which need to be evaluated in a further study".[15]

2.4. Conclusions

In these four separate studies of guinea pigs it is evident that each study adopted a distinctly different method for measuring and recording STT data. It is this author's opinion that the key difference between the results of the four studies is mainly attributable to the differences in how the tear wetting measurement values were recorded in each study particularly when wetting did not reach the notch or extend beyond the notch. Another observation by this author is that none of these studies conducted a baseline test to calibrate their STT strips versus some standard such as distilled water or saline.

One very positive innovation was the development of the modified STT strips and the corresponding mSTT measurements. One can conclude that a standardized method for recording low values (≤ notch) is necessary in Schirmer tear testing, particularly in smaller companion animals or exotic pets that have smaller eye globes and smaller palpebral fissure lengths. A smaller modified STT strip would greatly facilitate this. The ability to set definitive reference values using a new standardised STT measurement methodology using modified widths would better enable recognition of signs of ocular disease in guinea pigs.

3. Own Investigations

3.1. *In Vitro*

3.1.1. Materials and Methods

Hamilton Microliter syringes (1µl, 5µl, 10µl, 15µl)

Glass Petri Dish,

Distilled water (22^{o}C), Room Temperature (23^{o}C)

Disposable latex examination gloves,

Stopwatch,

Thermometer.

Eickemeyer tear test strips Lot No: MIPL/A1/05

- 20 regular unnotched 5mm x 35mm strips
- 20 modified 2.5mm x 35mm strips (cut manually)

Method

For the unlimited fluid source a medium-sized glass petri dish was filled with distilled water. Strips were held using forceps and submerged up to the zero mark on the tear strip for one minute. Wetting values were recorded at both 30 second and 1 minute intervals. This was performed five times for each group of strips.

When testing under limited fluid conditions Hamilton microliter syringes (1, 5, 10 and 15µl) were used to draw up and apply set values of distilled water to the base of each strip. This was performed five times for each of the four µl values. Wetting values were recorded after 30 seconds and 1 minute had elapsed.

Figure 1: Schirmer tear test strips transected into two 2.5mm wide/ 35mm long strips.

3.1.2. In Vitro Results

Table 4: Summary of the results of the *in vitro* investigation.

	Normal Strip	30sec	1min	Modified Strip	30sec	1min
Unlimited volume distilled water	**#1**	19	26	**#1**	20	27
	#2	20	28	**#2**	21	29
	#3	19	26	**#3**	19	28
	#4	19	27	**#4**	19	27
	#5	18	27	**#5**	21	28
1µl	**#1**	1	1	**#1**	5	6
	#2	-1	-1	**#2**	3	4
	#3	1	2	**#3**	4	5
	#4	0	1	**#4**	3	4
	#5	0	0	**#5**	3	4
5µl	**#1**	6	7	**#1**	13	15
	#2	6	7	**#2**	15	16
	#3	7	8	**#3**	13	15
	#4	7	8	**#4**	14	16
	#5	6	7	**#5**	14	16
10µl	**#1**	14	15	**#1**	20	26
	#2	13	15	**#2**	20	26
	#3	15	17	**#3**	21	26
	#4	14	15	**#4**	21	29
	#5	15	17	**#5**	21	25
15µl	**#1**	19	23	**#1**	23	30
	#2	20	25	**#2**	23	31
	#3	19	23	**#3**	25	34
	#4	20	24	**#4**	24	33
	#5	19	22	**#5**	23	29

Figure 2: Graph exhibiting wetting of STT and mSTT strips with unlimited volume of distilled water.

