A modified plastic culture flask for microscopic observation of fungi

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Abstract

A plastic culture flask modified for the direct microscopic observation of fungi is described. The use of this flask allows for in situ observation of fungal morphology and monitoring of development of different stages of fungal growth. The flask provides a safe, rapid, and inexpensive culture technique for the clinical laboratory.

Introduction

The identification of fungi isolated from clinical specimens is dependent upon microscopic identification of sporulating structures. Maintenance of the structural integrity of these sporulating structures during preparation for microscopic examination can be a problem. An ideal method for examination is direct microscopic observation of a fungus in situ during growth without mechanical disaggregation of sporulating structures from use of inoculating loops, needles or other instruments. Direct observation has the advantage of allowing a fungus to grow undisturbed so that conidiogenesis or other growth characteristics may be studied.

A problem which occurs with in situ observation is that the thickness of most glass and plastic culture vessels hinders high magnification observation. Other investigators recognizing this problem have devised various culture chambers (2, 3, 5) and other slide methods (1, 4, 6) to microscopically observe fungi while growing in place. However, many of the methods described do not address adequately the problem of laboratory safety when pathogenic fungi are being cultured. This report describes the development, use, and evaluation of a culture chamber made from a plastic tissue culture flask for direct microscopic observation of fungi. The flask is modified using common inexpensive laboratory materials, can be used at high magnification without alteration, and provides reasonable safety to laboratory personnel.

Materials and methods

A hole was made in the top side of a 30 ml plastic tissue culture flask\(^1\) approximately 2 cm from the bottom using a hot #16 stainless steel (Morton) tube closure\(^2\) (Fig. 1). The tube closure was heated in a gas flame and then applied to the plastic flask. Gentle pressure resulted in a hole being melted through the plastic wall of the container. Care was taken that the tube closure did not penetrate too far to melt into the opposite wall and was removed quickly to prevent build-up of excessive melted plastic around the hole. The plastic ridge which formed around the hole as a result of the melting process was scraped away with the edge of a glass microscope slide. Plastic scrapings resulting from this procedure were blown away with a jet of com-

1 Costar Plastics, 205 Broadway, Cambridge, MA 02139. Cat# 3025.
pressed air. A No. 1, 22 by 22 mm glass cover slip was carefully placed over the hole and xylene applied to the edges of the cover slip using a cotton swab. The xylene moved under the glass cover slip by capillary action and in a few minutes bonded it to the plastic.

The flask was sterilized overnight with ethylene oxide. A thin layer of clear fingernail polish was applied around the edge of the cover slip prior to use as an additional safety seal.

Ten milliliters of melted potato dextrose agar or equivalent medium was added to the flask and al-

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Fig. 1. Construction diagram of modified plastic culture flask for microscopic observation of fungi.

Fig. 2. Low power magnification view of *Microsporum gypseum* strain Pohm growing on cover slip of modified plastic culture flask containing potato dextrose agar X100 unstained.