Hosting a Modified Hanging Drop Training Clinic

An instructor guide for teaching microscopic diagnosis of American foulbrood and European foulbrood using the Modified Hanging Drop. Prepared by Michigan State University.

For additional details on the Modified Hanging Drop or other honey bee medicine information, visit www.BeesNeedVets.com.

Overview

This guide provides instructors with the knowledge and structure needed to confidently teach the Modified Hanging Drop (MHD) method. The MHD is a rapid, slide-based diagnostic technique used to visualize bacterial morphology, specifically:

- *Melissococcus plutonius* (causative agent of European foulbrood, EFB)
- *Paenibacillus larvae* (causative agent of American foulbrood, AFB)

The guide includes clinic planning logistics, safety considerations, troubleshooting, and instructor support. It is suitable for instructors of all experience levels, including those who have never led a microscopy clinic.

Audience

This guide is designed for:

- Veterinarians, veterinary technicians, veterinary students
- Extension educators
- Apiary inspectors
- Beekeeping instructors
- Advanced beekeepers interested in teaching diagnostics

Learning Objectives

By the end of the clinic, participants should be able to:

- Select appropriate brood samples for the MHD
- Prepare slides using the MHD workflow
- Use compound microscopes with oil immersion
- Identify *M. plutonius* and *P. larvae* based on morphology
- Differentiate pathogens from debris and common contaminants
- Interpret results and troubleshoot common issues

Clinic Planning & Logistics

A successful clinic requires thoughtful planning of space, equipment, staffing, and participant flow. Instructors should plan participant numbers around constraints of their setup rather than relying solely on interest, such as microscope availability, table space, staffing limitations, lab coat availability, and sample quantity.

Space Requirements

A clean lab or classroom with stable tables and access to microscopes with 100x oil immersion are essential. Heat lamps must be placed on surfaces that can tolerate prolonged heat exposure. Consider lab level requirements for your location; institutions may require BSL-1 or BSL-2 spaces.

A projection system or microscope camera dramatically improves teaching by allowing participants to watch the instructor's slide viewing in real time.

Microscope Capacity

Microscope availability may determine group size and pacing.

- Ideal: 1 microscope per participant
- Minimum: 1 microscope per 2 participants

When microscopes are shared, instructors should plan for the additional time needed for microscope usage.

Staffing

A well-staffed clinic can ensure smooth participant support, which includes:

- Lead instructor familiar with MHD
- 1-2 assistants for microscope support
- Optional apiary assistant for live sample collection.

MSU's typical setup includes 3-4 instructors/assistants for up to 20 participants, with samples prepared in advance.

Additional Considerations

Instructors should confirm:

- Parking instructions and access codes
- Lab access restrictions (key card, limited hours)
- Safety and accessibility accommodations

Communicate these details in advance to prevent delays and confusion.

Sample Options

Prepared Samples

Prepared samples offer consistency and predictable teaching outcomes. Instructors can prepare multiple samples of EFB, AFB, and negative controls the day before the clinic. Verify each sample using the MHD to ensure quality. Consider labeling samples “A”, “B”, and “C” to allow students to diagnose them independently.

Samples can be prepared in several ways depending on clinic size and teaching goals. One approach is to collect multiple larvae into a microcentrifuge tube or a Whirl-Pak with Phosphate Buffered Saline (for example, 10 larvae into 10 mL PBS) and macerate them thoroughly to create a uniform suspension. This pooled sample provides enough material for multiple slide preparations and ensures consistent results across participants. Alternatively, individual larvae may be prepared in separate tubes.

Samples from an Infected Frame

Samples may also be obtained directly from a brood frame, requiring less preparation and allowing participants to see clinical signs in person. This works best for small groups or field-lab hybrid clinics.

Larvae can be macerated within the cell using a wetted swab. Single larvae may only provide enough bacteria for a few slide preparations; macerate multiple larvae or pull them into a sample tube when needed.

Equipment and Supplies

Field Supplies

- Brood frame with visible signs of AFB or EFB
- Sample tubes (e.g., 10 larvae in 10 mL PBS)
- Toothpick, forceps, or lab spatula for collection

Lab Supplies (Per sample)

- Nitrile gloves
- Microscope slide + cover slip
- Distilled water
- Cotton swab
- Self-closing forceps or tight clothespin
- Infrared heat lamp
- **Type A** immersion oil *(Type B will not work)*
- Carbol fuschin stain (Ziehl-Neelsen stain) *Note human health hazard
- Blotting paper (e.g., Kim Wipes)
- Compound microscope with 100x oil immersion

Demonstration Tools (Recommended)

- Microscope camera

- Projector or monitor
- Reference sheets showing morphology and common contaminants

Pre-Clinic Checklist

- Verify equipment availability and functionality
- Set up safety materials
- Plan room layout
- Stage backup supplies
- Provide parking/access instructions
- Prepare accessibility accommodations

Clinic Setup

Stations

Each participant should have access to two stations:

1. **Heat Lamp / Rinsing Station** (Shared among up to 4 participants)
    a. Heat lamp
    b. Forceps/clothespins
    c. Markers (for labeling samples/clothespins)
    d. Carbol fuschin stain
    e. Swabs
    f. Distilled water
    g. Rinsate collection container
    h. Blotting paper (e.g.., Kim Wipes)
    i. Sample tubes or brood frame
2. **Microscope Station**
    a. Microscope
    b. Immersion oil
    c. Reference guides

Each participant will need disposable gloves. Consider a setup near the entrance where participants can obtain items such as gloves, lab coats, and reference guides. Alternatively, these can be placed at each microscope station.