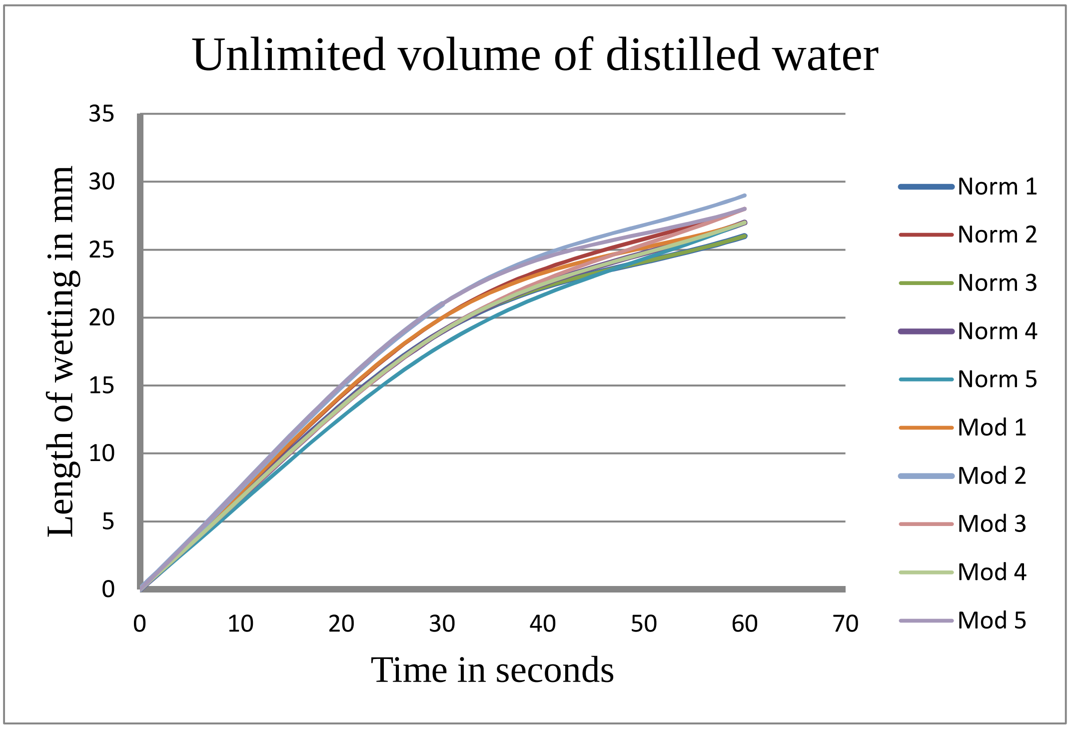


Figure 3: Graph exhibiting wetting of STT and mSTT strips with 1µ of distilled water.

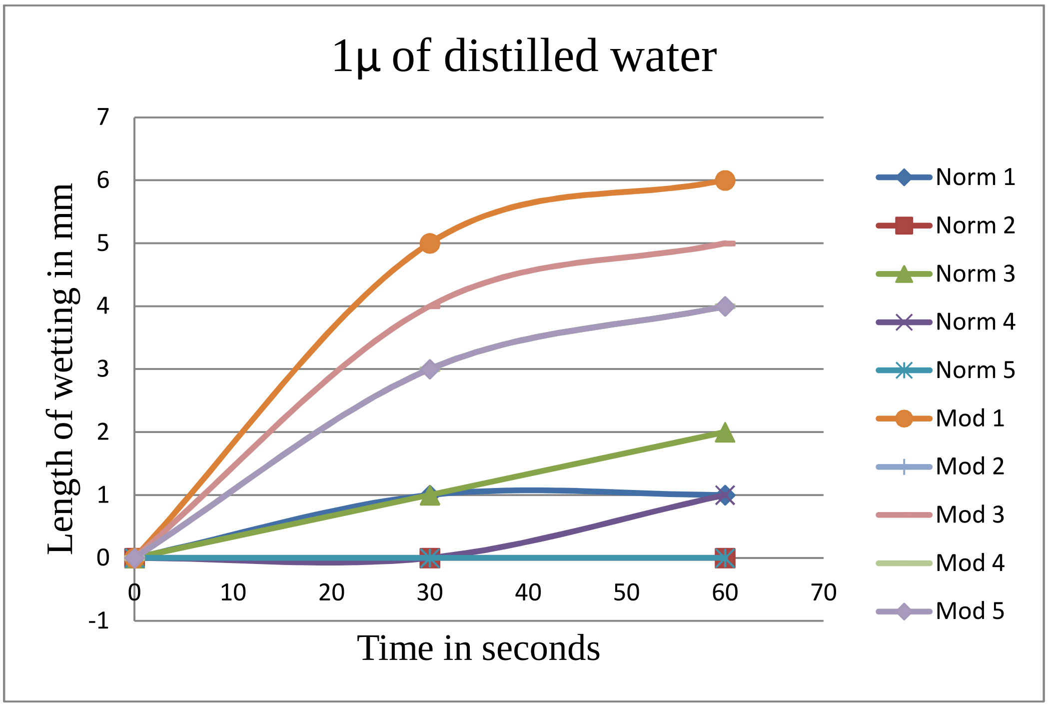


Figure 4: Graph exhibiting wetting of STT and mSTT strips with 5µ of distilled water.

