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Slide Making and Prototype of a Dropper Device for Chromosomal Preparations

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ABSTRACT Slide making is the most critical aspect of good banding preparations. Slide making is best done after the cells have been in fixative at 4ºC overnight. Variables affecting preparation includes relative humidity, room temperature, drying time and number of cells per slide. Increased height is often used to increase spreading. We have designed a simple Dropper device for our slide preparations. This facilitates easy operation and well spread chromosomes are obtained. This device is made of stainless steel with a broad base where a main rod is fixed. Two side rods are fixed with clamps, attached to the main rod. Both can be raised or lowered according to the requirement. The upper one is provided with a pipette-holder where the pipette with the material can be inserted. The lower one is provided with a grooved plate with a ball and a socket to adjust the angle. The slide is pushed gently from the plate lengthwise as the material is dropped from the pipette.We have been using this for our lymphocyte culture slide preparations and we are quite satisfied of its use.

INTRODUCTION

The air-drying slide making methods we have today evolved in two steps. The first was when Rothfels and Siminovich (1958) accidently allowed some *in situ* harvested slides of monkey cultures to dry out by evaporation and they found that the chromosomes were spread out in a single plane without mechanical force. Nowell (1960) who described the use of PHA for lymphocyte culture used the squash method of slide preparation. It was Moorhead et al. (1960) who modified Rothfels and Siminovich's air-dry techniques for lymphocyte cultures to give the slide making method we still use.

Early slide drying methods involved manipulations to improve spreading such as flaming cell preparations. One such method was to dip slides in 95% alcohol, drop the fixed cell on to the alcohol and pass the slide through a Bunsen burner or alcohol flame. Another was to drop slides in dry ice-cooled water, drop cells on the water, ignite the fixative in a flame and dry the slides on a hot plate. In the early 1970's to accommodate the new banding methods, most laboratories learned to use air-drying methods instead of flaming. Slides were made by dropping the fixed cells on to wet slides and allowing them to dry by evaporation.

Various methods were developed to improve air-drying methods for slide making and most of these deals with relative humidity, which is linked to air temperature. Spurbeck et al. (1996) who measured cell spreading (cell volume) in relation to increasing humidity and temperatures and found a positive correlation between increased humidity and spreading at a set temperature, up to a certain threshold. The authors also report that warmer air yields more spreading due to the ability of warm air to hold more moisture. However warm air created in winter by building heating system will drive moisture out of the air, and these warm air conditions will usually yield less spreading.

There is correlation between chromosome spreading and height (Gibas and Jackson 1985). Increased heights of dropping cells on slides were often used to increase the metaphase spreading. Sometimes cell drops may miss because of increased height/missing coordination with both hands/new person handling the sample. When cell suspension is too less, a single drop is very precious eg. – FNA material. Keeping above points in mind – to achieve best results with small quantity of sample without wasting a drop of cell suspension, we have designed a dropper device for the first time to facilitate easy operation of slide making procedure.

MATERIAL AND METHOD

Lymphocyte cultures were set up by the modified method of Arakaki and Sparkes (1963) using PHA (supplied by Shreyas Solutions, Bangalore). The fixed cells obtained were loaded into a disposable Pasteur pipette and inserted into the pipette holder of the dropper device. Cold wet slides were taken and drained on to a paper towel leaving a thin layer of water. The slide is placed lengthwise into the grooved plate, which is adjusted at an angle of 30º and gently pushed while the material is dropped from the pipette at a height of 9”. The slide is flooded with a little fixative to improve water removal.

The dropper device is made of stainless steel with a broad base measuring 9”x7½". A center rod with a height of 25” is fixed on the base. One upper rod with a pipette holder measuring 6” in length is fixed on the main rod with a clamp. The lower one with a grooved plate measuring 4”x 4” with a ball and socket and screw to adjust the angle is fixed in the clamp. Both rods can be raised and lowered according to the requirements. The material is drawn into a pipette and the pipette is inserted into the pipette holder. The slide is pushed gently from the plate lengthwise as the material is dropped from the pipette (Fig. 1).

RESULTS AND DISCUSSION

Well spread chromosomes are obtained as the angle of the slide and the height from which the cells are dropped are controlled (Fig. 2).

Normally one should be able to drop fixed cells with a convenient height on to a clean wet slide at 20-30º angle on a paper towel, drain the excess water, flood the slide with a little fixative to improve water removal and make the slide dry consistently and dry the slide flat to get good preparations.