Room Layout

Arrange heat lamp stations along the perimeter to prevent crowding. Place staining and rinsing supplies in clearly marked areas to avoid cross-contamination. Designate waste collection areas.

Designate an area for the sample tubes and/or brood frame if shared among multiple stations.

Teaching the MHD Method

This section outlines how to structure and teach the Modified Hanging Drop method.

Clinic Format Options

Clinics can be adapted to different teaching environments, timeframes, and group sizes.

Option A: 2-Hour Diagnostic Clinic

- 15 min - Overview of AFB/EFB
- 15 min - Live slide preparation demonstration
- 60 min - Hands-on slide preparation + microscopy
- 30 min - Interpretation, case examples, Q&A

Option B: Half-Day Intensive Clinic

Includes all components of Option A, plus:

- Extended practice time
- Comparison of MHD vs. culture vs. PCR
- Additional case examples
- Optional microscopy modules, such as Nosema testing, tracheal mite microscopy, and anatomy dissections (all not included in this guide)

Option C: Field & Lab Hybrid

- Collect brood samples in the apiary
- Prepare and examine slides indoors

Agenda and Teaching Workflow

1. Welcome & Orientation
    a. Introductions, outline goals, review safety & biosecurity.
2. Biology of AFB & EFB (optional or brief)
    a. Pathogen characteristics, transmission route, clinical signs vs. microscopic confirmation, comparison of MHD vs. PCR/culture.
3. Live Demonstration
    a. Instructor performs full MHD workflow with projected microscope view.
4. Hands-on Practice
    a. Participants prepare multiple slides using known positives, known negatives, and optional mystery samples. Instructors circulate to assist with technique, microscope adjustments, and troubleshooting.
5. *Optional: Holst Milk Test*
    a. During heat-fixing period, demonstrate the Holst Milk Test to fill down time. Shows results after incubation.
6. Discussion

    a. Review findings, pathogen morphology, common contaminants (pollen grains, debris), and when to repeat sampling or escalate to PCR/culture.
7. Wrap-Up
    a. Answer questions, gather feedback, and provide reference materials.

Hands-on Practice: Full Workflow

This is the core technique participants must learn.

Prepare the Slide

1. Place a small drop of distilled water on a clean cover slip.
2. Wet a sterile swab with distilled water.
3. Thoroughly macerate the brood sample in the sample tube with the wetted swab.
    a. If pulling samples straight from the frame, macerate the larva within the cell with the wetted swab.
4. Gently touch the swab into the drop of water on the cover slip just until the drop becomes slightly cloudy. Transferring too many bacteria may cause the slide to be difficult to read.
5. Hold the cover slip in self-closing forceps or tight clothespins and label it. (We find that tight, wooden clothespin work well, allow participants to label their sample with initials and sample #, and do not get overly hot under the heat lamp.)
    a. Labeling can be done directly onto wooden clothespins or by placing a labeled piece of paper under the participant's forceps/clothespins.
6. Place the forceps/clothespin with the slip under the heat lamp to heat-fix the sample to the slip. There is no set distance that the slip needs to be from the heat. It may depend on your setup. The further the lamp is from the sample, the longer it may take to heat-fix.
7. While the cover slip is heat-fixing, label a microscope slide with participant's initials and sample ID.
8. Gently spread Type A immersion oil on the slide just larger than a cover slip's size. This can be done using the oil's immersed glass bar attached to the cap or by placing a drop of oil and then spread it with a clean, gloved finger or swab handle.
9. Once the cover slip is fully heat-fixed, it will appear completely dry and as a cloudy circle on the slip (usually 10-20 minutes).

Stain the Sample

10. With the cover slip still in the forceps/clothespin, flood the fixed sample on the slip with carbol fuschin stain. This is done by adding enough drops of stain to cover the sample and holding it horizontally to not pour off the stain.
    a. It may be best to do this over a container to catch any stain that drops.
11. After 30 seconds, gently and briefly rinse the slip with distilled water using a squeeze bottle or small trickle from a faucet. Be sure to catch the rinsate in a container for proper disposal.
12. Leave a little bit of water on the slip. [There needs to be enough water that when inverted in the next step, a drop hangs from the slip, thus the name of the method.]

