Droplet Analysis Methods

Droplet analysis methods are used to assess both droplet characterization and droplet deposition associated with ultra-low volume (ULV) insecticide applications. Droplet characterization is performed prior to a field trial to calibrate spray equipment and confirm that the generated droplet spectrum meets operational specifications. During the field trial, droplet deposition is assessed by collecting droplets on sampling substrates placed within the treatment area, allowing evaluation of the droplets that are deposited under actual field conditions. Analysis of these samples provides quantitative measures of droplet size, density, and spatial distribution, which are critical for interpreting spray performance and efficacy. The following describes three commonly used droplet analysis methods.

DropVision is a droplet analysis software developed and managed by Leading Edge, Inc (Daytona Beach, FL), available through a paid license. It provides comprehensive analysis of the droplet spectrum generated by ground and aerial spray equipment. The software works in conjunction with a microscope and digital camera setup, analyzing droplets that are collected on 1-inch Teflon-coated or magnesium oxide slides or 3mm rods.

Leading Edge, Inc. offers the DropVision Fluorescence option for areas where significant background particles may complicate the analysis of chemical droplets. This system involves mixing a fluorescent tracer into the chemical before the field trial, allowing for clear differentiation between the fluorescent chemical droplets and background particulate. To accommodate this feature, additional microscope hardware is required, specifically designed for fluorescent droplet analysis. The fluorescent tracer commonly used for field trials is Tinopal.

The microscope and camera are connected to a computer that has DropVision software. Images of the slides or rods are systematically captured, and the software displays the droplets in the microscope’s field of view. Users can verify the automatic

identification of the chemical droplets that the software indicates, as well as manually adjust the droplet identification to select or deselect droplets.

DropVision allows the user to define either a set number of droplets to analyze or a pre-determined number of images to capture before generating a final report. The report includes several key outputs. DV 0.1, DV 0.5, DV 0.9: These metrics indicate the distribution of the droplet sizes. The DV 0.1 value indicates the droplet diameter below which 10% of the spray volume is contained. The DV 0.5, also known as the volume median diameter (VMD) is the droplet size at which 50% of the spray volume is composed of smaller droplets, and DV 0.9 represents the droplet diameter below which 90% of the spray volume is contained. DropVision also reports the total number of droplets collected, the number of droplets larger than 32 and 48 microns, the total area analyzed, and the droplet density in drops per mm^2.

ADrop is a droplet analysis program developed and managed by Valent BioSciences (Libertyville, IL), available free of charge upon completion of an end-user license agreement. Users must procure and assemble the appropriate equipment including a microscope and camera attachment. The software supports analysis of droplets collected on both 1-inch slides and 3mm rods, which are read systematically using the software.

A minimum of 10 images are taken of the slides or rods. After the images are collected, they are run through the ADrop program in ImageJ, which generates a CSV file containing the droplet data. This CSV file can then be uploaded to the ADrop app, accessed via a web browser, to generate graphs, statistical summaries, and a comprehensive downloadable report.

Unlike DropVision, ADrop does not allow for manual selection or deselection of droplets identified by the software. This limitation necessitates the use of a fluorescent tracer, such as Tinopal, during semi-field trials to distinguish chemical droplets from background particles.

ADrop provides key outputs in its final droplet summary, including the total number of droplets collected, droplet density in drops per mm^2, and both a VMD and Number

Median Diameter (NMD). These metrics include DV 0.1, DV 0.5, and DV 0.9. The inclusion of the NMD in addition to the VMD offers an additional summary of the data to better characterize the spray cloud during a field trial.

Manual Droplet Analysis is a technique used to characterize spray droplet deposition by visually examining and measuring droplets collected on slides. Droplets are counted and measured manually using a microscope. Although more time-intensive than automated methods, manual droplet analysis provides a low-cost and accessible approach for assessing spray quality and verifying application performance in field trials.

