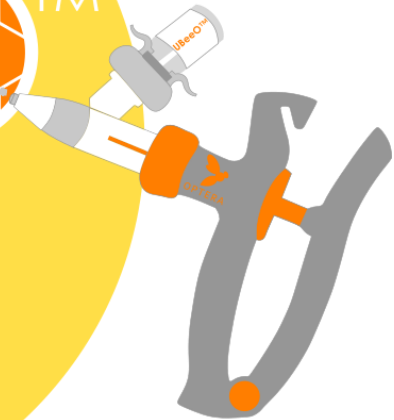




UBeeTM

INSTRUCTION MANUAL



hello@opterabees.com
www.opterabees.com
(336) 223-6130
@opterabees



Table of Contents

What's in your UBeeO™ Kit?.....2-3

UBeeO™ Safety.....4-5

UBeeO™ Testing: Prep Guide.....6-8

UBeeO™ Testing: Step-by-Step Guide

- 1) Priming the UBeeO™ applicator 9-10**
- 2) Selecting the UBeeO™ test frame 11**
- 3) Isolating the UBeeO™ test area 12**
- 4) Performing the UBeeO™ test 13-14**
- 5) Collecting UBeeO™ results 15-16**
- 6) Calculating UBeeO™ Scores 17-19**

How did your colonies do?.....19-20

Troubleshooting Tips.....21-24

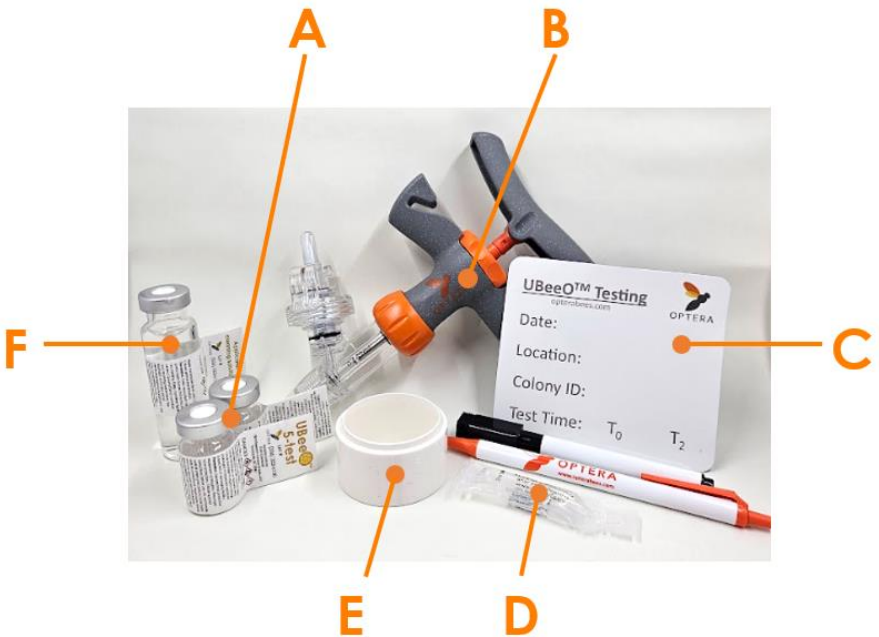
UBeeO™ Applicator Care and Maintenance Guide.....24-26

UBeeO™ Data Sheet27

What's in your UBeeO™ Kit?

Your UBeeO™ Kit contains:

- A. Vials of UBeeO™ mix
- B. A UBeeO™ applicator (see Applicator Care and Maintenance Guide for parts)
- C. A dry-erase board & dry-erase marker
- D. Applicator lubricant
- E. A UBeeO™ test ring
- F. A vial of applicator cleaning solution