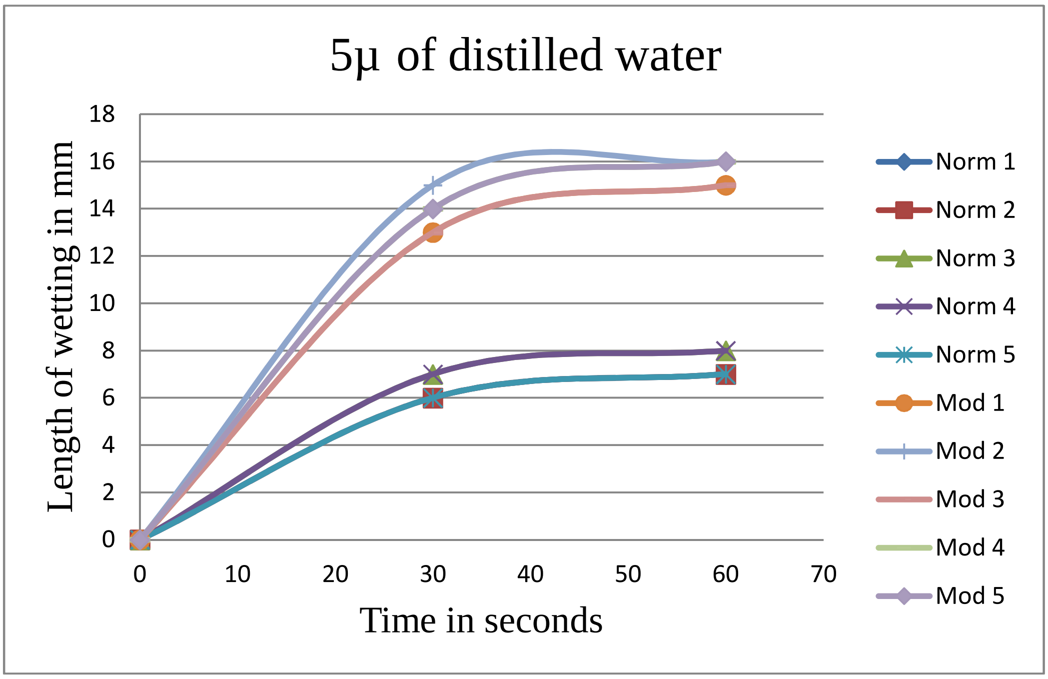


Figure 5: Graph exhibiting wetting of STT and mSTT strips with 10µ of distilled water.

