Indoor Airborne Microbial Concentration and Dry Eye
Supplement Online Material

Ocular surface testing procedure in the order they were performed

1. Tear osmolarity. Tear osmolarity (TearLAB, San Diego, CA) testing was performed once in each eye prior to instillation of eye drops. The osmolarity hand-piece was held over the outer 1/3 of the inferior conjunctivae to sample the inferior tear meniscus. Patients were asked to look up and nasally during the testing.

   Inflammadry testing (RPS, Tampa, FL). A tear sample was collected by exposing the lower palpebral conjunctiva and gently dabbing the fleece of the sample collector temporally to nasally approximately 6-8 times, allowing the patient to blink between dabs to ensure saturation. The sampling fleece was glisten or turn pink when an adequate sample is collected and then was snapped into the test cassette prior to immersion of the absorbent tip into the buffering solution for approximately 20 seconds or until a purple wave appears in the cassette window. The cap was then replaced over the absorbent tip and the applicator was laid flat for 10 minutes before interpretation of test results. Results were recorded as absent pink line (negative) or present pink line. The intensity of the pink line was further graded on a scale of 0-3 (none, faint, pink, fuchsia).

2. Skin assessment. The facial skin was assessed and the presence or absence of skin conditions was graded. These include rosacea (marked as present if skin telangiectasia, pustules, rhinophymia present) and/or seborrheic dermatitis.

3. Eyelid laxity. The presence of lower eyelid laxity was determined by the snap back test (0=laxity within normal limits, 1=a delay of two to five seconds for the lower lid to return to its native state, 2=persistent separation necessitating a blink to return to the normal state). Upper eyelid laxity was determined by the lid distraction test (0=laxity within normal limits, 1 i= 7-10 mm of distraction, or ~50% eversion of eyelids; 2 = greater than 10 mm of distraction or 100% eversion of eyelids).

4. Conjunctival staining. The examiner gently retracted the upper lid and 5 µl of preservative free lissamine green was placed on the superior bulbar conjunctivae. The upper lid was released and the subject was allowed to blink normally for 15 seconds. The patient's head was positioned in the headrest of the slit-lamp instrument, making sure the patient was comfortably supported with their forehead in full contact with the headrest band. The temporal and nasal conjunctivae were examined and graded for the presence of staining on a 0 to 3 scale.

5. Tear film break up time (TBUT). The examiner gently retracted the upper lid and 5 µl of preservative free fluorescein was placed on the superior bulbar conjunctivae. The upper lid was released and the subject were allowed to blink normally for 15 seconds. The patient's head was positioned in the headrest of the slit-lamp instrument, making sure the patient is comfortably supported with their forehead in full contact with the headrest band. The patient was instructed to blink three times naturally, then stare and NOT BLINK. The investigator monitored the integrity of the tear film and, using a stopwatch, measure the time from the last blink until one or more black (dry) spots appear in the precorneal tear film. After the 1st measurement, the patient was instructed to blink naturally 3 additional times and a 2nd measurement was taken. The procedure was then repeated a 3rd time. After a 60-second rest period, the entire procedure was repeated for the left eye.

6. Corneal staining. Corneal staining was assessed using the NEI standard scoring scale assessing 5 areas of the cornea. This was done directly after TBUT testing. A grade (0-3) was assigned to each section of the cornea and a total score (0-15) was generated by summing the 5 section scores.

7. Conjunctivochalasis: Conjunctivochalasis was graded as absent or present in each area of the lower eyelid (temporal, central, nasal) based on the obliteration of the tear film by conjunctivae in the region of interest.

8. Other eyelid measures: The degree of upper and lower eyelid telangiectasia was scored on a scale of 0 to 3 (0=none; 1=mild; 2=moderate; 3=severe) as was the degree of lower eyelid meibomian orifice plugging (0=none; 1=less than 1/3 lid involvement; 2=between 1/3 and 2/3 lid involvement; 3 greater than 2/3 lid involvement). The presence of fibrosis in the inferior eyelid was marked as absent or present. Inferior palpebral conjunctival hyperemia was marked as absent or present. Papillary changes

in the inferior conjunctivae were marked as none, present and mild, or present and severe. Meibomian gland drop out of the inferior MG was measured via noncontact meibography (a technique that uses transillumation to evaluate degree of area loss of glands according to the Meiboscale).