- A. Each **UBeeO™ vial** contains enough **UBeeO™ mix** to test either **5 or 10 colonies**. The **UBeeO™ mix** contains pheromones that trigger honey bee hygienic response. These pheromones are applied to a small test area of capped brood, and the treated frame is returned to the colony for two hours. The response of adult bees to the treated cell caps will allow you to calculate the colony's **UBeeO™ Score**, informing your selective breeding and mite management strategies.
- B. The **UBeeO™ applicator** is used to apply the **UBeeO™ mix** to the capped brood cells, ensuring accurate, contactless dosing during **UBeeO™** testing. Three full compressions of the applicator handle equal one **UBeeO™ test**.
- C. The **UBeeO™ dry-erase board and marker** allow you to easily record and photograph the **UBeeO™ test** information that is required to calculate the **UBeeO™ Score** once testing is complete.
- D. **Applicator lubricant** is used to keep the O-ring in your applicator working properly. Refer to the **UBeeO™ Applicator Care and Maintenance Guide** at the end of this instruction manual for directions on lubricant application.
- E. The **UBeeO™ test ring** isolates the **UBeeO™ test area** from the rest of the brood frame by confining the **UBeeO™ mix** to the test area during application. The impression left in the wax caps by the test ring enables easy identification of the test area at the end of the two hour testing period.
- F. The **applicator cleaning solution vial** contains ethanol and should be used before and after **UBeeO™** testing to keep your applicator working properly.

UBeeO™ Safety

The **UBeeO™ mix** contains pheromones that are dissolved in **hexane**, a chemical solvent found in many consumer products. Hexane evaporates quickly once sprayed onto the test area of capped brood cells. While properly administered **UBeeO™** tests are safe for the colony, beekeepers must take safety precautions during testing to prevent exposure to the **UBeeO™ mix**.

Danger! Hexane is flammable, a health hazard, and a skin irritant. **DO NOT EAT, DRINK, or SMOKE while using UBeeO™.** Always **use appropriate personal protective equipment** such as gloves and eye protection when working with **UBeeO™.** Always **work outdoors or in a well-ventilated space.** Never point the **UBeeO™** applicator at people or animals. **Store UBeeO™ in a cool, safe place, away from heat and flames. KEEP AWAY FROM CHILDREN.**

If you have any safety questions or concerns, please contact Optera prior to product use.



Flammable

Flammable liquids, category 2



Health hazard

Aspiration hazard, category 1
Reproductive toxicity, category 2



Irritant

Skin irritation, category 2
Specific target organ toxicity following single exposure, category 3

IF EXPOSED/CONCERNED, seek medical attention.

IF ON SKIN immediately remove all contaminated clothing and rinse skin with soap and water.

IF IN EYE protect unexposed eye and flush exposed eye gently with water for 15-20 minutes. If able/applicable, remove contact lenses during flushing.

IF INHALED immediately move to area with fresh air. Loosen clothing as necessary and situate individual in a comfortable position.

IF SWALLOWED rinse mouth thoroughly. Do not induce vomiting. Have exposed individual drink sips of water.

UBeeO™ DISPOSAL. UBeeO™ is highly volatile, and thus evaporates quickly from unsealed containers. DO NOT THROW SEALED UBeeO™ VIALS IN THE TRASH. To dispose of unused, sealed containers, follow all local guidelines regarding proper disposal of hazardous waste.

The **applicator cleaning solution** contains **ethanol** and functions to flush out the hexane from the applicator. Hexane can cause damage to the internal components of the **UBeeO™ applicator** if exposed to the **UBeeO™ mix** for long periods of time. Safety precautions discussed above should also be taken when using the **applicator cleaning solution**. See the **UBeeO™ Testing: Step by-Step Guide** and the **Applicator Care and Maintenance Guide** for more information on how and when to use the **applicator cleaning solution**.



Flammable
Flammable liquids, category 2



Irritant
Eye irritation, category 2A

UBeeO™ Testing: Prep Guide

Storing your UBeeO™ vials

Store your UBeeO™ vials in the UBeeO™ Kit and/or vial box and keep in a cool, dark, dry location. Sealed UBeeO™ vials have a shelf-life of one year when stored at room temperature or cooler. Inspect the UBeeO™ vials closely prior to use to ensure that the seal is intact. Read more about UBeeO™ storage and safety in the UBeeO™ Safety section.

Recommended UBeeO™ testing conditions

For the most accurate UBeeO™ results, be sure that:



Local nectar flow is on. The ideal time to test with UBeeO™ is during spring to mid-summer nectar flows. Results may be unreliable if testing is conducted during the fall flow or a nectar dearth. Spring testing allows you to implement your selective breeding and management plan earlier. If testing during a dearth is necessary, feed 1:1 sugar syrup at least two days prior to UBeeO™ testing.