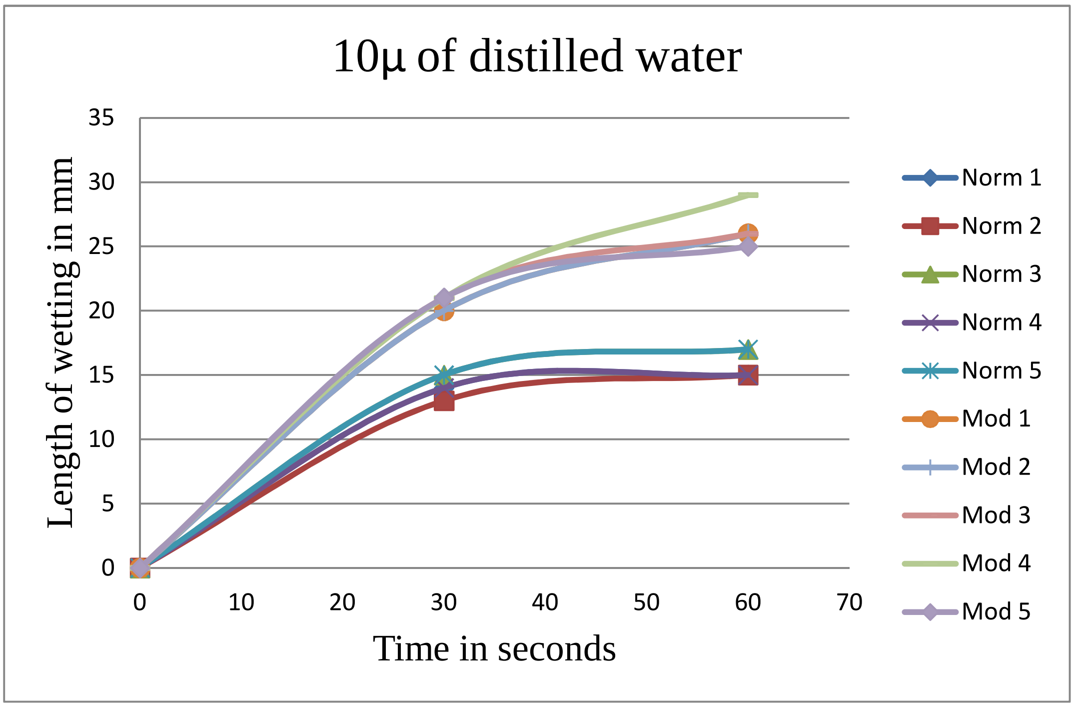
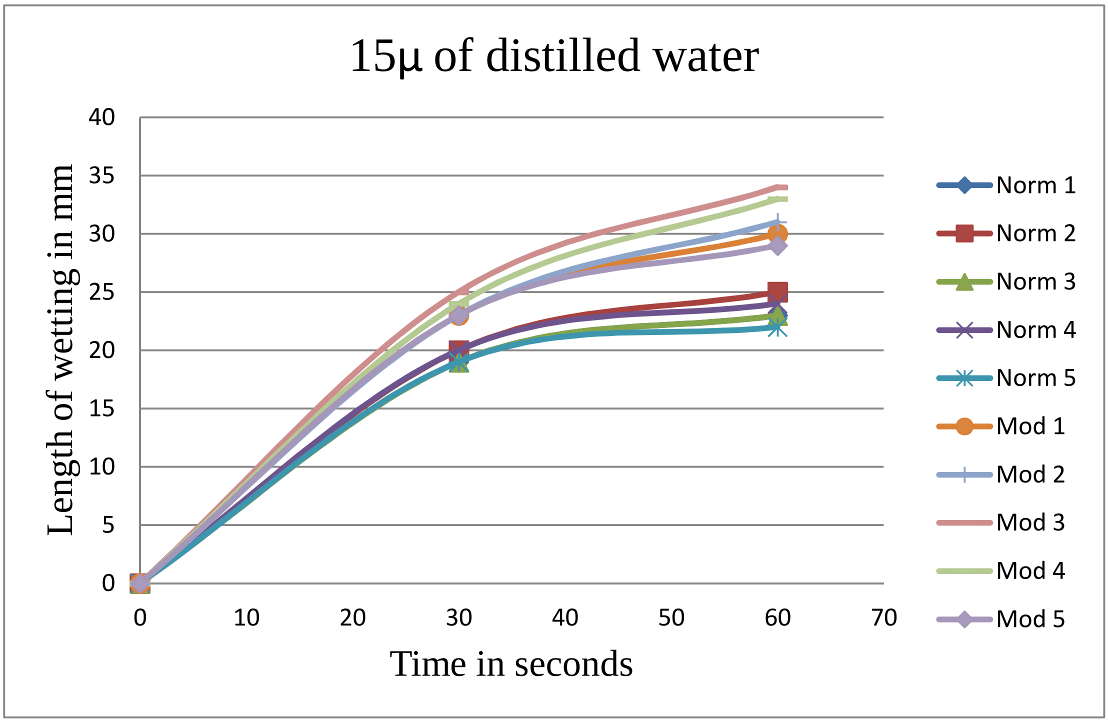


Figure 6: Graph exhibiting wetting of STT and mSTT strips with 15µ of distilled water.



3.2. *In Vivo*

3.2.1. Materials and Methods

Animals

Twenty mixed breed Guinea pigs were enrolled in this study. 10 females and 10 males were selected at random from the Guinea Pig Village population at the Budapest Zoo and Botanical Gardens.

The females were all adults with an age range of roughly 11 months to 5years and an average body weight of 735.4g. Adult sows typically weigh between 700-900g. The males selected however were all juvenile. The plan was to have all adult males also but circumstances on the day at the zoo were such that only this batch of juvenile males was available for the study. The males were aged approximately 2 to 6 months with an average body weight of 423.8g which is a little less than half the average weight for an adult male guinea pig. An adult male boar typically weighs in the range of 900-1,200g. This unplanned change introduced an interesting result in this case and is discussed below.

No topical medications were used in the guinea pigs eyes prior to or during the test. The population consisted of healthy guinea pigs with no visible signs of ocular problems and which had not undergone any recent surgical procedures.

