## Sample Preparation

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

- Use the 4-section counting chamber (Fig. 1) to minimize count time. 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.


**Figure 1.** Counting chamber with 4 divisions.

- Using the transfer pipette, transfer 1 mL of the first sample into the first counting chamber. Rinse the pipette with deionized (DI) water at least one time between samples. Dispose of rinse water in the waste jug.

- After transferring 1 mL of each sample into its respective chamber, cover the slide with the slide cover and let the samples settle for 5–10 minutes. Remember to keep the samples in order.

- While the samples are settling, set up the notebook (Fig. 2).
    - Record the analysis date, staff initials, the collection date (underline this date), and volume of sample used. The standard volume used is 1 mL.
    - Below the analysis details, list the site names with sample time and title the count column by species (*K. brevis*).

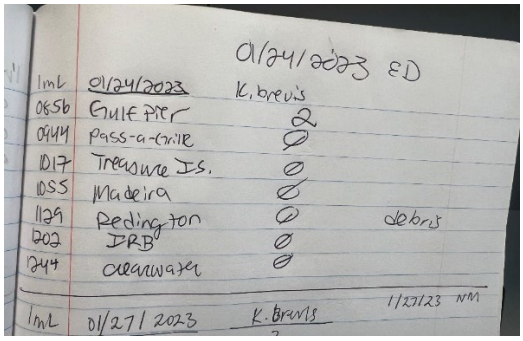


**Figure 2.** Sample notebook setup.

## Microscope Setup

- 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.

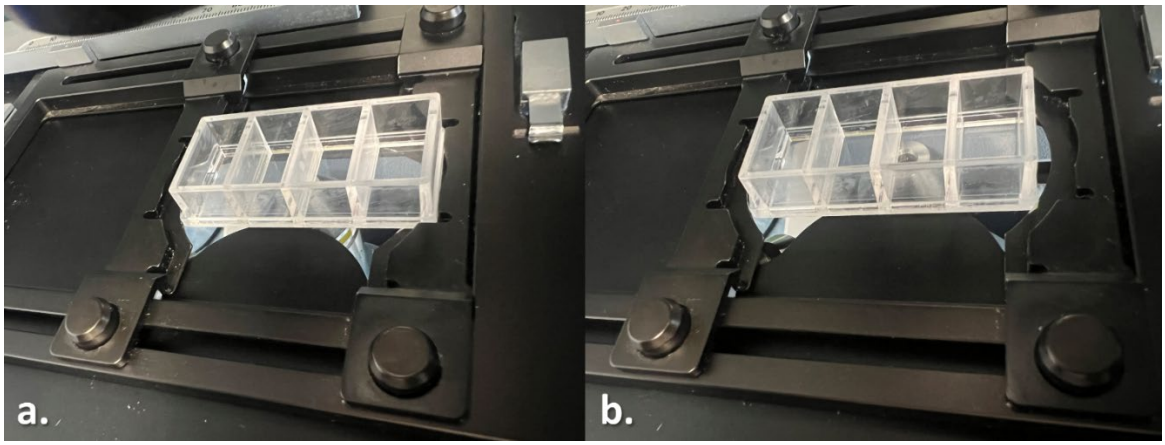


**Figure 3.** Horizontal chamber orientation if flush with the stage clips, which yields a blind spot a); horizontal chamber orientation to eliminate the blind spot b). Note the gap in b).

- Turn on the microscope. While at the 100x magnification (yellow dial) use the coarse tuner (thicker knob) to focus the slide (Fig. 4).