Manual Droplet Analysis Protocol

Equipment needed

- Compound microscope equipped with 10x objective and 10x eyepieces.
- 1 mm eyepiece micrometer (ocular reticle) ruled in a scale of 100 divisions (Figure A).

Fig A. 1 mm eyepiece micrometer

- A NIST certified stage micrometer, 1 mm long and ruled in 0.10 and 0.01 mm increments (Figure B).

Fig B. Stage micrometer

1. **Eyepiece micrometer calibration**
    1.1. Place the stage micrometer on the microscope and view at 10x magnification.
    1.2. Superimpose the reticle over the stage micrometer and measure the entire length of the ocular micrometer (Figure C).

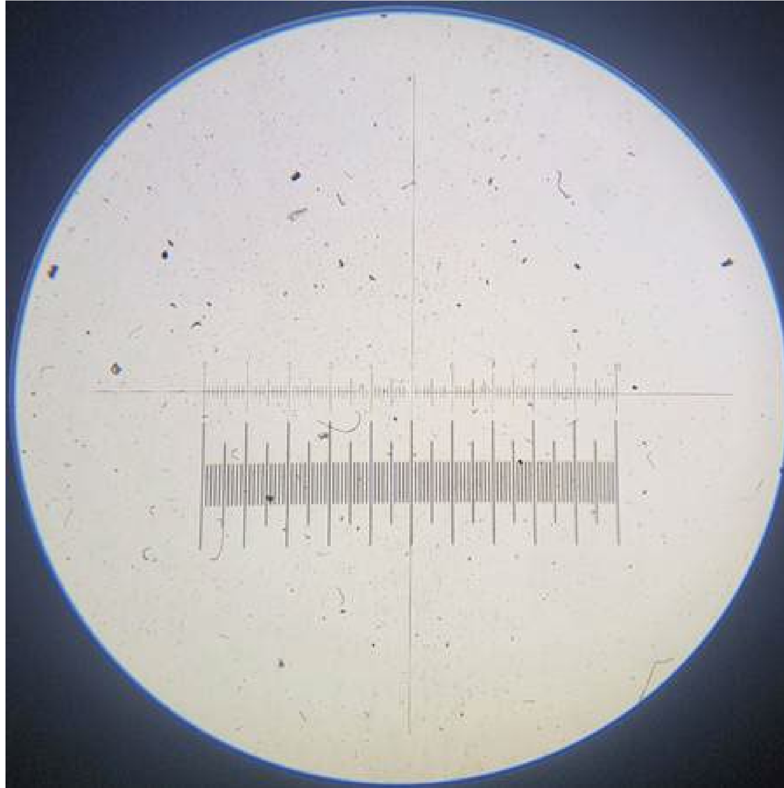


Fig C. Reticle superimposed over stage micrometer

    1.3. Divide the length of the reticle by its number of eyepiece divisions.
    1.4. As pictured in Figure C, the stage micrometer is below and the total length is 1000 µm (1 mm, each division is 0.01 mm or 10 µm). The reticle is above and the 100th eye piece division (EPD) aligns with the 1000 µm mark on the stage micrometer. Therefore, each EPD of the reticle is 10.0 µm.
    1.5. The µm/EPDs is specific to the objective lens that was used and unique to each microscope. Recalibration is necessary if the reticle, eyepiece, or objective lens is replaced.

2. **Measuring droplet size on a slide**
    2.1. Place the slide on the microscope with Teflon-coated slide facing upwards.
    2.2. Adjust the focus so that the edges of the droplets are clear and sharp.
    2.3. Superimpose the reticle over the droplet and count the number of eyepiece divisions that fit inside the droplet.

2.4. Each eyepiece division represents a number of microns that was determined in the calibration step. The diameter of an impinged droplet needs to be adjusted to account for its spread on the slide, which is a function of the surface tension of the ULV formulation. Each ULV formulation has its own spread factor. Below is a list of spread factors for commonly used ULV products.