Equipment

Standard sterile Schirmer strips used were **Eickemeyer** tear test strips Lot No: MIPL/A1/05.

Modified Schirmer strips were obtained by manually cutting the standard commercial strips in half with scissors after first scoring a central line with a metal ruler and blunt edge of a scalpel blade while the strips remained in their original sealed package. This gave two equal sized strips measuring 2.5mm x 35mm long, see Figure 1.

Disposable latex examination gloves, stopwatch and thermometer.

Method

For all ophthalmic testing and clinical data acquisition, each Guinea pig was immobilised and gently restrained by an experienced zoo handler, with care taken to ensure the animal was comfortable. Testing was delayed if any animal became too difficult to physically restrain, or if they became excessively agitated or vocal. All examinations and measurements were performed by the same single individual (the author) to avoid any discrepancies. The time of day (10am-1pm) and room temperature (17^{o}C) were also recorded.

The sequence of procedures performed in the study was:

(i) Ocular inspection (including blink frequency observation),
(ii) Schirmer tear test (STT$_I$) and modified tear test (mSTT$_I$),
(iii) Each guinea pig was weighed.

The initial brief ophthalmic inspection of each animal revealed normal ocular findings. No swelling or redness of palpebrae, staining of fur, pus or excess tearing was noted. Before opening the individually wrapped packets of unmodified strips, the strips were first bent while still in their wrappers. The purpose of this was to maintain the sterility of test strips and not to expose the strips to any superficial foreign material prior to insertion in the guinea pig eye.

After bending the distal end, the strips were inserted into the medial lower lid of the eye into the conjunctival pocket. They were gently held in this position for 60 seconds. The measurement of the amount of wetting was immediately recorded in millimetres on withdrawal of the strip from the eye.

The STT was performed first in both eyes in a randomly selected order, alternating between left or right first. After both eyes were tested, the guinea pig was returned to a small holding pen to rest and refresh its eyes while the rest of the animals were tested. After one set of strips was used in all 10 animals (of one gender), these animals were then tested again using the modified strips. The mSTT was performed in the same manner as the STT and the wetting area was measured and recorded in mm after one minute. The second eye was measured using the same technique.

Figure 7: L to R: Guinea pig restraint, STT, mSTT.

3.2.2. In Vivo Results

The results of the ophthalmic diagnostic testing are summarized below.

Table 5: Summary of the results of the *in vivo* investigation in females.

FEMALES	**Female Body Weight grams**	**STTI Right Eye mm/min**	**STTI Left Eye mm/min**	**mSTTI Right Eye mm/min**	**mSTTI Left Eye mm/min**	**Both Eyes STTI mm/min**	**Both Eyes mSTTI mm/min**
Minimum	666	1	-1	2	2	-1	2
Median	739	3	4	9	7.5	3	8.5
Maximum	794	6	9	16	18	9	18
Mean	735.4	3	3.9	9.2	8.7	3.45	8.95
Std. Deviation	43.33	1.633	2.846	3.853	4.218	2.305	3.94
Std. Error of Mean	13.7	0.5164	0.9	1.218	1.334	0.5154	0.881
P value	0.6446	0.691	0.9275	0.663	0.1173	0.518	0.3683

Table 6: Summary of the results of the *in vivo* investigation in males.

MALES	**Male Body Weight grams**	**STTI Right Eye mm/min**	**STTI Left Eye mm/min**	**mSTTI Right Eye mm/min**	**mSTTI Left Eye mm/min**	**Both Eyes STTI mm/min**	**Both Eyes mSTTI mm/min**
Minimum	321	0	1	0	1	0	0
Median	413.5	2	1	2	2	1.5	2
Maximum	633	4	2	3	4	4	4
Mean	423.8	1.7	1.3	1.7	2.2	1.5	1.95
Std. Deviation	95.36	1.16	0.483	0.8233	1.033	0.889	0.945
Std. Error of Mean	30.16	0.3667	0.1528	0.2603	0.3266	0.1987	0.2112
P value	0.0717	0.6133	0.1873	0.2878	0.7517	0.0577	0.8897

For normally distributed data, results are reported as mean ± SD and comparisons between means were made using unpaired T test results (2-tailed). For non-normally distributed data, results are reported as median ± Interquartile Range (IQR) and comparisons between medians were made using the Mann-Whitney test. Correlations were made using Pearson's correlation co-efficient. Within this project a P value <0.05 was determined to have reached significance. Data was entered into an excel spreadsheet as described previously. Statistical analyses were performed in GraphPad Prism version 6.0b for Mac OS X and multiple regression analysis was performed using GraphPad InStat version 3.10 32 bit for Windows (GraphPad Software, La Jolla, California, USA, www.graphpad.com).