Colony has been queenright for at least 7 weeks. UBeeO™ measures the mite and disease resistance traits of the queen through the hygienic performance of her worker offspring. If a test colony is queenless or has recently requeened, the UBeeO™ Score will apply to the old queen and may not accurately reflect the colony's hygienic capabilities or management needs. Since older nurse bees perform hygienic behaviors, it is

critical to wait at *least* 7 weeks after queen introduction before UBeeO™ testing.

Strategizing your UBeeO™ workflow

Testing for mite resistance is simple with UBeeO™. Here are our tips for staying organized to get the most out of your UBeeO™ testing:



Give your test colonies unique ID numbers. If your test colonies are not already named, we recommend labelling them before testing. Unique ID numbers for each colony will allow you to keep track of your test colonies during and after UBeeO™ testing.



Set up your UBeeO™ work station. We recommend setting up a portable solid surface (like a folding table or truck bed) near your test colonies, where you can store the UBeeO™ Kit and conduct UBeeO™ tests.

Testing more than 10 colonies? Here's how you can make UBeeO™ testing even simpler:



Plan for UBeeO™ vial changes. Each UBeeO™ vial will test either **5 or 10 colonies**. Make sure to check the labels carefully, and to bring the appropriate number of vials for all the colonies you plan to evaluate in your apiary. Remember to store extra vials in a cool place out of direct sun light until you are ready to use them.



Work in teams. To improve efficiency, have one beekeeper stay at the work station to run UBeeO™ tests and record data, and a second beekeeper transport frames between the work station and the hives.



Plan your testing route ahead of time. If your test colonies are located across multiple yards, be sure you'll have enough time to travel back to the first colony before the 2-hour test period is up.

If you have any questions before you get started with UBeeO™ testing, please reach out to us at hello@opterabees.com.

UBeeO™ Testing: Step-by-Step Guide

1) Priming the UBeeO™ applicator



If you are working in a team, the beekeeper collecting brood frames may proceed to step 2) Selecting the UBeeO™ test frame while the workstation beekeeper is priming the UBeeO™ applicator.

- 1.1 Remove the spike cover from the UBeeO™ applicator.
- 1.2 Invert the UBeeO™ applicator so that the spike is facing downwards. Center the cleaning solution vial under the spike and press the spike through the vial septum.



- 1.3 Pinch the applicator vial clips to secure the vial onto the applicator. Turn the applicator right side up, so that the vial is above the applicator barrel.
- 1.4 Point the UBeeO™ applicator slightly upwards (about a 30° angle) and compress the handle to fill the barrel of the applicator with the cleaning solution. Continue compressing the handle (usually 2 or 3 times) until the barrel is full. A small bubble inside the barrel is normal and is not a concern.



If you have trouble with priming, refer to our [Troubleshooting Tips](#).

- 1.5 Invert the [UBeeO™ applicator](#) so that the spike is facing downwards. Pinch the applicator vial clips to remove the [applicator cleaning solution](#) and replace it with a [UBeeO™ vial](#).
- 1.6 As before, turn the applicator right side up and point it slightly upwards (about a 30° angle). Compress the handle 2-3 times to replace the cleaning solution in the barrel with [UBeeO™ mix](#) (don't worry – you will still have plenty of [UBeeO™ mix](#) left for testing). Note that **once a vial is pierced, all the UBeeO™ mix in that vial must be used within 3 hours**. Your primed [UBeeO™ applicator](#) is now ready for testing!

2) Selecting the UBeeO™ test frame

Avoid using your smoker during UBeeO™ testing as smoke can dull worker bee senses and may affect your UBeeO™ results. If smoke is necessary, apply small amounts consistently across all test colonies, and avoid smoking the area around the UBeeO™ test frame, especially when returning the frame to the colony after UBeeO™ mix application.



- 2.1** Choose a worker brood frame that contains at least some solid areas of capped brood that are aged **purple-eye or younger**. Avoid testing areas of brood that are within a few days of emerging, as the UBeeO™ mix can cause these bees to emerge during the test period, resulting in inaccurate UBeeO™ scores.



To verify the age of brood by eye color, use your hive tool to uncap a couple of cells on different areas of the frame. Don't worry! The bees will recap these cells later.

