

An improved quantitative protargol stain for ciliates and other planktonic protists

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With 3 figures and 1 table in the text

Abstract

An improved quantitative protargol stain (QPS) is described. Records of entire protozooplankton communities can be obtained by a four hour procedure. Permanent slides prepared with this improved method can be examined using high resolution oil immersion lenses employing bright field, phase contrast or DIC. The numerical results of the improved QPS are comparable to settling chamber counts. Lugol fixed plankton samples may be treated by QPS as well.

Introduction

One essential problem for aquatic biologists interested in the ecology of protozoa is the accurate identification and enumeration of different taxa. Although live observation is still essential for the determination of protists, the clear identification of most species also requires an advanced cytological examination. Especially for ciliated protozoa taxonomists have introduced several methods for species identification, ranging from light microscopical staining procedures to scanning electron microscopy. A compilation of current methods is given by FOISSNER (1991). Most of these methods are rather time consuming and cannot be employed for quantification of ciliates. Therefore they are not often applied in ecological field studies. On the other hand the more common quantitative techniques like the settling chamber method (UTERMÖHL 1958) give good estimations for the abundance of ciliates, but only limited information about species composition.

The quantitative protargol stain (QPS) of MONTAGNES & LYNN (1987) is a very promising step forward to bridge the gap between quantitative and taxonomic methods. This technique is based upon the protargol silver impregnation (BODIAN 1936, 1937, TUFFRAU 1967, WILBERT 1975), one of the most valuable cytological stains used in ciliate taxonomy, combined with a filtration method for cell enumeration. However, the QPS has not yet been established

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in ecological investigations. This is probably due to the difficult and very time consuming procedure. The QPS method described here has been modified in some important details regarding the procedure of MONTAGNES & LYNN (1987) thus reducing the total time of preparation from 15–27 hours to less than 4 hours. The quality of the stain is reproducible and ranks equally to established protargol techniques used in ciliate taxonomy.

Method

The preparation of permanent quantitative protargol slides includes more than twenty single steps. With regard to the clarity of description the whole procedure is subdivided into six major operations:

- (1) Water samples are **fixed** to preserve and stabilize the planktonic organisms.
- (2) The plankton is **concentrated** on a cellulose membrane by **filtration**.
- (3) To avoid loss of cells during the next steps of preparation, the cells are **covered** and **attached** to the filter by a **thin layer of agar**.
- (4) The agar is **hardened** by formaldehyde to prevent scratches on its surface and to make it resistant against heat.
- (5) Fast **impregnation** in warm protargol solution.
- (6) The stained filters are **dehydrated** and **mounted** in Canada balsam resulting in permanent slides.

1. Fixation

Plankton samples (volume depending on the abundance of plankton, e.g. 5–10 ml for eutrophic lakes) are fixed with fresh concentrated Bouin's fluid:

saturated solution of picric acid	15 ml
formalin (37%)	5 ml
glacial acetic acid	1 ml (add just before use)

Note: It is recommendable to use buffered formaldehyde (e.g. Merck Nr. 3999) for preparing the fixative. Fixation time should never be less than 15 min in order to stabilize the cells and prevent breaking of cells during filtration. A good ratio of fixative to sample is 1 + 5 for freshwater, but even much lower concentrations of Bouin's fluid (1 + 50) will provide acceptable results. An inadequate fixation (unsuitable fixative or fixation time) may lead to poor silver stains. Nevertheless, even Lugol fixed plankton samples can be treated with protargol if a postfixation with Bouin's fluid (at least 30 min) is applied before filtration. In this case Hollande's modification of Bouin's fluid (see LEE et al. 1985) is recommended:

Picric acid	4 g
cupric acetate	3.5 g
formalin (37%)	10 ml
glacial acetic acid	5 ml (add just before use)
distilled water	100 ml

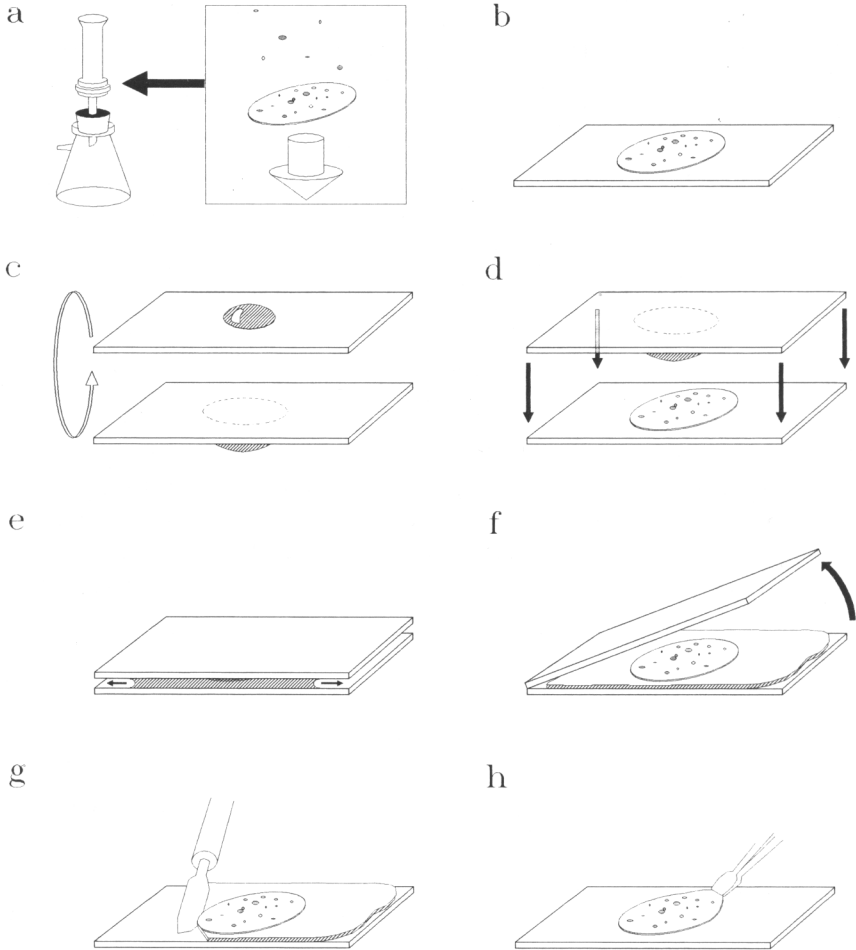


Fig. 1 a–h. Agar embedding of membrane filters for protargol stain.

2. Filtration

If necessary (large sampling volumes), the fixed material is concentrated by settling. Samples are carefully filtered through $0.45\text{--}0.8\ \mu\text{m}$ membrane filters (diameter 25 mm) composed of cellulose nitrate or mixed esters of cellulose (Fig. 1 a). A filtering device with a fritted glass base (Sartorius, Millipore) has to be used. Subfilters may be advantageous for the even distribution of organisms on the filter (MONTAGNES & LYNN 1987). To avoid cell damage, filtration has to be stopped immediately when all liquid is removed from the filter.

Note: Filters made of cellulose nitrate are very rigid and should therefore be favoured. Filter membranes composed of mixed esters of cellulose are often deformed by filtration or become warped during the following steps of preparation. Gridded filters