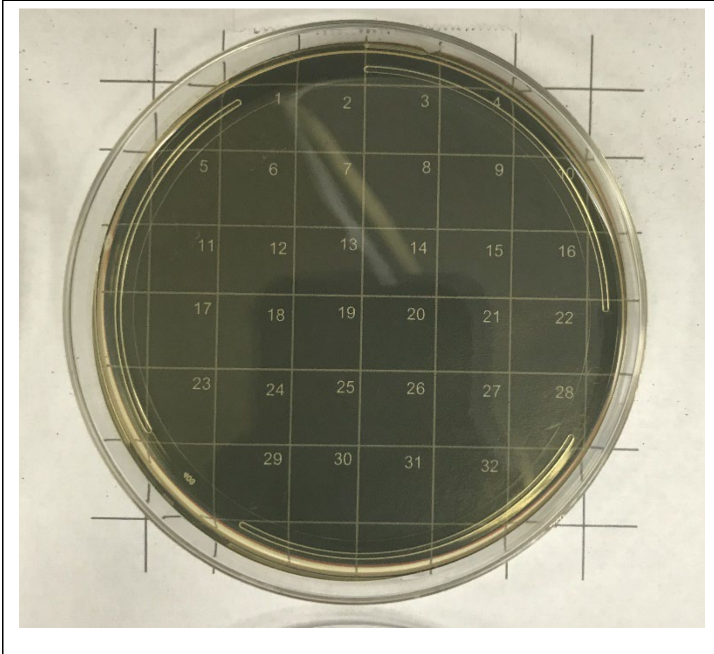
9. Ocular pain rating: Subjects were asked to rate the intensity of ocular pain at that moment (defined as any ocular sensation the patient construes as painful: dryness, itching, burning, stinging, aching, shooting, etc) using a numerical rating scale anchored at “0” for no ocular pain sensation to “10” indicating the worst ocular pain sensations. 10 μl of proparicaine was then placed in the inferior fornix of each eye. After a 15 second wait, individuals were asked to rerate their ocular pain using the same scale.
10. Schirmer strips were placed in the outer 1/3 of the lower conjunctivae and the length of wetting after 5 minutes was recorded in each eye. Excess anesthetic was gently wiped from the eyelids prior to introducing the schirmer strips. After 5 minutes, the Schirmer strips were removed from the eye and placed in individually labeled Eppendorf tubes and placed on ice. Right and left eyes were separately stored. The tubes were preserved in -80°C.
11. Tear collection: 50μl of balanced salt solution was placed in the inferior fornix of each eye. Three capillary tubes were then placed in the inferior fornix and as much as 30 μl of the ocular wash was removed and placed in individually labeled Eppendorf tubes and placed on ice. Right and left eyes were separately stored. The tubes were preserve in -80°C.
12. Meibum quality was rated on a scale of 0 to 4 (0=clear; 1=cloudy; 2=granular; 3=toothpaste; 4=no meibum extracted). Two cotton tipped applicators were dipped into anesthetic. At the slit lamp, the patient were asked to look up and one cotton tip was placed behind the inferior eyelid and one in front. These 2 applicators exerted pressure on the inferior eyelid and the quality of extracted meibum graded on the scale above. The extracted meibum was collected with the applicator that was initially behind the eyelid. These applicators were used to assess meibum quality in the right and left eyes. After collection of meibum from both eyes, the tip of the applicator was broken and placed in an Eppendorf tube, which was initially placed on ice and then transferred to - 80°C.

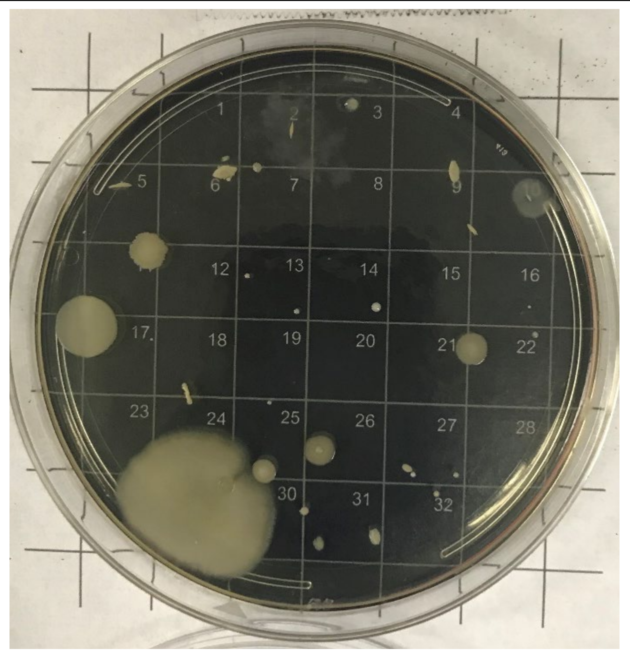
Home Air Sampling Protocol and Microbial Colonies Characterization