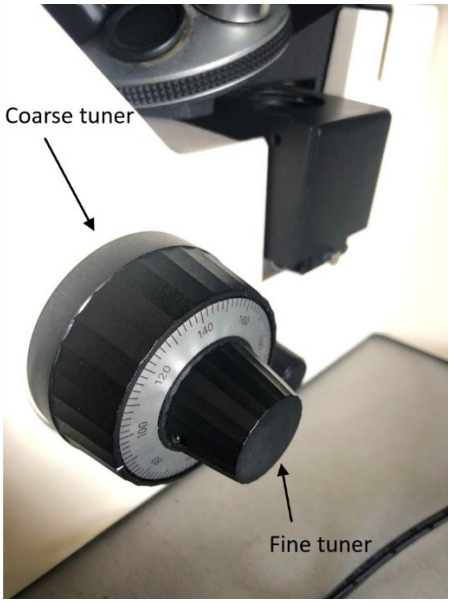


**Figure 4.** Photo showing the coarse and fine tuners.

- The left eye piece has a scale bar (Fig. 5a) and the right has a grid (Fig. 5b). For *K. brevis* samples, count everything visible in the plane of view along with what is seen in the grid.

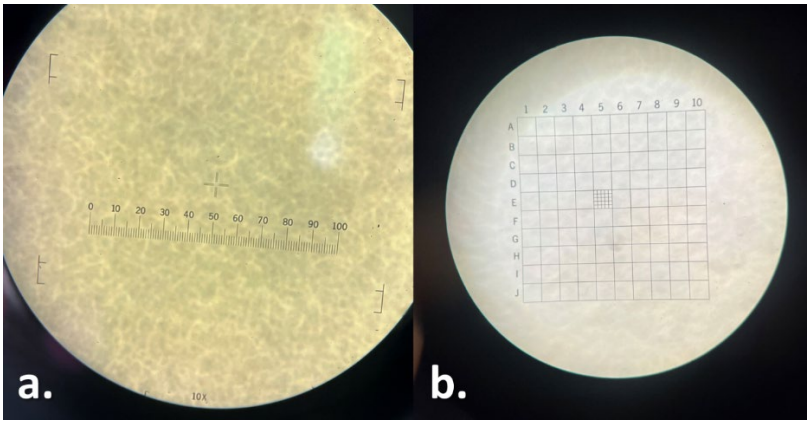


**Figure 5.** Scale bar seen in the left eye piece a); grid seen in the right eye piece b).

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

- Once focused, use the directional wand (Fig. 6) 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 6.** Photo showing the stage directional wand.

- Focus the sample while set to the lowest magnification and proceed to focus at the 200x magnification (green dial). It is recommended to only use the fine-tuning knob while focusing in at 200x magnification.

## Sample Counts

- Starting in the upper-left corner, avoid any air or water bubbles, begin counting the *K. brevis* cells in the sample using a hand-held counting device. 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. 7a and 7b).