Figure 8: Scatter graph exhibiting wetting of STT and mSTT strips in both eyes in females.

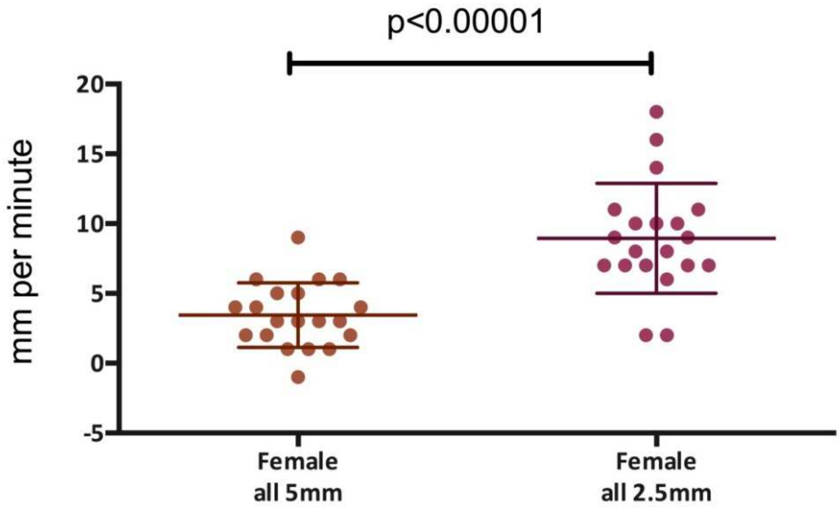


Figure 9: Scatter graph exhibiting wetting of STT and mSTT strips in both eyes in males.

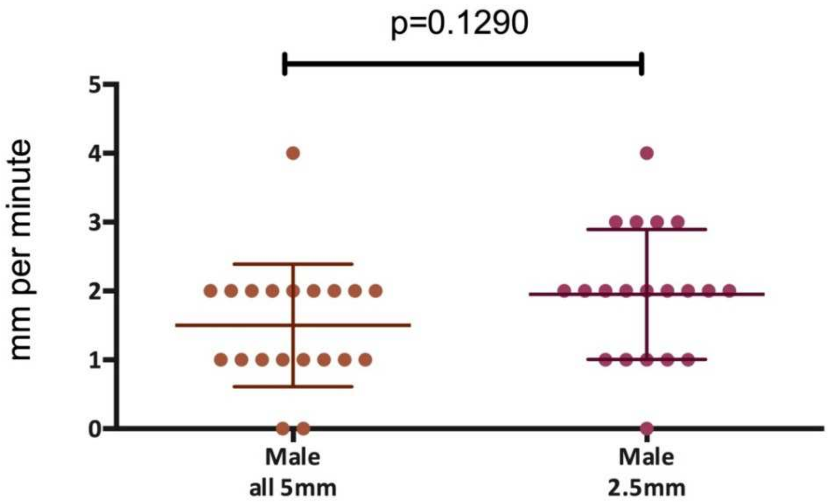
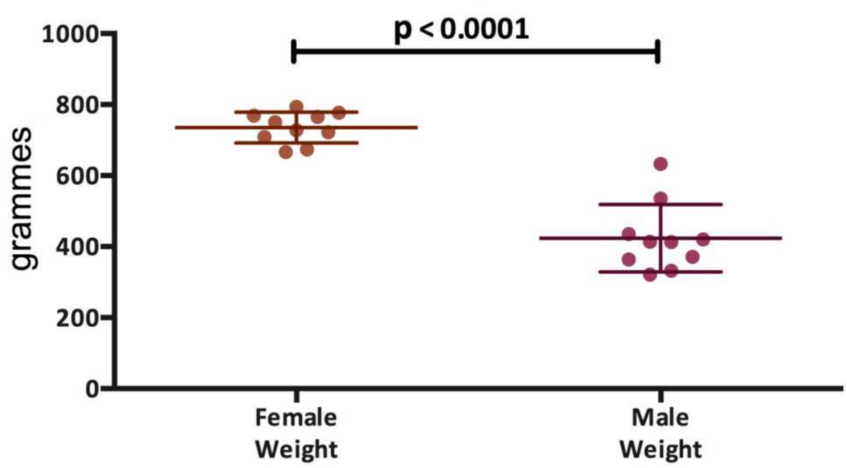


Figure10: Scatter graph exhibiting weight distribution of females and males.