- 2.2** Make sure the queen is not on the selected brood frame then shake or brush the bees off. Walk the frame back to your work station and lay the frame down on a flat or slightly inclined surface.

3) Isolating the UBeeO™ test area

3.1 Choose a mostly capped brood area on the test frame. With the cutaway side of the UBeeO™ test ring facing downwards, **gently** press and twist the test ring into the brood until the bottom of the thick part of the test ring is flush with the surface of the brood cells. Be careful not to apply too much pressure, as you do not want to injure the brood underneath.



3.2 Use your dry erase marker to fill in the information on your UBeeO™ dry-erase board and circle **T₀** to indicate that you are beginning the UBeeO™ test. Use your smartphone or tablet to photograph the dry-erase board. This will enable you to identify which colony each test area photo is paired with during scoring.



3.3 Photograph the UBeeO™ test area you selected in step 3.1. Be sure that the photo is in focus with enough light to show detail on the wax caps. The photo should be framed



on all sides by the UBeeO™ test ring as shown in photo 3.3.

4) Performing the UBeeO™ test

4.1 Tilt the frame upwards at a slight angle (~30°) and center the UBeeO™ applicator just above the plane of the top of the test



ring. Compress the handle of the applicator to spray one full barrel of UBeeO™ mix onto the test area. Allow the handle to retract and pause for 15-30 seconds to allow drying of the test area. Deliver a second spray, wait another 15-30 seconds, and then deliver a third and final spray to the test area. Once you have delivered **three full sprays**, lay the frame back down until the test area appears completely dry (15-30 seconds). Mark the top of the frame above the test area with a thumbtack or permanent marker to easily identify it at the end of the testing period.

4.2 Return the frame to the test colony. If possible, always place the test frame in between two other brood frames. This helps ensure that nurse bees quickly find and attend to the test frame.

4.3 Record the colony ID # and the time of frame return (**T0 Time In**) in the **UBeeO™ Data Sheet** provided at the back of this manual. Add two

hours to **T0 Time In** to determine when to retrieve the frame for scoring (**T2 Time Out**).

4.4 Repeat steps **2) Select the UBeeO™ test frame – 4.3** for up to two hours from the first **T0 Time In**, at which point you must stop and begin pulling frames for scoring.

4.4.1 Between tests, rest the applicator vial-side down to prevent extended contact between the **UBeeO™ mix** and the applicator spike. Please note that the **UBeeO™ mix** may leak from the vial if the vial is left upside-down on the spike for an extended period of time.



4.5 If you are testing **more colonies than there are tests in your vial**, you will need to replace the empty **UBeeO™ vial**.

4.5.1 To disengage the **UBeeO™ vial** from the **UBeeO™ applicator**, invert the applicator then apply pressure to the applicator vial clips to release the vial. Pull the vial off the spike and discard the empty vial.

4.5.2 Repeat step **1) Priming the UBeeO™ applicator** using a **UBeeO™ vial** (no cleaning solution is needed between consecutively used **UBeeO™ vials**) and

continue testing the remainder of your test colonies.

5) Collecting UBeeO™ results

- 5.1** Retrieve the marked UBeeO™ test frame exactly **two hours** after it was returned to the test colony in step **4.2**. After the two-hour test is complete, you may use the smoker as desired. Check to make sure the queen is not present before shaking or brushing the bees off the frame. Walk the frame back to your UBeeO™ work station and lay the frame down on the flat or slightly inclined surface.
- 5.2** Locate the UBeeO™ test area under the frame marker using the indentation left in the wax by the **UBeeO™ test ring**. Carefully place the test ring back into the exact position used at **T₀**. No pressure is needed.
- 5.3** Use your dry erase marker to fill in the information on your **UBeeO™ dry-erase board** and circle **T₂** to denote the end of the UBeeO™ testing period. Use your **smartphone or tablet** to photograph the dry-erase board.

5.3
UBeeO™ Testing
opterabees.com
OPTERA
Date: 5/15/22
Location: Optera
Colony ID: 46
Test Time: T₀ (T₂)

5.4 Use your **smartphone** or **tablet** to photograph the test area with the **UBeeO™ test ring** in place. Make sure that the photo is in focus and oriented correctly, as shown to in photo 5.4. Double check the lighting is adequate in your photo before returning the frame to the test colony.