Protocol – Agar Petri Dish Characterization. Both nutrient and soy agar dishes were prepared and stored in refrigerator in 2° to 5° C temperature before the sampling. After sampling both nutrient and soy agar petri dishes were incubated for 48h at 37.5° C temperature and 5% CO_2. After 48 hours, each dish was placed on a 32 and photographed (**Figure S1**). Using an automated random generating and data capturing system that our team has developed eight random numbers were selected. Microbial colonies were counted in the selected boxes (**Figure S2**). The some of counted colonies was multiplied by four. Distinct number of colonies based on their shape, color and morphology were also recorded. Growth perception was judged based on the following criteria: no growth, low = 1 to 3 boxes with the colonies, moderate = 4-10 boxes, high = 11-16 boxes covered, very high = 17+ boxes covered. If a colony fell across two boxes, it was counted in the box with 50 % of its area. Examples of blank, moderate, high and very concentration are presented in Figure 1S.

Figure S1. Example blank, nutrient and soy agar petri dishes with low and high concentration of microbial communities. A. Field blank nutrient agar petri dish. B. Nutrient agar petri dish after 48h of incubation with moderate concentration. C. Soy agar petri dish with very high concentration, and D. Nutrient agar petri dish with medium high concentration.

A. Field blank nutrient agar dish Placed on a 32-box grid	**B.** Nutrient agar dish 48 after incubation (moderate concentration)





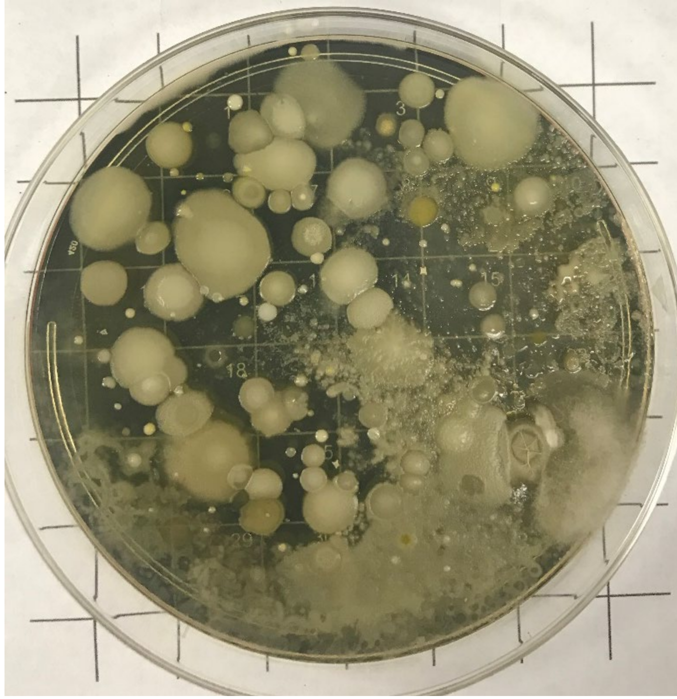
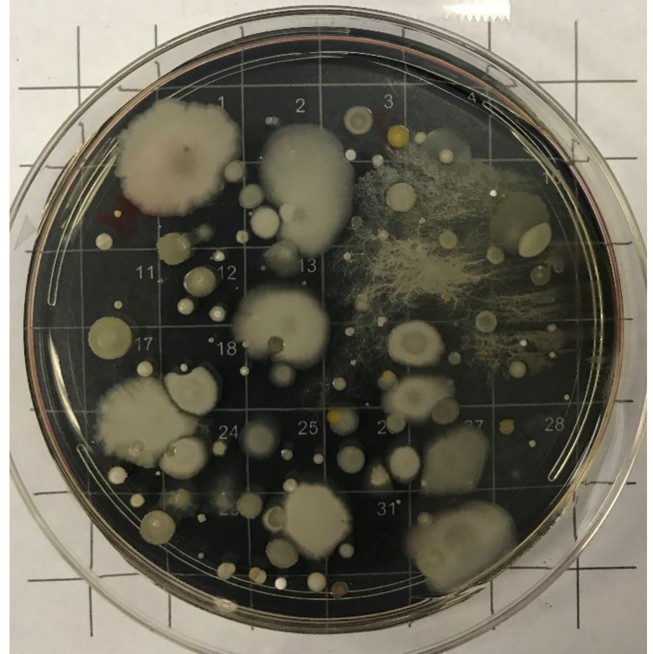
C. Soy agar petri dish after 48h of incubation (very high concentration)	**D.** Nutrient agar petri dish after 48h of incubation (very high concentration)
	

Figure S2. Online data capturing and storing system. The system randomly selected a box number for each of the six rows and additional two random boxes not already used. Microbial colonies in each of the selected boxes were counted and their sum was then multiplied by 4 to assess the total number of colonies entered in the database (see **dryeye.miami.edu** for details; the data capturing system is secure, but access can be provided upon request).

