STANDARD OPERATING PROCEDURES

FOR

Karenia brevis COUNTING PROCEDURE

AND SAMPLE HANDLING

Updated 10/20/2025

PINELLAS COUNTY PUBLIC WORKS DEPARTMENT

DIVISION OF ENVIRONMENTAL MANAGEMENT

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Pinellas County Division of Environmental Management (DEM) Standard Operating Procedures (SOPs) are adapted from published methods or developed by in-house technical experts. Most of the methods are based on Florida Fish and Wildlife Commission (FWC) and/or Florida Department of Environmental Protection (FDEP) methods, which are referenced throughout the document. This document is intended primarily for internal DEM use. This SOP document should not replace any official published methods.

Any reference within this document to specific equipment, manufacturers, or supplies is only for descriptive purposes and does not constitute an endorsement of a particular product or service by the DEM. Additionally, any distribution of this SOP does not constitute an endorsement of a particular procedure or method.

Although the DEM will follow these SOPs in most instances, there may be instances in which an alternative methodology, procedure, or process will be used.

Personnel performing monitoring tasks must be trained and certified internally according to DEM standards. Use of equipment and reagents are subject to approval by the monitoring QA Officer and/or the Environmental Monitoring Coordinator. Training will be done through annual audit exercises in the laboratory and field to familiarize personnel with operation, calibration, and maintenance. Staff must be familiar with the SOP document and user's manuals, when applicable.

Sample Preparation

- Preserve each sample with Lugol's solution by adding three drops into the vial and then gently inverting the vial to homogenize it.
    - Lugol's does stain skin and clothing, so use caution when handling.

- Counting chambers must be clean and smudge-free. Use a cotton swab and a small amount of lens cleaner to clean the chamber. Make sure the chamber is dry, as water droplets can obscure the sample.
    - Use the 4-section counting chamber (Fig. 1) to minimize count time.

Figure 1. Counting chamber with 4 sections.

- Line up sample vials in order of site location from south to north.

- Using a glass 1 mL pipette, transfer 1 mL of the first sample (southernmost location) into the first counting chamber section. *****During bloom conditions when a high concentration of K. brevis is observed, ½ mL of sample should be used in each chamber for more accurate counting.*** *If needed, add ½ mL of DI water into the chamber along with the ½ mL of sample to fully cover the area with liquid.*

- Rinse the pipette with deionized (DI) water at least one time between samples. Dispose of this rinse water in the waste jug.

- Proceed with putting 1 mL (*or ½ mL if bloom conditions*) of each subsequent sample into each chamber section. Once all four sections have a sample in them, cover the chamber with the slide cover and let the samples settle for approximately 20 minutes.
    - Remember to keep the samples in order.

- While the samples are settling, set up the notebook (Fig. 2).
    - Write the collection date, counter's initials, and volume of sample used. The standard volume used is 1 mL, but ½ mL may be used if there is a high concentration of *K. brevis*.
    - List the site names in order of location from south to north and sample time. The count results will be written to the right of each site name.

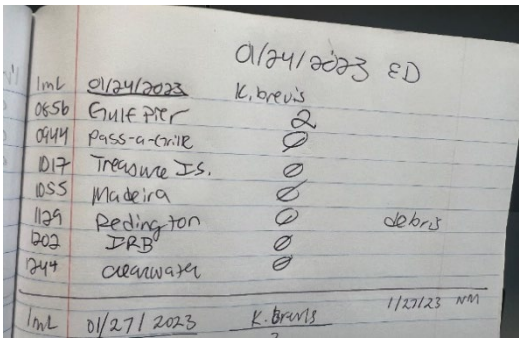

Figure 2. Sample notebook setup.

Microscope Setup & Sample Counts

- After allowing the samples to settle, uncover the microscope and place the prepared counting chamber on the microscope stage. Orient the chamber horizontally between the stage clips.
    - Avoid blind spots when the chamber is sitting on the top part of the stage clips (Fig. 3a) by placing the chamber to allow a gap (Fig. 3b). The chamber should still fit snugly between the clips.