Product	Spread factor
Biomist 4+4[1]	0.67
Anvil 10+10[1]	0.63
Anvil 2+2[1]	0.57
Aqua Anvil[1]	0.65
AquaHalt[1]	0.66
Duet[1]	0.60
Duet HD[1]	0.645
Merus 3.0[1]	0.633
Zenivex E4[2]	0.60
DeltaGard[3]	0.60
Permanone 30+30[3]	0.61
Fyfanon[4]	0.69
Dibrom Concentrate[5]	0.72

[1]Registered trademark of Clarke Mosquito Control Products

[2]Registered trademark of Central Life Sciences

[3]Registered trademark of Envu

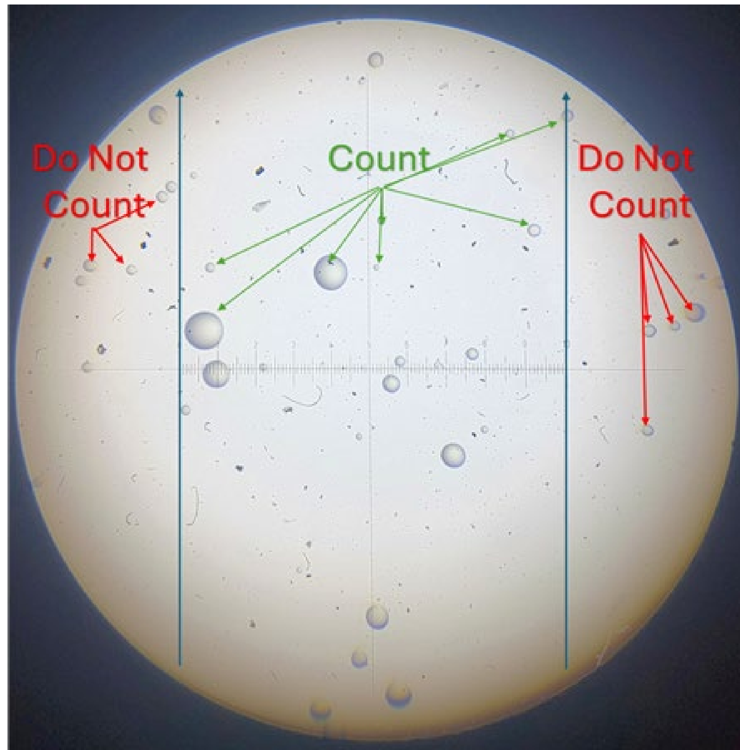
[4]Registered trademark of FMC

[5]Registered trademark of AMGUARD

3. **Reading slides to determine DV50 and droplet density**

3.1. Move the slide so that the edge of the slide is in view. Select a portion of the slide free of debris and fingerprints.

3.2. Starting at one edge of the slide, move the slide across the field of view in a straight line and record the number of eyepiece divisions in each droplet that passes through the reticle. Droplets that are outside the reticle should be ignored.



3.3. This can be done most efficiently by using two people, one person using the microscope and calling out the number of EPDs for each droplet and one person tallying on paper as follows:

EPDs	Number of Droplets
1	###
2	### ### //
3	### ### ###-///
4	###-/
5	///

3.4. Once the other edge of the slide comes into view and crosses the reticle, a "pass" has been completed. If less than the desired number of droplets has been counted (a minimum of 100 droplets is recommended) move the slide far enough laterally so that none of the previously counted droplets will be in view and complete another pass.

3.5. Repeat as needed until the minimum number of droplets have been counted and note the number of passes on the droplet tabulation form.

3.6. Once a pass has been started it must be completed, and it must be conducted from edge-to-edge. The number of passes and the width of the slide will be used to determine the total area that droplets were counted within, which is needed to determine the droplet density.

