THE USE OF THINPREP® TECHNIQUE FOR THE EVALUATION OF CONJUNCTIVAL CYTOLOGY

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ABSTRACT

Background: Conjunctivitis is an inflammation of the membrane that lines the eyelids and covers the outer surface of the eye and is the most common ocular problem seen each year by practicing optometrists and ophthalmologists. Different forms of conjunctivitis are classified as either infectious or non-infectious. Identifying cell types can help to characterize the origin of the conjunctival inflammatory response as either infectious or non-infectious so the patient can be properly treated. Two non-invasive methods, a traditional scrape and smear and a new application of the ThinPrep® technique, have been developed for evaluating the cells of the eye. *Methods:* The patients' palpebral conjunctiva was swabbed with both techniques, testing slightly different areas to evaluate for leukocytes. The purpose of the study was to determine if there was a significant difference between the two techniques in evaluating the conjunctiva of the eye for cell cytology. *Results:* The results indicated that the ThinPrep® technique was significantly different from the traditional scrape and smear when comparing the total number of cells present ($U = 1491.50$, $p=0.0362$), cell preservation, and detecting the presence of neutrophils. However, the results did not show a significant difference when comparing the presence of monocytes, macrophages, eosinophils, and basophils. *Conclusion:* The new application of the ThinPrep® technique proved to be a reliable tool for evaluating the eye for cell cytology.

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Introduction:

Conjunctivitis refers to a broad group of conditions caused by an inflammation of the membrane that lines the eyelids and covers the outer surface of the eye. It is the most common cause of "red eye" in adults and children and is the most common ocular problem seen each year by practicing optometrists and ophthalmologists.[1] Conjunctivitis is characterized by many different symptoms such as eye pain, swelling, excess tearing, mucous discharge, redness and itching. Many of these symptoms are associated with the causes of conjunctivitis.[2] Conjunctivitis can be classified as either infectious or non-infectious. Infectious conjunctivitis accounts for 70% of all cases and is caused by a bacterium or a virus and is considered to be very contagious. Bacterial conjunctivitis accounts for 72% of the infectious cases and is usually caused by species of staphylococci, streptococci, neisseria, haemophilus or chlamydia. Bacterial conjunctivitis is characterized by a rapid onset of conjunctival hyperemia, lid edema, and yellow, mucopurulent discharge.[1,3] Viral conjunctivitis occurs in the remaining 28% of the infectious cases and is usually caused by the rhinovirus, adenovirus, or herpes simplex. It is extremely common and the diagnosis can usually be made clinically, so viral culture and laboratory investigation are rarely utilized. Signs found clinically with viral conjunctivitis are follicles, preauricular adenopathy, scanty discharge and profuse tearing.[3] Non-infectious conjunctivitis accounts for 30% of all cases and can be caused by allergies, chemical exposure, or underlying diseases such as rheumatoid arthritis, lupus, or Crohn's disease.[1] Allergic conjunctivitis is characterized by extreme itching, mucoid white stringy discharge, swollen lids, bulbar conjunctival chemosis and burning.

Diagnosing the proper cause of conjunctivitis is usually done by clinical evaluation and on the basis of signs and symptoms.[2] However, obtaining conjunctival cytology scrapings can help to better characterize the origin of the conjunctival inflammatory response.[4] Laboratory testing should be obtained from patients that do not respond to treatment or have chronic or recurrent conjunctivitis.[1] Scrapings of the conjunctiva are seldom done, mostly because they are expensive and time consuming.[2] Some non-invasive methods of assessing the type of conjunctivitis have been developed. These include a traditional scrape and smear and more recently a new application of the ThinPrep® technique.

The traditional scrape and smear is the most commonly used technique for obtaining cells from the eye for cytological evaluation. The procedure is relatively easy to perform and requires the use of a sterile cotton swab. The scrape is performed by gently pulling on the patient's lower lid, exposing the palpebral conjunctiva and making a firm, uniform swipe of the cotton swab across the exposed area. The cotton swab is then smeared onto a specimen slide, where it can be spray fixed with 90% alcohol to be stained into a Giemsa stain, Wright stain or a Papanicolauo stain.[5] In this tissue stain, conjunctival epithelial cells are present along with a relative number of other cell types such as neutrophils, monocytes, eosinophils or mast cells that can help to properly diagnose the origin of the conjunctivitis.[3]