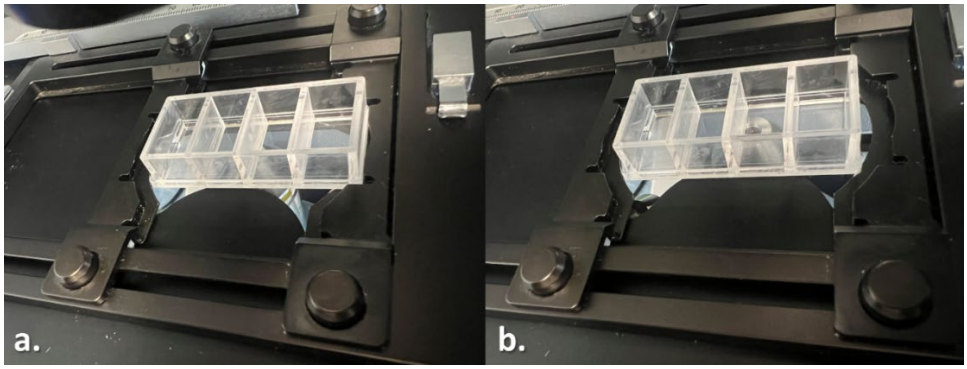

Figure 3. Horizontal chamber orientation if flush with the stage clips, which yields a blind spot (a); horizontal chamber orientation to eliminate the blind spot (b). Note the gap in (b).

- Turn on the microscope. While at the 100x magnification (**yellow** dial) use the coarse adjustment (thicker knob) to focus the slide.

- Adjust the eye pieces to comfortably view the sample. Use lens cleaner with a lens tissue to ensure eye pieces are smudge-free.

- The left eye piece has a scale bar (Fig. 4a) and the right has a grid (Fig. 4b). For *K. brevis* samples, count everything that is visible in the circular plane of view, not just what is seen in the grid.

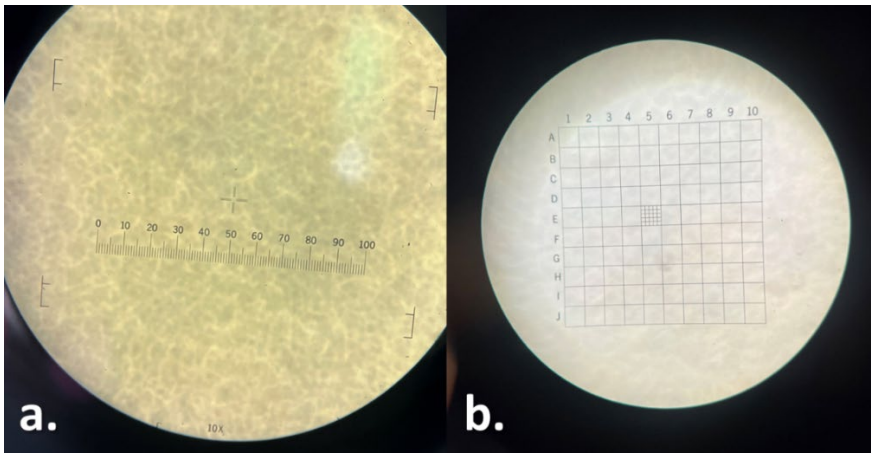

Figure 4. Scale bar seen in the left eye piece (a); grid seen in the right eye piece (b).

- Once focused, use the directional wand (Fig. 5) to move around the slide and perform a quick scan of the slide to get a rough idea of the cell concentration. The top dial moves the slide up and down; the bottom dial moves the slide side-to-side.

Figure 5. Photo showing the stage directional wand.

- Focus the sample at the lowest magnification and then proceed to focus at the 200x magnification (**green** dial). *Only* use the fine-adjustment knob once at 200x magnification. Do not use the coarse-adjustment knob at this magnification.

- Starting in the upper-left corner, begin counting the *K. brevis* cells in the sample, keeping track of the counts using a hand-held tally counter (clicker). Use the directional wand to move across the first row, or column, depending on preference. Once the end of the row or column is reached proceed to the second row or column. It helps to use a cell as a reference point when scanning. **Note:** At 200x, each slide consists of about 10 columns and approximately 18 rows. Follow this "lawn mower" method to scan the entire slide (Figs. 6a and 6b).