5.5 Repeat steps **5.1-5.4** with your remaining test colonies. Once you have photographed the results for your last test colony, follow the instructions in the **Applicator Maintenance and Care Guide**. You are now finished collecting UBeeO™ results! Proceed to the next step to calculate your **UBeeO™ Scores**.

6) Calculating UBeeO™ Scores

Now that you have the **T0** and **T2** photos of your test colonies, you can calculate your **UBeeO™ Scores** from the comfort of your home or office!

Here are a few tips to get you started:



Check out our UBeeO™ scoring resources.

Visit our website for walkthrough scoring tutorials, the **UBeeO™ Score Sheet**, and more.



Back up your UBeeO™ data.

We recommend backing up your UBeeO™ photos and data sheets to a digital folder in a secure location.

This will keep your data safe and enable you to refer to old data so that you can easily track progress from season to season.

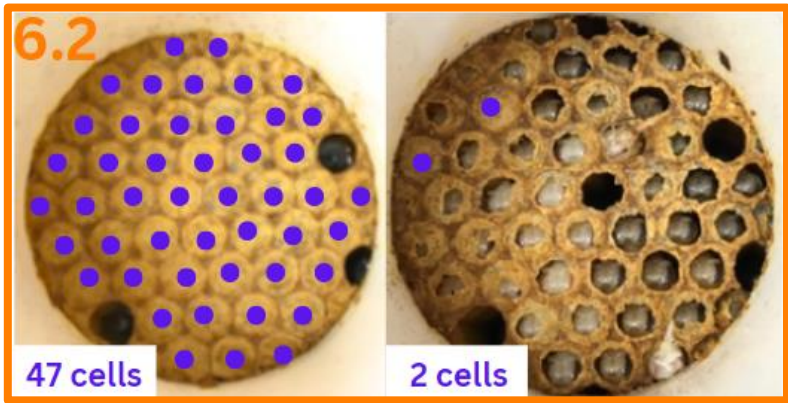


Download free apps to make scoring easy.

Check the app store on your device for photo collage and counting apps to arrange photos side by side and facilitate cell counting.



6.1 Arrange the **T0** and **T2** photos from your first test colony side by side. Count all **capped brood cells that fall at least 50%** inside the test area in the **T0** photo. Record the cell count directly into the **T0** column in the **UBeeO™ Data Sheet** (provided at the end of this manual, digital versions available on our website).



6.2 Count the **capped brood cells** remaining inside the test area in the corresponding **T2** photo. Be sure that the cells you are counting are consistent between the **T0** and **T2** photos. In other words, if you did not count a cell along the test area perimeter at **T0**, do not count it at **T2**, even if it appears in the **T2** photo to be at least halfway inside the test area. Record the cell count directly into the **T2** column in the **UBeeO™ Score Sheet**.

6.3

$$\left(1 - \left(\frac{2 \text{ cells}}{47 \text{ cells}}\right)\right) = 96\%$$

6.3 Calculate the **UBeeO™ Score** for each colony using the formula:

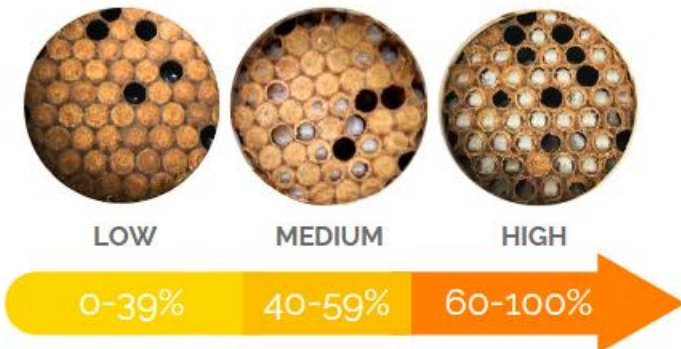
$$(1 - (T2 \text{ capped} / T0 \text{ capped})) \times 100$$

If using the **UBeeO™ Score Sheet** downloaded from our website, this calculation will be done for you automatically.

6.4 Repeat steps **5.1-5.3** for the remaining photos of your test colonies.

How did your colonies do?

