



HYGIENIC BEHAVIOUR PIN TEST ASSESSMENT PROTOCOL

INTRODUCTION

OVERVIEW

The Pin-Kill Brood (PKB) assay is a field test used to evaluate hygienic behaviour in honey bee colonies. Hygienic behaviour refers to the ability of worker bees to detect, uncap, and remove unhealthy or dead brood from the hive.

This behaviour is recognized as an important component of honey bee social immunity and may contribute to:

- Reduced varroa mite reproduction
- Improved brood disease resistance
- Increased colony resilience

The PKB assay simulates unhealthy brood by pin-killing capped pupae and measuring the colony's removal response over a 24-hour period.

WHY HYGIENIC BEHAVIOUR MATTERS

Research has shown that hygienic colonies may:

- Detect and remove varroa-infested brood
- Interrupt mite reproductive cycles
- Remove diseased brood more efficiently
- Reduce impacts from brood diseases such as chalkbrood, European foulbrood, and other brood abnormalities.

Selecting breeder queens from hygienic colonies can support long-term stock improvement programs.

IMPORTANT ACCURACY CONSIDERATIONS

QUEEN AGE

Colonies should contain the same queen for a minimum of 7 weeks prior to testing to ensure worker bees represent the queen's genetics.

BROOD AGE

Correct brood age is critical. Test only capped brood with pink to light purple-eyed pupae.

TIMING

Frames must remain in the colony for exactly 24 hours to ensure consistent and comparable results.

PRECISION PINNING

Minimal capping damage improves accuracy by ensuring bees respond to unhealthy brood rather than visibly damaged cells.

MATERIALS & EQUIPMENT

MATERIALS REQUIRED

- 0.3 mm insect pins or PKB tester
- Paint pens
- Push pins
- Colony labels
- Frame labels
- Hive tool
- Bee brush
- Datasheet
- Smartphone/camera
- Standard beekeeping PPE
- 70% Ethanol-soaked sponge for sanitation

SELECTING COLONIES

COLONIES SELECTED FOR TESTING SHOULD:

- Be healthy and queenright
- Contain strong brood patterns
- Have active brood production
- Be representative of the breeding population

AVOID:

- Weak colonies
- Recently requeened colonies
- Colonies under severe stress
- Colonies with poor brood viability

DAY 1:

SETTING UP THE ASSAY

Step 1 — Select a Suitable Frame

Choose a brood frame with:

- Solid capped brood,
- Minimal empty cells,
- Appropriate pupal stage.

To verify brood age:

- Gently uncap several cells,
- Confirm pupae have pink to light purple eyes.

Step 2 — Label the Frame

Label:

- Colony ID
- Test areas (A and B) with colony ID for photo
- Frame orientation.

Mark arrows on the top bar indicating which side contains the test areas using the paint pen.

Step 3 — Mark Test Areas

Circle two separate areas of 50 capped cells each using a paint pen. A small number of empty cells within the test area is acceptable.

Step 4 — Pin-Kill Brood

Insert a fine insect pin into the centre of each capped cell until resistance is felt at the cell base.

Important:

- Keep punctures small
- Avoid tearing cappings
- Maintain consistency between cells

Avoid damaging frame base by stopping pin when resistance is felt. Photograph the completed frame before returning it to the hive.

Step 5 — Return Frame

Return frame to original colony and record:

- Date
- Time
- Colony ID
- Observer information

DAY 2:

ASSESSING RESULTS

Return exactly 24 hours later.

Step 1 — Remove frame

Brush bees gently from the frame and inspect both test areas.

Step 2 — Record Results

Photograph the frame with labels visible.

Count:

- ✓ cells completely cleaned out
- ✗ do not count uncapped cells with dead brood still present

CALCULATING HYGIENIC BEHAVIOUR

$$\text{Hygienic Score (\%)} = \frac{\text{Removed Cells}}{\text{Total Pin-Killed Cells}} \times 100$$

Calculate:

1. Area A score
2. Area B score
3. Average both scores for final colony result

INTERPRETING RESULTS

Score	Interpretation
95–100%	Highly hygienic
80–94%	Moderate hygienic response
Below 80%	Low hygienic behaviour

TROUBLESHOOTING & BEST PRACTICES

COMMON SOURCES OF ERROR

Issue	Possible Cause
Low removal rate	Incorrect brood age
High variability	Uneven brood development
Excessive uncapping	Damaged cappings
Inconsistent results	Timing errors
Poor repeatability	Timing not exactly 24 hrs; queen was not present for 7 weeks beforehand; supercedure between tests

BEST PRACTICES

- ✓ Test colonies under similar conditions
- ✓ Use consistent timing
- ✓ Standardize scoring methods
- ✓ Repeat tests over time
- ✓ Compare colonies within same yard

USING HYGIENIC BEHAVIOUR IN BREEDING PROGRAMS

To reduce disease transmission risk:

- Sanitize tools between yards,
- Sanitize pins with 70% ethanol before testing the next hive
- Avoid transferring brood comb,
- Follow operation biosecurity protocols.

BIOSECURITY CONSIDERATIONS

Hygienic behaviour should be considered alongside:

- Honey production
- Wintering ability
- Temperament
- Brood pattern
- Varroa monitoring data

Repeated testing over multiple seasons improves confidence in breeder selection decisions.