ThinPrep® was originally developed by Cytyc© for gynecology purposes and today is the most widely used technology for cervical cancer screening. Since its development in 1996, ThinPrep® processor has expanded as an automated slide preparation unit that can be used for non-gynecological specimens.[6] Recent clinical trials

have applied the ThinPrep® method to ocular surface disorders, mostly for dry eyes. The study indicated that the ThinPrep® processor has excellent diagnostic sensitivity and good cell preservation.[7]

When evaluating a conjunctival sample for cell cytology from either the ThinPrep® or the traditional scrape and smear, a relative number of cells present can distinguish between the different forms of conjunctivitis. A relative number of neutrophils, macrophages, or monocytes present would indicate a patient that has a form of infectious conjunctivitis.[8] Mast cells and eosinophils would indicate an allergic conjunctivitis.[5] Fungal elements identify fungal infections and yeast is a sign of a yeast infection. Identifying different cell types can help to identify the origin of the conjunctivitis as either infectious or non-infectious so the patient can be properly treated.[4]

The primary objective of this study was to determine whether there was a significant difference between the ThinPrep® technique and the traditional scrape and smear in evaluating the conjunctiva of the eye for cell cytology and cell preservation.

Materials and Methods:

The 50 study subjects were patients 18 years of age or older not wearing contact lenses (to rule out contact lens infections) that presented with conjunctivitis. Each patient had the same eye evaluated by both the ThinPrep® technique and the traditional scrape and smear technique at the same time, testing slightly different areas of the palpebral conjunctiva. Swabbing the same eye using both techniques was done to ensure that the conjunctivitis was of the same origin and because the infection can be a unilateral

condition as well as bilateral. For a control, patients with no conjunctivitis were swabbed with both techniques to evaluate the types of cells present.

To obtain the conjunctival sample for both techniques, the medial and lateral aspect of the palpebral conjunctiva of the lower lids of the conjunctiva were scraped by either the traditional scrape and smear technique or the ThinPrep® technique. The location was randomized as to medial or lateral with each patient. The slides were then assessed for different cell types to determine whether there was diagnostic agreement with the ThinPrep® automated smear in the diagnosis of conjunctivitis.

The ThinPrep® technique uses a sterile plastic spatula to scrape across the patient's palpebral conjunctiva. The collected sample was then placed directly into a 30 ml PreservCyt Solution Vial® and labeled. The vials were sent to the cytology department at the Dickinson County Healthcare System to be prepared. The specimen was placed in the automated ThinPrep® 2000 processor along with a ThinPrep® filter. Under the control of the instrument's microprocessor, the ThinPrep® filter rotated within the sample vial, separating debris and dispersing the conjunctival cells without adversely affecting the appearance of the cells. A gentle vacuum used negative pressure pulses generated by the ThinPrep® 2000 processor to collect cells from the exterior surface of the filter. After all cells were collected, the filter inverted and gently pressed against the ThinPrep® microscope slide where air pressure caused the cells to stick to the slide. The ThinPrep® 2000 constantly monitored the flow rate through the filter during this process to collect the cells for evaluation. The slide was then evaluated for the presence of different cells.[6] Cytological preparation and evaluation of the conjunctival sample was

done under the supervision of Mrs. Sara Trevillian, a professional cytologist in the lab at the Dickinson County Healthcare System in Iron Mountain, Michigan.

Cytological assessment of the smears from both techniques involved estimating the total number of cells present and how many neutrophils, macrophages, monocytes, eosinophils, or other cells were found. A non-parametric Kruskal-Wallis test was performed to compare between the sum of the ranks of the total number of cells present and the presence of neutrophils between the traditional scrape and smear and the ThinPrep® technique. Each slide was ranked based on the total number of cells present and the presence of neutrophils (Table 1 and Table 2). A comparison between the number of well preserved cells versus air-dried cells from both techniques was also done.

Table 1: Ranking the total number of cells present on each slide for the traditional scrape and smear and the ThinPrep® technique.