UBeeO™ Scores can fall into one of three categories:



LOW (0-39%) – Few cells uncapped, if any, indicates weak mite and disease resistance. A colony that scores in this range would not be a suitable candidate for selective breeding of UBeeO-linked hygiene. This colony is at-risk of high mite and disease loads and may require additional management to improve chances of overwinter survival.

MEDIUM (40-59%) – A moderate number of cells uncapped indicates some mite and disease resistance. A colony that scores in this range may be a suitable candidate for selective breeding of UBeeO-linked hygiene if no or few colonies with High UBeeO™ Scores are available. Mite loads for this colony should be monitored closely.

HIGH (60-100%) – Most, if not all, cells uncapped indicate strong mite and disease resistance. A colony that scores in this range is an ideal candidate for selective breeding of UBeeO-linked hygiene. This colony will likely be able to maintain mites and disease below treatment thresholds.

Visit our website (www.opterbees.com) for the most up-to-date information on interpreting your UBeeO™ Scores.

Troubleshooting Tips

PRIMING If you are having trouble priming the applicator (filling the barrel with solution when a vial is attached), you may need to fix your vacuum seal. The vacuum seal can be fixed by reattaching the barrel to the handle assembly with or without lubricating the piston O-ring. Unscrew the barrel collar to separate the barrel from the handle assembly. If the piston O-ring appears dry, add 1-2 drops of the **UBeeO™ applicator lubricant** to the O-ring. Rapidly reinsert the piston into the barrel, almost like you are trying to trap as much air as possible inside the barrel. Secure the barrel to the handle assembly by tightening the barrel collar.

CHOOSING A TEST AREA If it is difficult to find an area of brood large enough to perform a UBeeO™ test, you can use a brood frame from another colony. However, lack of brood may be an indication that it is not an ideal time to test your colony, so you may want to consider testing that colony at another time. You may use a test area with as few as 25 capped brood cells, however the accuracy of your UBeeO™ test decreases as you decrease the initial (T₀) capped cell number.

FINDING YOUR TEST AREA If you have trouble locating your test area after two hours has passed, try increasing the pressure you use when placing and twisting the test-ring into the capped brood. You want to leave a clear circular indentation in the wax, but do not want to press hard enough to injure the brood underneath. You can also use a permanent marker, thumbtack, or small piece of tape to mark the top of your test frame.

Place the marker so that it corresponds to the location of the test area (i.e. the center of the frame on side 1).

CAP DISTORTION DURING APPLICATION If you notice that cell caps become distorted or partially uncapped when applying UBeeO™, wait longer between sprays, allowing complete drying of the test area before another spray is applied.

BROOD REMOVAL UNDER TEST RING You may notice brood under the test ring being uncapped and/or removed more than brood in the center of the test ring. If this happens regularly, try decreasing the pressure you use when placing and twisting the test-ring into the capped brood. If the caps of brood cells that fall >50% inside the test ring are distorted by the test ring, do not include these cells in your T0 or T2 cell counts.

LOW SCORES If your UBeeO™ scores are lower than expected, make sure you are testing under the recommended conditions: the spring or summer nectar flow is on and the colony has been queenright for at least 7 weeks. If you are following these recommendations and your scores are low, it may simply be that your colonies are not as hygienic as you had hoped. The UBeeO™ test is more difficult to pass than other hygiene tests, especially those that involve removal of dead brood. While use of a smoker may decrease scores somewhat, this effect has been measured to be less than 20%, so should not result in a high UBeeO™ colony ($\geq 60\%$) testing low ($\leq 39\%$). If your *untreated* colony tests low on UBeeO™ but has low mite loads, it may have another pest-resistance mechanism, such as grooming. If you're still confident

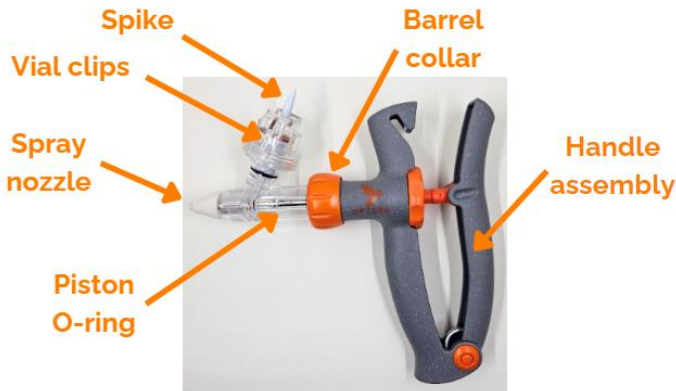
that the test should be higher, wait at least two weeks and retest. There are many variables that can affect colony performance, and we are still learning about how variables in and around the apiary may affect UBeeO™ scores.