3.3. Discussion

IN VITRO**:** The purpose of these tests was to observe the kinetics of strip wetting of the standard and the modified STT strips in both a limited and unlimited fluid supply situation. This was done primarily to check that the performance of these strips, such as the absorptive capacity, are identical and not compromised in any way due to modification. The *in vitro* test was performed using standard and modified test strips from the same batch.

Figure 2 illustrates that for the unlimited supply situation the performance of the standard and modified strips are essentially similar in that their graphs are generally overlapping (within the bounds of accuracy of this experiment). Figures 3 to 6 demonstrate that in the limited supply situation the graph for the modified test strip lies consistently above that for the standard strip. From this one can conclude that the modified strips enable extended wetting of the test strip thus enabling a better reading of STT values with a greater degree of accuracy.

The initial 30seconds of wetting demonstrated a fast uptake of fluid, followed by a more gradual absorption between 30seconds and 1 minute. This is illustrated well in the series of graphs above from Figures 2 to 6. These results are consistent with similar findings in more detailed studies conducted by Williams and Sullivan, (2010).[17]

IN VIVO**:** The average weight of female guinea pigs was 735.4grams while the average for males was 423.8grams. It transpired that the group of male guinea pigs selected for the project were all juveniles, aged in the range of 2 to 6 months. The average weight for the male group was less than half the average weight of a full grown adult male guinea pig (900-1,200g). It was not planned to use a juvenile group. The male and female groups therefore must be considered as two distinct population groups and their STT results interpreted separately rather than jointly as a total study group.

The Mean STTI for females measured with the standard strip was 3.45mm ± 2.3 and the Mean mSTTI for the modified strips was 8.95mm ± 3.94.

The Mean STTI for males measured with the standard strip was 1.5mm ± 0.89 and the Mean mSTTI for the modified strips was 1.95mm ± 0.95.

The results for the male guinea pigs were markedly different (lower) to that of the females. This is attributed to the fact that the males were all juveniles and is consistent with results found by da Silva et al[4] when STTs were measured for canine neonates and compared with adult canines. Their conclusion was that tear secretion in canine neonates, and possibly most species, is lower than that for adults of the species.

Results obtained for the standard STTI values in the adult female group in this investigation were very similar to those reported by Williams et al[17] (3.8 ± 1.3mm) and Coster et al[3] (Median 3mm), which corroborates their finding. Wieser et al[16] was the only study to report a value for STTI using a modified 3mm wide strip recording a mean of 9.65 ± 3.48mm. This compares closely with this study which measured a mean STTI of 8.95mm ± 3.94mm using the modified 2.5mm wide strip.

The STTI values reported in this study are for guinea pigs with healthy eyes and no visible signs of ocular disease. The results of this study provide some additional information on the use of a modified Schirmer tear test strip in conducting Schirmer tear tests in guinea pigs. Values found in this study reflect well with those previously reported in other studies, (see Table 3).

4. Summary

The kinetics of strip wetting of standard and modified Schirmer strips are exactly similar and clearly show an initial rapid absorption rate of fluid followed by a moderate absorption rate as the rate of absorption slows down.

The results of the study show that a modified Schirmer strip can be successfully used to measure tear production in guinea pigs and small rodents. The STTI and modified STTI measurements found in this study are broadly in line with similar findings by others. The modified strips can provide a greater degree of accuracy when performing STT measurements in small rodents. A commercially available modified strip would be preferable to manually modified strips as it would eliminate the variability factor and would be of great benefit to assist with ocular testing of small rodents.

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6. Abbreviations and Acronyms

BUT	Break Up Time
CTT	Corneal Touch Threshold
FS	Fluorescein Staining
IOP	Intraocular Pressure
KCS	Keratoconjunctivitis sicca
PFL	Palpebral Fissure Length
PRT	Phenol Red Thread Tear Test
STT	The Schirmer Tear Test
STTI	The Schirmer Tear Test, performed without topical corneal anaesthetic
STTII	The Schirmer Tear Test, performed with topical corneal anaesthetic
mSTT	Modified Schirmer Tear Test

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