Total Number of all Cells	Rank
0	0
<10	1
>10 but <50	2
>50 but <100	3
>100 but <200	4
>200 but <300	5
>300 but <500	6
>500 but <1000	7
>1000	8

Results:

A total of 53 patients had one eye swabbed with both the ThinPrep® technique and the traditional scrape and smear. Test subjects were 33 patients with conjunctivitis

and 20 patients were used as the control with no conjunctivitis. Six of the samples were not used in the data analysis due to lack of cells. The majority of the control patients had rare or no leukocytes present for both techniques. The results of the Kruskal-Wallis test comparing the sum of the ranks of the total number of cells present showed that the ThinPrep® technique and the traditional scrape and smear were statistically different from each other (U stat = 1491.50, p=0.0362). However, there was not a significant difference when comparing the number of leukocytes present between the two different techniques (U stat = 1181.50, p=0.8862).

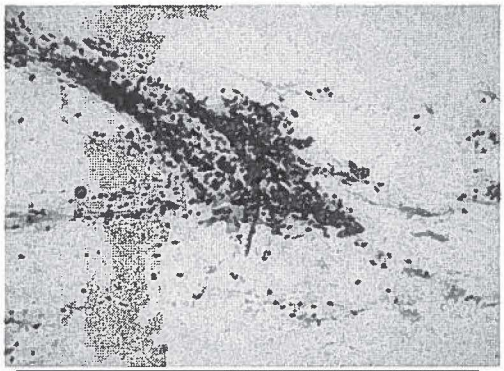

Figure 1: ThinPrep® sample on low power.

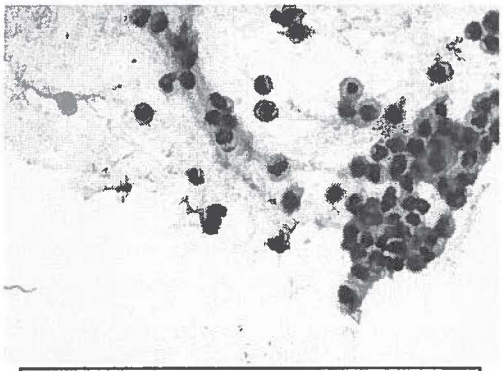

Figure 2: ThinPrep® sample on high power.

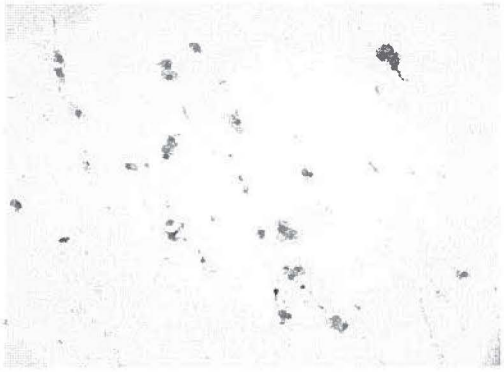

Figure 3: Traditional scrape and smear on low power.

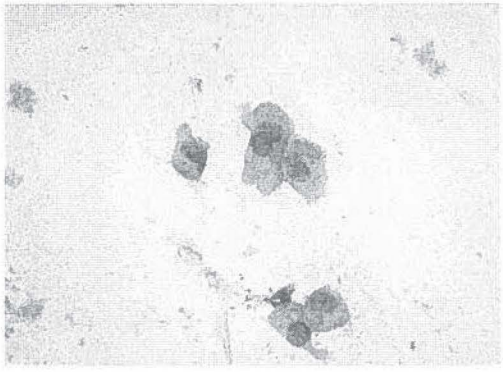

Figure 4: Traditional scrape and smear on high power.

