

A comparison of follicle mite (*Demodex*) collection methods.

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Abstract: *Demodex* is a genus of microscopic mites that live on human hosts, feeding on sebum and dead skin. They are classified as arachnids with an elongated abdomen that allows them to dwell in pores and hair follicles. The mites do not commonly cause any problems but a diseased state may arise if their population suddenly increases, leading to rosacea, blepharitis, and general dermatitis. Collecting the mites can be difficult and two collection methods were compared. The first method was spreading super glue onto a microscope slide and adhering it to the forehead for five minutes. The second was done by scraping the area on the forehead and transferring the debris onto another slide. For the eight participants, only one *Demodex* was found overall, using the super glue method. Our study concluded that, although mostly ineffective, the super glue method was best to collect and observe *Demodex* from human skin.

Keywords: Demodex, hair follicle mites, dermatitis

Hair follicle mites, belonging to the genus *Demodex*, are a common human ectoparasite living on the surface of the skin and in hair follicles. As arachnids, they have a clear cephalothorax with four pairs of legs and an elongated abdomen, which allows them to reside and feed within the pores of the skin. The two species most commonly found on human hosts are *Demodex folliculorum* and *Demodex brevis*, the former being the most abundant and conspicuous (Lacey et al. 2009). Both species are tiny, around 0.3 mm in length, commensalistic organisms which rarely produce symptoms in their hosts (Forton and Seys 1993). Their populations may increase with a higher abundance of food materials such as skin cells and, in particular, sebum (Rather and Hassan 2014).

Though it is uncommon for *Demodex* mites to take on a parasitic role in humans, pathogenesis may occur if population densities become unusually high. This is typically seen in patients with weakened

immune systems due to conditions such as HIV, lymphoma, or leukemia. Under normal circumstances, the human body produces immune responses that are sufficient to maintain *Demodex* populations at a healthy level (Elston 2010).

Demodex mites are most commonly associated with diseases such as rosacea, blepharitis, and general dermatitis. In rare cases, these mites have also been associated with androgenetic alopecia (male pattern baldness) and madarosis (loss of eyelashes) (Rather and Hassan 2014). One of the mechanisms thought to cause symptoms in hosts is the mechanical clogging of hair follicles and sebaceous ducts, which prevents the normal expulsion of dead cells and hair material from the follicle. Additionally, exoskeletons left behind by dead mites may also elicit responses by the immune system leading to the creation of granulomas. It is also possible that accumulations of waste excreted by the mites can cause inflammation

due to hypersensitivity of the host's skin (Lacey et al. 2009).

Currently, the role of follicle mites as vectors for other pathogens is not widely accepted, though they do offer a possible mode of transmission. Several types of potentially harmful bacteria have been found on the surface of these mites, and follicle to follicle transmission has been observed (Lacey et al. 2009; Elston 2010). The mites themselves can also be transferred from host to host through direct contact, making the transmission of bacterial infections from one individual to another a viable possibility (Lacey et al. 2009).

Due to the sparse and microscopic nature of *Demodex* mites, they are hard to collect and observe. The main method of collection is applying super glue to a microscope slide, waiting for it to dry on the skin, and then observing the *Demodex* under a microscope. However, this is sometimes ineffective and some argue that the best method is to scrape the *Demodex* from the skin and then transfer the mites and debris to a separate slide for observation.

Materials and Methods

When surveyed across 35 subjects, the average number of *Demodex* was 5 mites per square centimeter (Yun et. al. 2017). This served as an expected baseline.

Sampling Procedure

Eight college students were used as subjects for collecting samples. Approximately one square centimeter of cyanoacrylate, commonly known as super glue (Loctite® Brand, Westlake, Ohio), was applied on a glass slide and placed on the skin over the right eyebrow of every participant (Powell

2015, Lacey et. al. 2009, Yun et. al. 2017). The glue was allowed to dry for five minutes before being slowly peeled off and labeled for the specific participant. The area over the left eyebrow was squeezed and rolled with fingers until the skin was mildly irritated, and then scraped with a microscope slide. The material was then transferred to another slide by rinsing with ethanol. The slides were then labeled to identify which subject it came from and the treatment they received. This sampling procedure was repeated for all subjects.

Quantitation Procedure

The density of the *Demodex* population was determined by counting individual mites per square centimeter. This was done by viewing the collected samples under a microscope using 40-100x magnification. The difference in mite density was calculated between the scraping sample and the superglue sample of each participant. The difference in the number of mites per square centimeter was used to determine the efficacy of each collection method.

Results

Table 1. Number of *Demodex* per square centimeter for both collection methods.

Subject	Gender	Super Glue (#/cm)	Scraping (#/cm)
1	Female	0	0
2	Female	0	0
3	Male	0	0
4	Male	1	0
5	Female	0	0
6	Female	0	0

7	Male	0	0
8	Female	0	0

Of the samples obtained from the eight subjects, only one was found to have a single *Demodex* mite. This positive sample was taken using the superglue method. The subject for the positive sample was male. All of the samples from females for both sampling methods were negative for the presence of *Demodex* mites. The other males in the group also had negative samples for both sampling methods. Therefore, 12.5% of the sample population was found to have *Demodex* mites compared to the estimated 80-90% in the general population (Enginyurt et al. 2015).

Discussion

The results of the experiment were largely inconclusive due to the lack of *Demodex sp.* found. Because the lack of *Demodex sp.* found in this experiment contradicted the many studies that have shown that roughly 90% of humans have these mites, the experiment clearly can be improved upon (Enginyurt et al. 2015). One option is considering different methods of sampling. One method that we were not able to perform was dyeing the slides in order to increase the visibility of the mites. This method may have helped to eliminate any human error when searching for the translucent mites under a microscope. The fact that one mite was seen suggests that a lack of mites on the slide was the larger issue, rather than the difficulty of seeing them. A sampling method that may have improved the results would be plucking a fresh eyelash from the subjects to be viewed under a microscope. The base of eyelashes are common homes for *Demodex spp.* so, using an eyelash from each subject could have increased the number of *Demodex sp.*

found. This technique was not used in order to avoid the potential risk of pain for the subjects. The scraping method may have been improved by using a dull blade and scraping in the direction of hair growth. However, this method was not used because of the potential risk of injury and pain, and the potential risk of unwanted hair removal. The superglue method may have been improved by applying the superglue over areas with longer hairs where *Demodex sp.* are commonly found such as the eyebrows. This method was also not used because of the potential risk of unwanted hair removal. In addition to these methods, the experiment could have been improved by using a larger sample size. This was not possible in this case due to the limited amount of labor available to spend the time diligently scanning the microscope slides. When the subjects were questioned after the sampling, six of them reported that the superglue method was less painful in the end than scraping the facial skin with a glass slide. Based on the results in this experiment, it could be concluded that the superglue method is more effective and more comfortable for subjects. However, with only one *Demodex sp.* found this conclusion should not be widely accepted without more experimental efforts.

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