INCONSISTENT SCORES Test scores for the same colony (same queen) may be inconsistent over time, as numerous factors such as nectar flow, pollen quality, season, and recent testing may impact colony scores. We are still learning about how UBeeO™ scores might be affected by variables inside the hive and in the nearby environment. To accurately compare hygiene scores between colonies, try to reduce variability (i.e. smoker use, presence of hive feeders, and season) as much as possible. It is also a good idea to have the same people carry out the same tasks for each test. For example, have the same person apply the UBeeO™ spray to all test frames. Similarly, the same person should score the results for all tests. Since different people may do things in slightly different ways, consistency in task performance can reduce variability in your test results.

MITES IN MY HYGIENIC COLONY Typically colonies that score high on UBeeO™ maintain mite levels below treatment thresholds of 3 mites per 100 bees, even without chemical mite treatments or other beekeeper interventions. However, occasionally we find a high number of mites on adult bees in high UBeeO™ colonies. These mites are thought to be acquired when bees from the hygienic colony rob out nearby weaker, collapsing colonies. Because hygienic bees may be poor groomers, mites acquired while robbing may have

to make their way into brood cells before being detected and removed by hygienic bees. If you find a high mite load in a high UBeeO™ colony, retest for mites in 3-4 weeks, and you will likely find the mite population has decreased significantly.

UBeeO™ Applicator Maintenance and Care Guide



APPLICATOR CLEANING SOLUTION When not in use, the UBeeO™ applicator should be placed in the UBeeO™ Kit with the spike cover in place and stored in a clean, cool, dry location. The UBeeO™ mix contains compounds that may cause damage to the applicator if left in contact with applicator components for an extended period. Thus, the applicator should be cleaned after each testing session, or any time there is a pause in applicator use that lasts more than about 1 hour. For standard cleaning, the applicator should be flushed with the applicator cleaning solution provided in your UBeeO™ Kit. Flush your applicator by securing the applicator cleaning solution vial onto the spike with

the applicator clips and compressing the handle completely 3 or more times, as described in steps **1.2-1.4**. After removing the **applicator cleaning solution**, empty all remaining solution from the applicator barrel by compressing the handle until no more solution can be seen exiting the spray nozzle. You may also need to apply **applicator lubricant** to the piston O-ring, as discussed in the next section.



O-RING LUBRICANT If you have completed UBeeO™ testing for the season or are having trouble priming your applicator, you may need to lubricate the piston O-ring. To access the piston O-ring, unscrew the orange barrel collar to separate the barrel from the handle assembly. Apply 1-2 drops of **applicator lubricant** to the piston O-ring. Then reattach the barrel to the handle assembly, and secure by tightening the barrel collar.

COMPLETE APPLICATOR CLEANING If you are still having trouble priming your applicator or would like to clean your applicator more thoroughly, you can disassemble and clean the applicator using warm soapy water. To clean your applicator with warm soapy water, remove all applicator attachments, including the spike and

spray nozzle. Unscrew the orange barrel collar to separate the barrel from the handle assembly. Submerge each component in warm soapy water (use standard dish soap), and then rinse thoroughly by submerging in clean warm water. Prior to reassembling the applicator, dry all applicator components completely and then apply 1-2 drops of **applicator lubricant** to the piston O-ring.

UBeeO™ Data Sheet

Test Date	Colony ID	T0		T2	
		Time In	# Capped Cells	Time Out	# Capped Cells
		__ : __		__ : __	
		__ : __		__ : __	
		__ : __		__ : __	
		__ : __		__ : __	
		__ : __		__ : __	
		__ : __		__ : __	
		__ : __		__ : __	
		__ : __		__ : __	
		__ : __		__ : __	
		__ : __		__ : __	