U-DRY | Home Survey(s) Sampling | Home Setup Images | Bioaerosol Results | Upload Aerocet | Register PRECISE | Home Visit Complete | Proje Hom

BIOAEROSOL IMPACTOR (PETRI DISH) RESULTS

Your Initials:

Date and time placed in **INCUBATOR**: mm/dd/yyyy --:-- --

Date of **Scrapping:** mm/dd/yyyy --:-- --

A. PATIENT INFORMATION

Select or Selected Patient's ID: TRJ001_1

BOX NUMBER randomly selected for counting colonies for each row
Ln-1:**4**; Ln-2:**5**; Ln-3:**14**; Ln-4:**19**; Ln-5:**28**; Ln-6:**29**
Use the **first two distinct numbers** not already used earlier
23; **1**; **2**; **19**; **26**; **18**;

B. **NURTRIENT Petri Dish Information:**

Total count of colonies: # of colonies -11 if not ap

Number of **DISTINCT** colonies based on color and morphology # distinct colonies -11 if r

Concentration perception (Low to High):

C. **SOYA Petri Dish Information**

Total count of colonies: # of colonies -11 if not ap

Number of **DISTINCT** colonies based on color and morphology: # distinct colonies -11 if r

Concentration perception (Low to High):

C. **PETRI DISH IMAGE(S)**

☑ **KEEP this BOX CHECKED if both images in one file:**

Select Nutrient and Soya Plate Image
(allowed formats : PNG, JPG, TIF) Choose File No file chosen

<<< SUBMIT DATA>>>

<u>Protocol used for conducting home visit and air sampling</u>.

Call/text participants 24 h before the home visit: Call the participant 24h before the scheduled visit to confirm the home visit.
Call/text message before leaving the laboratory for the home visit: Reconfirm participant's availability for the scheduled visit and also repeat the above message. If answer is "YES", cancel home visit until further notice.

Please follow along with the instructions and check-off each task after completion, and submit with data log.

BEFORE YOU LEAVE		
☐	Sign-in (sheet in office)	*names and time
☐	Collect lunchbox from hood, Grab clipboard with checklist	*ensure that 3 filters and both bioaerosols are inside
	Check for these before you leave:	
☐	Face masks	*wear face mask covering mouth and nose at all times in the lab and during the home monitoring
☐	Hand gloves	*wear hand gloves at all times in the lab and during the home monitoring
☐	Eye goggles	
☐	Face shields	
☐	Disinfectant wipes	
☐	70% Alcohol spray	
☐	Microbiome (MB) PM Filter	
☐	Indoor PM Filter (PTFE pore size 0.4um)	
☐	Outside PM Filter (PTFE pore size 0.4um)	
☐	MB PM Filter holder	*make sure to disinfect all equipment using 70% alcohol inside biosafety cabinet in laboratory before leaving to participant's home
☐	Indoor PM Filter holder	
☐	Indoor PM Filter holder	
☐	Outside PM Filter holder	
☐	Indoor Air-o-cell	
☐	Outside Air-o-cell	
☐	Nutrient Agar plate	
☐	Soy Agar Plate	
☐	1.5 ml conical tubes for Schirmer test	
☐	Precise	