Many laboratories prefer to drop fixed cells

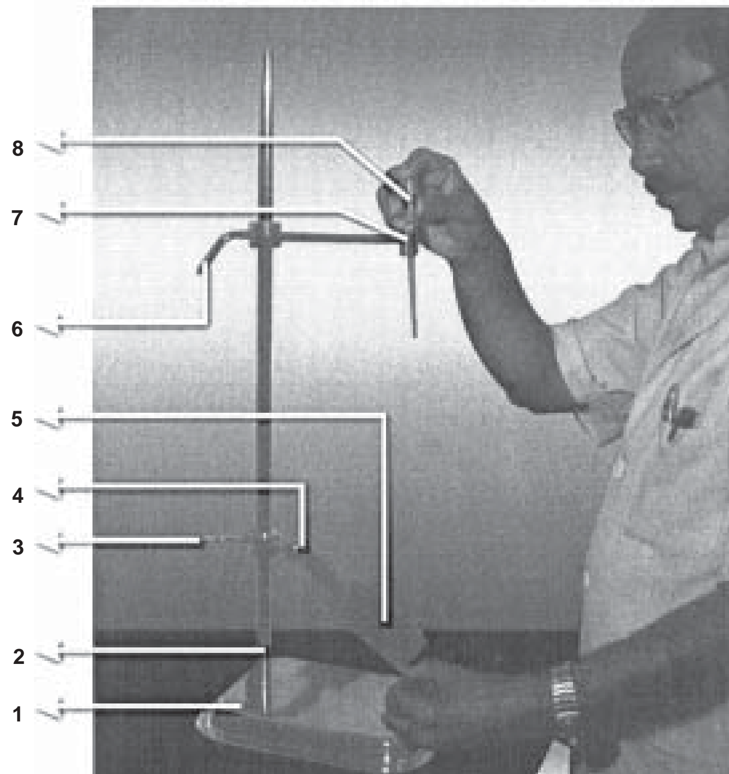

Fig. 1. Droppper - Device
1. Base 2. Center rod 3. Clamp to hold plate 4. Ball & Socket to adjust angle 5. Plate to hold slide 6. Clamp to hold pipette 7. Pipette holder 8. Pipette

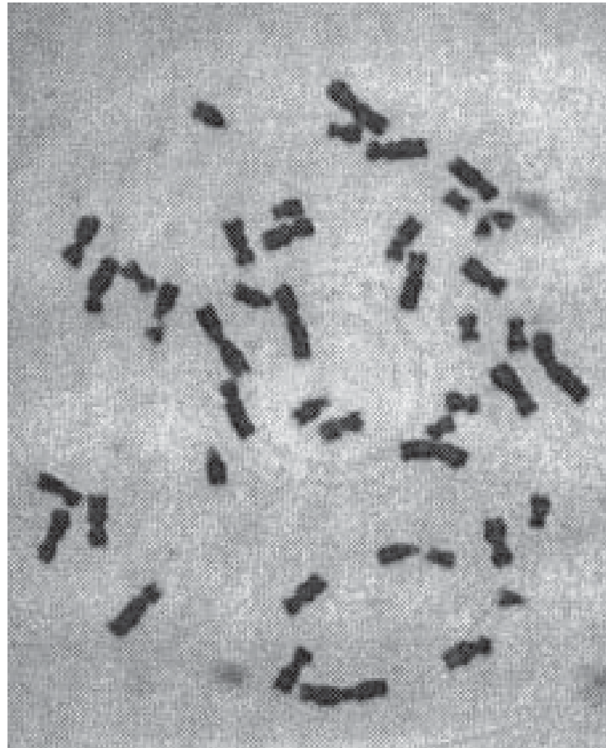

Fig. 2. Metaphase Spread

on wet slides, which facilitate spreading due to the immediate retraction of the water meniscus as soon as the fixative hits it. Holmquist and Motara (1987) report that by dropping cells on to wet slides, the energy of dehydration from fixation is returned as a change in the free energy of mixing between fixative and water, which spreads the cells.

Wet slides may further facilitate spreading control by the use of different temperatures of water coating to speed up or slow down drying time. Cold, wet slides will slow drying, increasing spreading, whereas higher room temperature or warm wet slides will accelerate drying time. The thickness of the water film may be varied by draining the slide on a paper towel more or less thoroughly and this can help control spreading by using a thicker film of water on very dry days.

Cell drying speed can be increased or decreased by changing the airflow over the slide. This can be accomplished by blowing on the slides, by waving them in the air, or by using a blower or an aquarium pump. A common mistake in slide making is applying too many cells on the slide so that there are lots of metaphases from which to choose. Crowded cells do not spread and band as well as optimally diluted cells because of physical crowding and possibly other factors such as extra cellular RNA and protein. Cell crowding also slows drying time.

Cells should not pool at one edge of the slide but should be in uniform circles that are about 20 mm in diameter and well centered. Greater tilt angles may speed up the drying process as the upper end of the slide compared to the lower end giving somewhat uneven inconsistent slides.

The ideal relative humidity for slide making can vary owing to differences in temperature, technique and specimen type. Lundsteen and Lind (1985) suggest 20ºC and 45% humidity with the optimum range between 40% and 50%. Spurbeck et al. (1996) found 50% humidity and 25ºC to be the optimum set points and they lowered the humidity to 35% if chromosome scattering is a problem. Drying time should be roughly 30-45 seconds.

The dropper device we have designed controls the height and angle of the slide.

Increased height is often used to increase spreading. The spreads are more uniform if the angle is kept at 20-30º. We have found the height of 9" to be ideal. With this device the height and angle can be adjusted according to the requirements of individual laboratory. This is also designed in such a way that the slide can be pushed gently as the cells are dropped from the pipette. We have been using this for our lymphocyte culture slide preparations and we are quite satisfied of its use.

This is the first time a dropper device has been made to facilitate slide preparation. By using the dropper device, one can achieve best chromosome preparation even from a less amount of cell suspension.

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