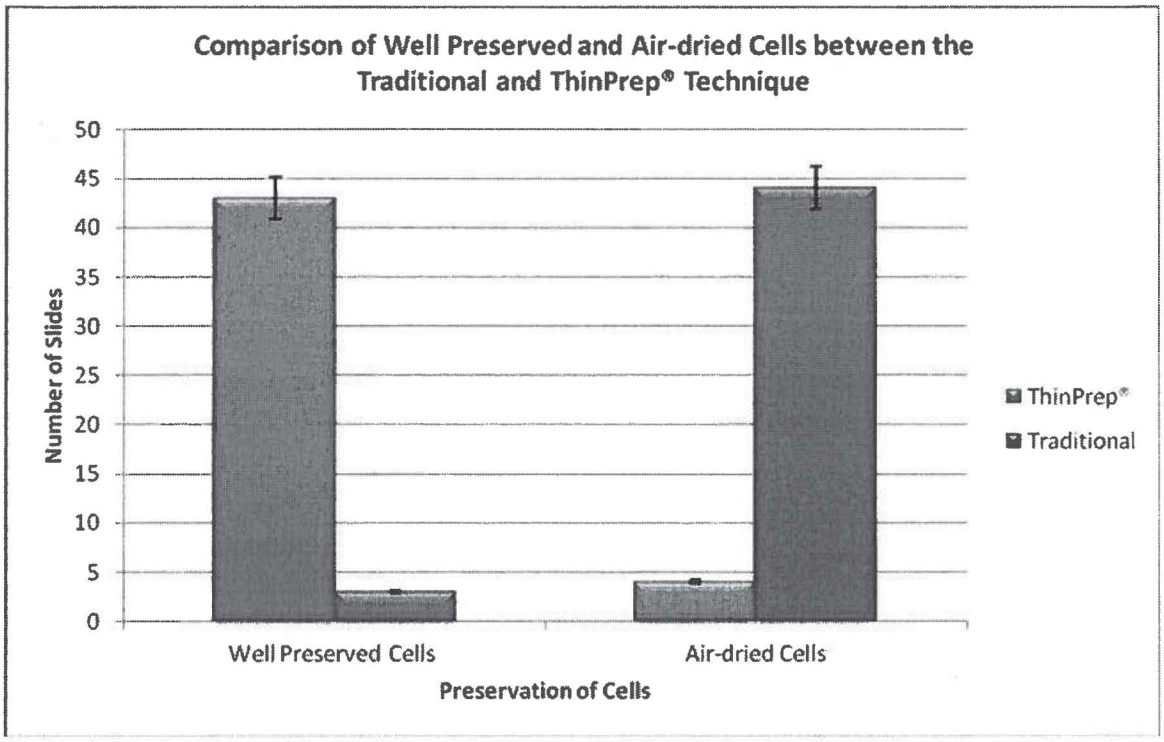

Figure 5: Comparison of cell preservation for both techniques.

The ThinPrep® technique showed a greater number of slides with well preserved cells as compared to the traditional scrape and smear (Figures 1-4). The ThinPrep® technique had 43 slides with well preserved cells as compared to the traditional scrape and smear with only 3 slides with well preserved cells. The traditional scrape and smear yielded the highest amount of slides with air-dried cells with 44 as compared to only 4 slides with air-dried cells from the ThinPrep® slides (Figure 5).

