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Preserving Phytoplankton Samples with Lugol's for FlowCam Analysis

SUMMARY

Aquatic scientists often need to store natural samples for a period of time before processing them using FlowCam. Lugol's preservation is routinely used for this purpose[1,2]. Here we provide instructions on how to use Lugol's as a preservative with room temperature storage conditions until a technician has the time to run them on FlowCam using AutoImage mode (Lugol's preserved samples are not recommended for Trigger mode).

This technical note covers the following topics:

- How to prepare Lugol's-preserved aquatic samples
- Results from experiments, where samples were stored for up to 42 days at room temperature
- Additional considerations when using Lugol's as a preservative to store aquatic samples and how to run them on FlowCam

Samples tested:

- Natural freshwater samples taken with a plankton net from a drainage pond in Scarborough, Maine at 43.58, -70.35 (Summer 2021)
- Natural marine samples taken with a plankton net from Long Island, Casco Bay, Maine 43.69, -70.16 (Summer 2021)

SAMPLE PREPARATION

This technical note contains data from monitoring the Autoimage mode concentration in Lugol's preserved samples over time. Samples preserved with Lugol's were tested against samples where no preservative was used.

1. Anachemia Product Number 48396[3] was used to preserve samples in Lugol's.
2. Sample was diluted to a final concentration of 1% (500 µL into 50 mL).
3. Samples were stored in 50 mL centrifuge tubes (VWR, Catalog #89079-494) at room temperature.
4. Triplicate samples were run using Autoimage mode to measure the count and concentration over a period of 3 months.

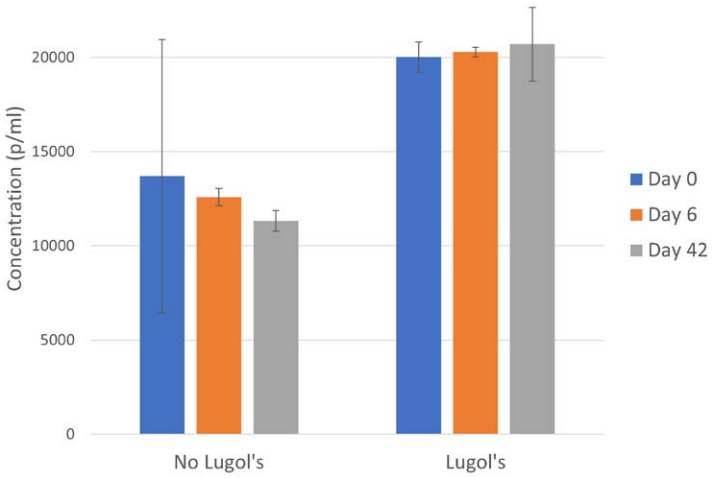
FLOWCAM PROCEDURE

The FlowCam instrument used in these experiments was equipped with a color camera. The software for data acquisition and data analysis was VisualSpreadsheet, and samples were processed using Autoimage mode. A 10X objective, FOV 100 flow cell, and 1 mL syringe were used.

ANALYSIS

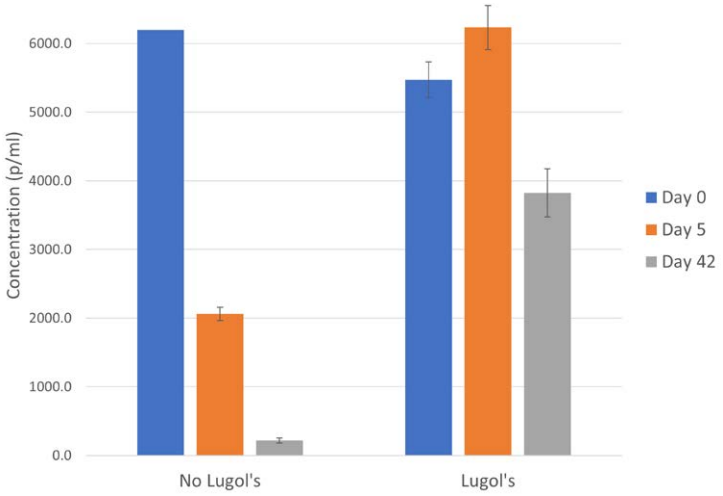
Graph 1 compares the concentration over time of a natural freshwater sample (Enterprise Pond) preserved with Lugol's. The error bars displayed are the standard deviation for triplicate runs. A noticeable drop off in concentration without Lugol's is observed after 1 week. After a 42-day period there was no discernible difference in concentration for the Lugol's preserved samples.

Graph 1 - Enterprise Pond



Graph 2 compares the same data for natural marine samples (Casco Bay, Maine). All samples were stored at room temperature in the dark for a 42-day period. The error bars are the standard deviation for triplicate runs. There was a noticeable drop in concentration after Day 5 when no preservative was used compared to a stable concentration when the samples were preserved with Lugol's.

Graph 2 - Casco Bay



IMPORTANT CONSIDERATIONS

- We recommend only using a Lugol's-preserved sample when processing the sample with Autoimage mode. We do not recommend using Lugol's when using Trigger mode. There was a dramatic difference in Trigger mode concentration when Lugol's was used (data not shown). If you want to use Trigger mode, we recommend preserving your samples in glutaraldehyde. Please see our technical note, "Using Glutaraldehyde as a Preservative in Samples with Phytoplankton".

- Lugol's is considered hazardous waste, so waste should be separated and disposed of according to local regulations.

- Data presented here suggests that particle concentration is maintained when using Lugol's as a preservative, but whether the actual phytoplankton diversity was maintained was not assessed.

- While many FlowCam users use different types of Lugol's (acidic, basic, or neutral), the type that you choose may be taxa-specific, so please check your literature to see what other researchers are using.

- Some scientists add to the concentration of Lugol's until the sample appears like tea. Over time the Lugol's will fade, and many scientists will add more Lugol's as time passes. This procedure was not tested in this study.

CONCLUSIONS

The main takeaway of this technical note is that freshwater and marine samples preserved in Lugol's can be stored for 42 days with minimal differences in total concentration. If you have samples to send to our Laboratory for analysis and you cannot send fresh samples, you may consider using this method for analysis to take place one month after shipping.

REFERENCES

1. Jakobsen H, Carstensen J (2011) FlowCAM: Sizing cells and understanding the impact of size distributions on biovolume of planktonic community structure. Aquat Microb Ecol 65:75–87. doi: 10.3354/ame01539

2. Zarauz L, Irigoien X (2008) Effects of Lugol's fixation on the size structure of natural nano–microplankton samples, analyzed by means of an automatic counting method. J Plankton Res 30:1297–1303. doi: 10.1093/plankt/fbn084

3. Anachemia Product Number 48396 can be purchased here: https://ca.vwr.com/store/product/en/11805568/lugol-s-iodine-solution-concentrate