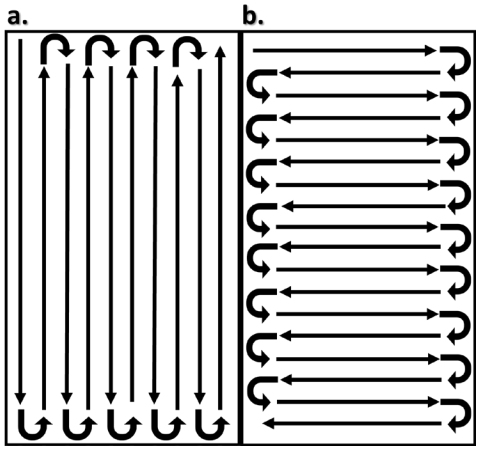

Figure 6. "Lawnmower" scanning utilizing a column method (a); and a row method (b).

- Once the entire sample has been scanned, record the total count of *K. brevis* cells in the notebook. Add comments with additional observations of interest. Repeat this process for all samples.

Sample Count Estimates

- If there is a high concentration of cells in the chamber (Fig. 7), you do NOT need to count the entire chamber section. Total counts can be estimated based on consistent row or column counts using a standard multiplier to calculate a volume count. Scan and count a few rows (not just one) to confirm consistency in distribution across the slide and record the calculated count in the notebook.
    - At 200x, each ¼ chamber section consists of about 10 columns and approximately 18 rows, so multiply accordingly to obtain the total count in the section.

- If the 1 mL sample has such a high concentration of *K. brevis* that there are overlapping cells which make it difficult to clearly and accurately count, replate the sample using only ½ mL per chamber. Allow the proper settling time again. ***If needed, add ½ mL of DI water into the chamber along with ½ mL of sample to fully cover the area with liquid.*

- If a slide is completely covered even at ½ mL sample per section and difficult to differentiate individual overlapping and layered cells, record the concentration as "TNTC," or "too numerous to count."

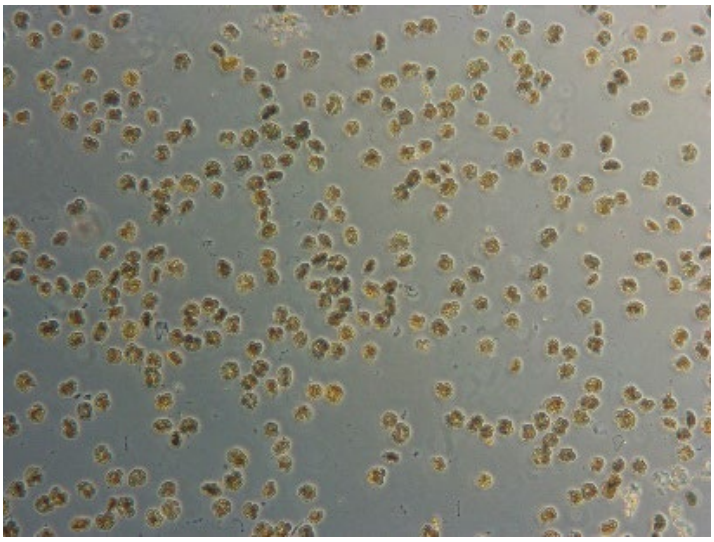

Figure 7. An example of a high concentration of *K. brevis*.

Sample Disposal

- After all samples have been counted, pour the sample down the lab sink while flushing with water, and put the dirty vial in the bin next to the sink.

- To clean vials, fill up the sink with water and a small amount of Liquinox and allow to soak for at least a couple of hours. After allowing the vials to soak, drain the sink and rinse each vial with tap water three times. Place the clean vials upside down in the silver drying rack next to the sink.

- Place the used counting chambers in the small plastic bin next to the lab sink and allow to soak in tap water with a small amount of Liquinox for at least a couple of hours. Then, rinse well and allow to air dry.