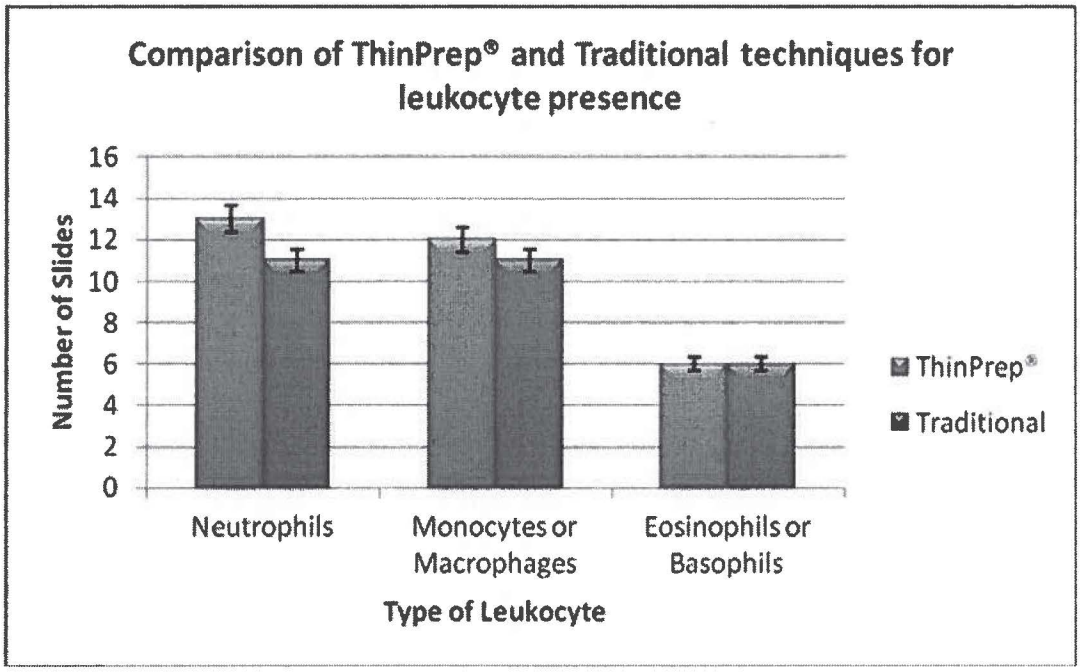

Figure 6: Comparison of leukocyte presence for both techniques.

The ThinPrep® technique demonstrated 13 slides with neutrophils present compared to the traditional scrape and smear that showed 11 slides. When comparing the slides with monocytes and/or macrophages, the ThinPrep® technique yielded 12 slides while the traditional scrape and smear showed 11. The results for slides containing eosinophils and/or basophils were consistent for both techniques with 6 slides (Figure 6).

Discussion:

When comparing the results of the total number of cells present, it was indicated from the Kruskal-Wallis non-parametric test, that the ThinPrep® technique was significantly different from the traditional scrape and smear. The ThinPrep® technique had more cells present on the majority of the slides, which helps to prevent false negative tests when examining slides for different cell types to find a definitive diagnosis for the origin of conjunctivitis. One possible explanation to the difference in the number of cells

present could be the different instruments used to obtain the samples. The ThinPrep® technique used a sterile plastic spatula, whereas the traditional scrape and smear used a sterile cotton swab. The sterile plastic spatula could have picked up more cells than the sterile cotton swab, or possibly some of the cells did not come off of the sterile cotton swab.

Comparing the preservation of cells between the ThinPrep® technique and the traditional scrape and smear, ThinPrep® showed superior results over the traditional scrape and smear for well preserved cells. When diagnosing patients, cytologists optimally require slides with the most well preserved cells so they can make an accurate diagnosis regarding the origin of conjunctivitis. This study supported recent findings of clinical trials that have applied the ThinPrep® method on ocular surface disorders, such as dry eyes. Study results indicate that the ThinPrep® processor has great diagnostic sensitivity and good cell preservation.[7] One possible explanation for the difference between the techniques could be the media on which each one was stored or how the slides were fixed. For the traditional scrape and smear, cells were smeared directly onto a slide with a sterile cotton swab and then spray fixed with 90% alcohol, whereas for the ThinPrep® technique cells were placed directly into a 30 ml PreservCyt Solution Vial® to be stored until they were processed by the ThinPrep® 2000 processor. Another possible explanation for the variation in cell preservation could be attributed to the difference in time between sample collection and analysis. The average length of time was approximately one to two weeks with a maximum of three weeks and a minimum of two days.

When evaluating the different leukocytes present between the ThinPrep® technique and the traditional scrape and smear, the ThinPrep® technique was significantly different in the detection of neutrophils, which would be indicative of bacterial conjunctivitis. The different between techniques was insignificant in the detection of monocytes, macrophages, eosinophils, and basophils. For each sample pair (1 ThinPrep® technique and 1 traditional scrape and smear of the same patient), there was no change in the type of leukocyte present; only whether or not it was present (Figure 6). The traditional scrape and smear failed to yield any leukocytes on three total samples compared to the ThinPrep® technique due to poor cell preservation and overall decreased number of cells present. The majority (80.7%) of the non-control samples were diagnostic of an infectious form of conjunctivitis. The remaining 19.3% were of non-infectious origin indicating an allergic form conjunctivitis.

To further improve on this study, the data should be collected over a longer period of time, and a greater sample size would be ideal for interpreting the results. Analyzing the sample in a uniform time frame could also standardize the data collected.

The ThinPrep® technique proved to be a reliable tool for evaluating the eye for cell cytology. Professional cytologist Sara Trevillian, preferred the ThinPrep® technique over the traditional scrape and smear, due to enhanced cell preservation and reduced time requirements. The ThinPrep® technique also reduces human technical error, because the ThinPrep® 2000 processor automatically fixes the slide with the collected cells. The study could be improved, by collecting more data over a longer period of time, using different instruments for collecting cells on the palpebral conjunctiva, or testing for other ocular problems.

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