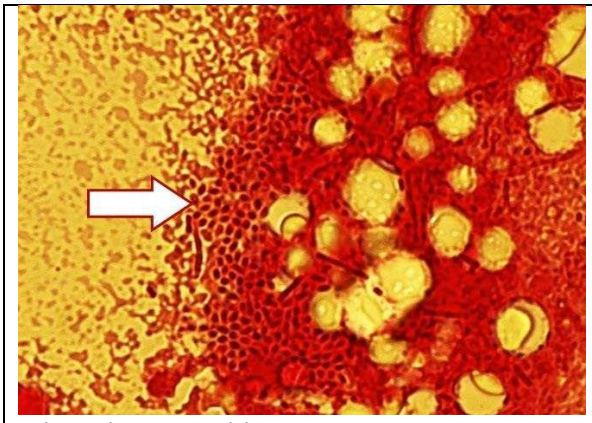
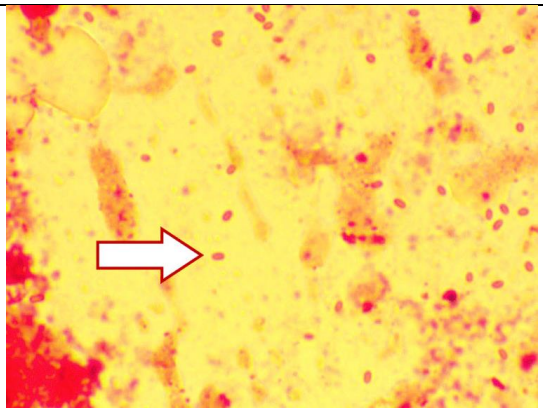
Assemble the Slide

13. Carefully invert the slip and place it onto the oiled microscope slide with the sample/water side down.
14. Blot excess water with blotting paper.
15. Place the slide on the microscope and add a drop of immersion oil to the top of the cover slip.

Microscopy

16. View under the microscope. Start at low magnification to locate the sample, then adjust to 100x.

Microscopy & Findings

 *Photo by Sam Abban, USDA.*	 *Photo by Sam Abban, USDA.*
Melissococcus plutonius (EFB) • Small cocci (spherical-shaped) • Arranged in chains or pairs	*Paenibacillus larvae* (AFB) • Large, rod-shaped ovals • Exhibit Brownian motion

Staining Quality

- Good staining: Clear contrast, visible morphology
- Over-staining: Dark background, obscured structures
- Under-staining: Faint, washed-out

Common Contaminants that may obscure microscopy findings include pollen grains, tissue fragments, and other debris.

For additional details, videos, and further microscopy instructions, visit www.BeesNeedVets.com.

Safety & Biosecurity

Because MHD clinics involve infectious brood material, chemical stains, and shared equipment, instructors must implement safety and biosecurity practices. Consider each of the following to help ensure participant safety, prevent cross-contamination, and support compliance with institutional and state requirements.

PPE

At a minimum, all participants should wear disposable gloves, closed-toe shoes, and long pants during the clinic. Lab coats provide additional safety and are often required by organization policies. Safety goggles may not be necessary when handling small amounts of carbol fuschin but should be available if individuals request them.

Cross-Contamination Prevention

AFB and EFB bacteria are not harmful to humans but should be handled carefully to prevent contamination, especially during field clinics.

Carbol Fuschin Handling

This stain is a carcinogen; careful handling is essential. Use labeled containers, avoid spills, and collect rinsate for proper disposal. Educate participants about risks and safe handling. Follow all institutional chemical safety requirements.

Waste Disposal

Follow label instructions and state or local regulations for proper disposal. **Even diluted chemicals should not be poured down a sink.**

AFB Handling and Transport

Regulations vary by state; instructors should verify local requirements and comply with state regulations when handling and transporting infected materials.

Accessibility

Support low-vision participants, adapt microscopy for mobility limitations, and provide digital microscope feeds when needed.

Instructor Resources

Optional Add-ons

Provide handouts or online references to support participants. Consider:

- Morphology reference sheets with descriptions and photos of each bacteria
- AFB and EFB regulatory information
- Microscopy quick-start guides
- Slide interpretation examples – photos and/or videos

Enhance your clinic with:

- Workflow cards or handouts
- Positive and negative slide sample library
- Continuing Education credits
- Pre/post clinic quizzes to assess knowledge gains and confidence levels
- Integration with a broader honey bee disease curriculum

Troubleshooting Guide

Issue	Reasoning	Solution
Not enough sample transferred to the cover slip	Sample macerated with a dry swab	Wet the swab before macerating samples
Slides barely contain visible sample or are empty	Stain was over-rinsed from the cover slip	Rinse *briefly* with gentle stream of water
Staining is faintly visible on slides	Slip did not contain enough stain for long enough	Stain should fully cover sample, hold horizontally while staining, and stain for the full 30 seconds. Add additional stain if needed.
Cloudy, debris-heavy slides that are difficult to interpret	Too much sample transferred to slip	Wet swab before macerating samples. Just barely touch swab to slip's water droplet to transfer sample.
Rods and other microbes are difficult to distinguish	Magnification and lighting insufficient for interpreting slides	Adjust microscope's lighting and magnification for clarity. Adjust fine focus to view through the oil layers.

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