4. Interpretation of Droplet Counts

4.1. Calculating droplet density

4.1.1. Determine the total area that was examined in mm^2 by with the following formula

$$\frac{(\mu m/\mathrm{EPD}) \times (\#\ \mathrm{of\ EPDS})}{1000} \times slide\ length\ (mm) \times \#\ of\ passes$$

Example based on values collected in section 2.4, reading a 1 inch slide, and completing 3 passes:

$\frac{10 \times 100}{1000} \times 25.4 \times 3 =$ 76.2 mm^2

4.1.2. Divide the total number of droplets counted by the total area examined

Example using tally chart from section 4.3 and area calculated in section 5.1.1.:

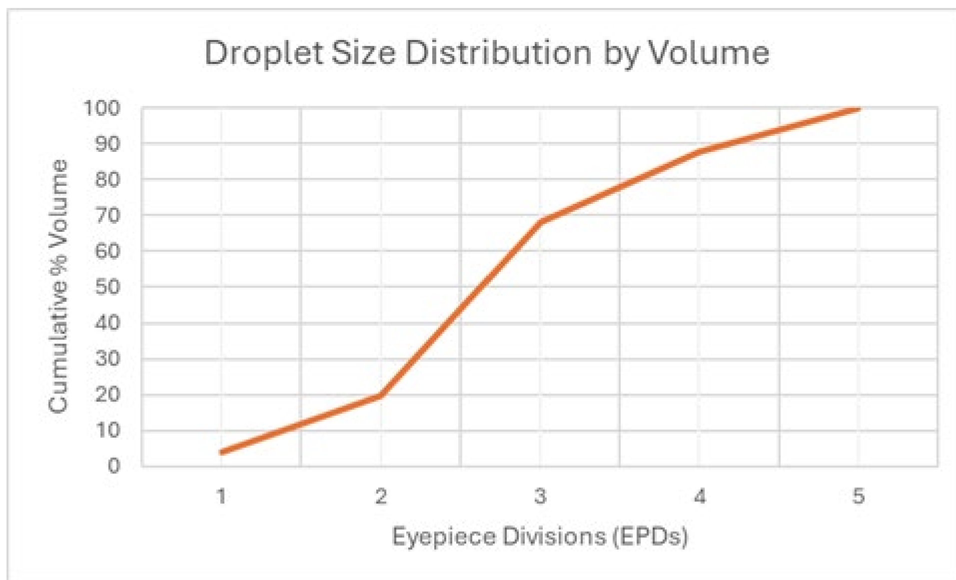
${}^{44\ droplets}/_{76.2\ mm^2} =$ 0.57 drops/mm^2

4.2. Determine DV_{10}, DV_{50}, and DV_{90}

4.2.1. Create the following chart and input the droplet tallies recorded in section 4.3

EPDs (D)	# of droplets (N)	EDPs X # of droplets $D \times N$	% of total volume $\frac{D \times N}{\sum D \times N}$	Cumulative total volume
1	**5**	5	4.1%	4.1%
2	**12**	24	19.7%	19.7%
3	**18**	54	44.3%	68.1%
4	**6**	24	19.7%	87.8%
5	**3**	15	12.2%	100.0%
Total	44	122	-	-

4.2.2. Plot the cumulative total volume (Y axis) against the corresponding EPD (x axis) as below:



4.2.3. To determine DV_{50} find the point where the line crosses the 50% mark and note the position on the X axis to the nearest 10th of an EPD.

4.2.3.1. Multiply the X axis value by the µm/EPD value determined in section 2.4 and by the spread factor (section 3.4) of the product that is being evaluated.

Example (using Duet spread factor):

$$\mathrm{DV_{50}} = EPDs \times {}^{\mu\mathrm{m}}/_{EPD} \times Spread\ factor$$

$$\mathrm{DV_{50}} = 2.6 \times 10.0 \times 0.60 = 15.6\mu\mathrm{m}$$

4.2.4. To determine DV_{10} and DV_{90} Repeat prior step using 10% mark and 90% mark.