☐	Call the patient: 1. Confirm Visit 2. Ask for Wi-Fi information for precise	*If the patient does not know their Wi-Fi information it can be done at the house with the patient. *If the patient declines to give Wi-Fi or the precise staying for 6 months then bring the data port and still record data but do not leave with them.
☐	Record start time mileage	
☐	Record start time mileage	
☐	Put on eye goggles, face shield, and hand gloves before arriving to participant's house (face mask should be on already)	
INDOOR SETUP		
☐	Record time of arrival	
☐	Call patient on phone once parked outside their home and discuss indoor monitoring location with them while maintaining distance of 6 feet apart	
☐	Setup tripod & pins	
☐	Place pumps beneath tripod	
☐	Connect Pumps to Rotameter in this order Left to right GREEN RED YELLOW BLACK	*pumps connect to top of rotameter
	CONNECT SAMPLES TO BOTTOM OF ROTAMETER	
☐	Microbiome to GREEN tubing to rotameter	
☐	Air-O-Cell to RED tubing to rotameter	
☐	Bioaerosol (impactor) to YELLOW tubing to rotameter	*note if you started with soy or agar in data log
☐	PM filter to BLACK tubing to rotameter	
	Aerocet	
☐	Turn Aerocet ON to MANUAL and COUNT and set Patient ID	
☐	Attach temperature probe and tubing to top of Aerocet 531	*check if temperature probe working. (Temperature and Humidity reading should be on collect sample screen)
☐	Turn on Data Port	
☐	Turn on Precise after Data port	*check **precise.miami.edu/um_wifi/** to verify machine is connected and uploading.

☐	If leaving a precise with patient then turn on second precise to be running simultaneously	*this precise is connected to their Wi-Fi and to be left with them
☐	If using Data port instead of patient Wi-Fi check data port to see if it is connected.	
☐	Turn on pumps	
☐	Set flow to 28.5	
☐	Start Aerocet	
☐	Start a 45 minute timer	
☐	Take 3 pictures of setup from different angles	
☐	Head outside to set up outdoor monitoring	
OUTSIDE SETUP		
☐	Record location in data log	
☐	Setup tripod and extension cord	
☐	Place pumps beneath tripod	
☐	Connect pumps to top of tripod Left to right YELLOW GREEN	
☐	Place samples on top of tripod	
	CONNECT SAMPLES TO BOTTOM OF ROTAMETER	
☐	Air-O-Cell to YELLOW tubing to rotameter	
☐	PM filter to GREEN tubing to rotameter	
☐	Turn on pumps	
☐	Set flow to 28.5	
☐	Start a 45 minutes timer	
☐	Take 3 pictures of setup from different angles	
TESTS AND DATA ENTRY		
☐	Perform Schirmer test	*make sure to use new gloves when doing this test. Project staff should be wearing all PPE

		previously mentioned and the patient should be wearing a face mask during this test.
☐	Store Schirmer test in ice jell box	
☐	Conduct survey	*follow up survey if second visit as well *administer survey through either a phone call to the patient while the study team is outside the house, or in person outside the house while maintaining at least a distance of 6 feet and everyone wearing PPE.
☐	Follow up survey	
☐	Weather from Weather.com recording in data log	*use patients zip code
☐	Start Log	
☐	AC Swab	
☐	AC Swab stored in labeled plastic bag	
☐	Kitchen Swab	
☐	Kitchen Swab stored in labeled plastic bag	
☐	Temperature Reading Inside Dry and Wet	*Wait 10 minutes before recording values
☐	Temperature Reading Outside Dry and Wet	*Wait 10 minutes before recoding values

INSIDE SETUP AFTER 45 MINUTES		
☐	Record Bioaerosol flow level	
☐	STOP Bioaerosol pump.	*YELLOW TUBING
☐	Take out Agar plate and cover it with lid	
☐	Agar plate sealed and stored in plastic bag	
☐	Replace the impactor with the second one	
☐	Place second agar plate into new impactor	*note in data log if SOY or NUT
☐	Connect the YELLOW sample tubing to new impactor containing second agar plate	
☐	STOP Aerocet	
☐	Change Aerocet to COUNTER and confirm patient id	
☐	Start bioaerosol pump	
☐	Start Aerocet	

OUTSIDE AFTER 45 MINUTES

☐	Record Air-O-Cell flow	
☐	STOP Air-O-Cell pump	*YELLOW tubing
☐	Remove Air-O-Cell and reseal with its coverings	
☐	Place Air-O-Cell into its respective plastic bag	

AFTER MONITORING IS COMPLETE		
☐	Dismount and disinfect equipment using provided wipes and 70% alcohol before placing back in car	

BEFORE LEAVING CAR		
☐	Record Mileage	
☐	Record Location of Car	
☐	Wipe inside area of car with disinfectant wipes and/or 70% ethyl alcohol	
IN LAB		
☐	Place Schimer in -20°C freezer	
☐	Place swabs and labelled Air-O-Cells in fridge	
☐	Place filters back into respective caskets under the clean hood and place in fridge	
☐	Clean all tools used for samples i.e impactors under the clean hood with ethanol.	
☐	Clean Agar Plates with Ethanol under hood	
☐	Relabel Agar Plates	
☐	Agar plates stored upside down in incubator at 37.5° C temperature and 5% CO_2	