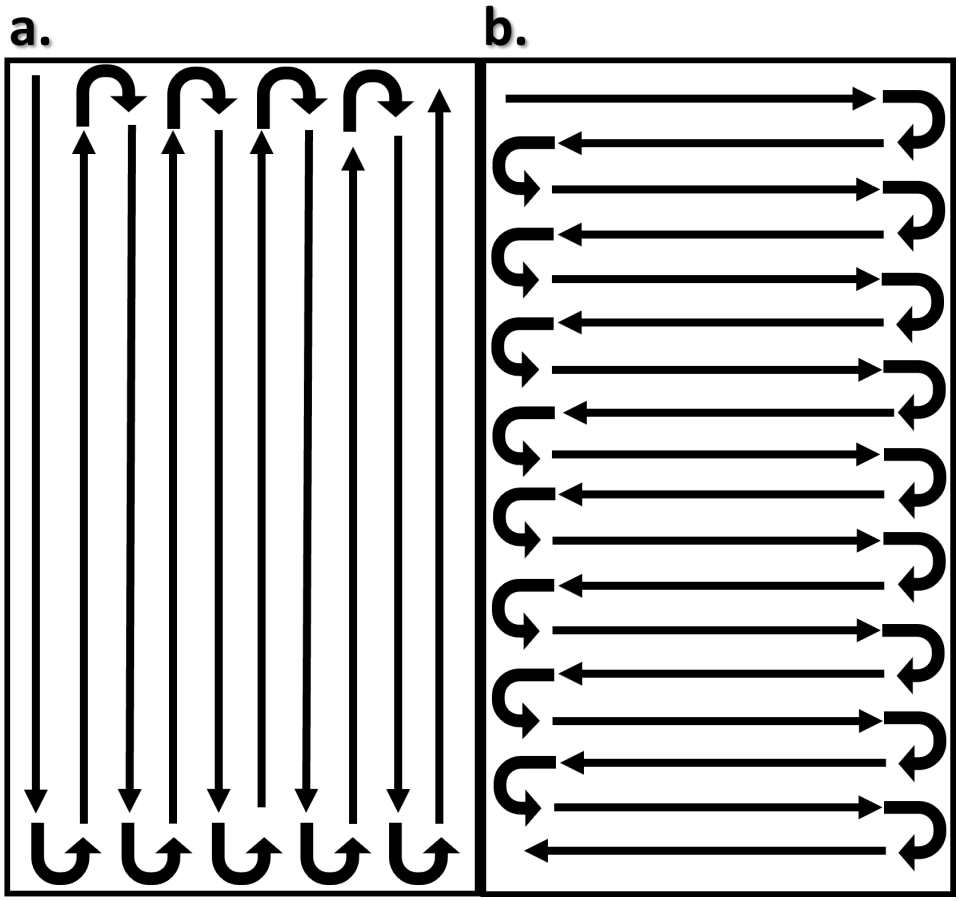


**Figure 7.** "Lawnmower" scanning utilizing a column method a); and a row method b).

- 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.

## Sample Count Estimates

- Samples with high concentrations of *K. brevis* cells can be estimated based on consistent row or column counts using a standard multiplier to calculate a volume count (Table 1). Scan and count a few rows to confirm consistency in distribution across the slide and record the calculated count in the notebook.

| 1 mL | at | 200x Magnification |
|---|---|---|
| Cell Count | per Scan | Multiplier |
| 250+ | 2 COLUMNS | 5 |
| 150+ | COLUMN | 10 |
| 150+ | 2 ROWS | 9 |
| 80+ | ROW | 18 |

- When a slide is completely covered and difficult to differentiate individual overlapping and layered cells (Fig. 8), record the concentration as “TNTC,” or “too numerous to count.”

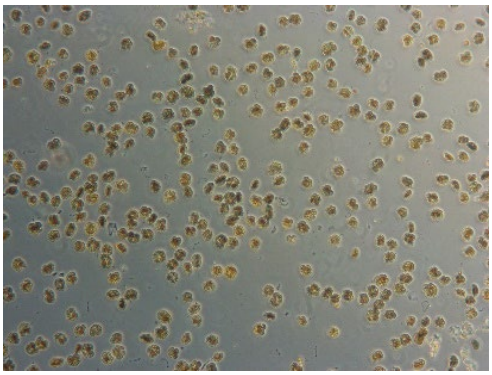

**Figure 8.** An example of a "TNTC" scenario.

## Concentration Status

- After each sample has been counted, add notation for any samples with a medium or high concentration, as determined by the Florida Fish and Wildlife Conservation Commission (FWC) concentration key (Fig. 9), with a red X. These samples will be put into the plastic bin in the cabinet above the microscope. Any samples with a background, very low, or low concentration count can be disposed.

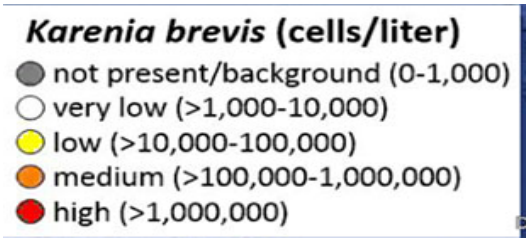


**Figure 9.** FWC key for cell concentrations

## Sample Disposal

- Counted samples, not being retained based on medium or high concentrations, can be disposed by dumping in the lab sink and used sample vials are placed in the bin next to the sink.

- To clean vials, fill up the sink with water with a small amount of phosphorus-free cleanser and allow to soak for a couple 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 any 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 phosphorus-free cleanser. Let chambers soak until any discolored chambers are clear.