DISCRETE CHLOROPHYLL READINGS PROTOCOL

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NOTE: The team leader needs to ensure that ALL watches are consistent in these procedures.

PREPARATION

1. Take a batch of samples from freezer and let them come to room temperature (~1hr).
   - Ensure samples have reached **room temperature**, (you can try touching other glass that has been sitting at room temperature) since readings are *very* temperature sensitive!
   - Always keep the samples covered (dark).
2. The fluorometer must be warmed up for at least 30 min prior to use. (it is usually left on)

RUNNING A BATCH 'O SAMPLES (run time ~1.25 hrs for 30 samples)

3. Start music.
4. Wear safety glasses and nitrile gloves.
5. Check the following settings:

WHAT	SETTING	WHERE (screen #)
pre-avg delay	10 sec	**1.63**
averaging period	15 sec	**1.63**
range	AUTO	**2.43**
cal st. val.	~130-165 for Turner AU-10 #5153	**3.2**
year	98 [s/n 5153 is NOT Y2K compliant]	**4.6**

6. **BLANKING**
   - If you forgot to prepare a blank during filtration, SHAME ON YOU! (preparing the blank during filtration is desirable because it ensures that the blank undergoes the same treatment as the sample). You can make one now by placing a filter into a scintillation vial and adding 10 ml of 90% acetone, and shake until the filter dissolves, then put in remarks of log sheet that you are doing this.
7. To remove smudges, wipe down cuvette with Kimwipe and insert into fluorometer.
8. Go to screen **2.11**
9. Once the reading is stable, press <**0**>.
10. Note the "%" value in comments on log sheet.
11. Go to **2.12** and set 'subtract blank' to NO.
12. Go to HOME screen and press <*****> to start avg.
13. Record value of blank in remarks, then remove, cover with parafilm and place it in a dark place (it will be re-read later).

SOLID STANDARD CHECK

14. Insert solid standard with 'H' on the left, and lightly twist top of cylinder fully clockwise.
15. Go to HOME screen and press <*****> to start avg, record in Rb column.
16. Turn solid standard around so 'L' is on the left, and lightly twist solid cylinder fully clockwise.
17. Go to HOME screen and press <*****> to start avg, record in Ra column, remove standard.
18. **Record room temperature and fluorometer temperature (screen 3.1)** in comments section of log sheet.
19. Check that solid standard values are not significantly different from the last time they were read.

SAMPLE READINGS

20. **Go to 2.12 and set ‘subtract blank’ to YES.**
21. Decant sample into cuvette (test tube) being careful to leave any particulates in the scintillation vial (you only need ~1 1/2" of liquid in the cuvette). [If vial is still just slightly cool, you can hold it in your hand for ~15 seconds while swirling to warm it to room temp.]
22. Remove smudges/fingerprints with Kimwipe, and insert cuvette into fluorometer.
23. From **HOME** screen
    - If display is '<999', allow reading to stabilize (~10 sec), and press <*> to start delay and averaging.
    - If display is ‘>999’, go to screen **3.2** and mentally average the value in 'fluorescense readout'.
        - If **3.2** still says ‘OVER’, you need to dilute:
            - See “chl_dilution_protocols.pdf” for the proper way to proceed
24. Record value (Rb) AND scale on log sheet.
25. Add **2** drops 10% HCl (!be careful not to let drops run down outside of cuvette!). If you accidentally add 3, note so in remarks, or re-read with what's left in the sample vial.
26. Allow reading to stabilize (~10sec), and press <*> to start delay and averaging.
27. Record value (Ra) AND scale on log sheet.
28. **Do not leave any blank spaces on the logsheet**, ensure to fill out the following sections: Vol acet, Rb & scale, Ra & scale, Initals & date/time
29. For each sample, start with a new cuvette and return to step 20.

    BLANK RE-CHECK
30. When finished with all samples, wipe blank cuvette (from step 12) with a Kimwipe and insert into fluorometer.
31. Go to **2.12** and set ‘subtract blank’ to NO.
32. Go to HOME screen and when stable, press <*> to start avg.
33. Record value of blank in remarks, then remove blank.
34. Check that blank value is not significantly different from the beginning of this batch.

    CLEAN UP
35. Recap HCl container.
36. Pour used acetone dregs into waste acetone container.
37. Place dry scint vials and test tubes into the waste lab glass container.
38. Place scint vial caps in lab waste container.
39. Double-check entries on logsheet.
40. Enter values into digital log.
