CONSERVATION OF ALCOHOL IN CYTOLOGY

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The Papanicolaou smear test has already established its importance in the diagnosis of cancer of the female genital tract and is now being routinely employed in General Hospitals, Family Planning Clinics, Rural Health Centres and even private clinics to screen both asymptomatic healthy and symptomatic women for the control of cancer of the uterine cervix. The 'Pap' smear test requires large quantities of absolute ethyl alcohol for fixation and staining. Though this is the fixation of choice and is being universally used to-day, there are some practical problems involved in its use.

The main problem of this fixative is that the ether-alcohol mixture is highly volatile and bulk of it evaporates causing considerable loss of the fixative. Again, being a liquid, much of it is lost due to spillage in transit from the clinics to the laboratory and back.

In our country, where absolute ethyl alcohol is expensive and difficult to get, it is a major handicap for mass screening programmes. It was therefore thought necessary that methods to conserve the ethyl alcohol should be explored. In the present investigation the use of 'Hair spray' for fixation and reutilization of the used alcohol was undertaken.

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Material and Method

The central laboratory of the Institute for Research in Reproduction (ICMR), Parel, Bombay, receives smears for examination, from its several Peripheral Family Planning Clinics. Two of these clinics are situated at a distance from the Institute and daily transport of smears is not possible. From the clinic which is at the Cama and Albless Hospital at a distance of 6 kilometres, smears are received once a week and from the second clinic at the Rural Health Centre, Nagothana (about 120 kilometres from Bombay) once a month. The ether-alcohol fixative evaporates from the containers during the long wait and the containers have to be checked regularly and the levels of fixative replenished. Large quantities of the fixative is also lost as a result of spillage during the long drives from the clinics to the laboratory. Initially we tried the glycerine mailing technique (Koss, 1968). But this proved to be very messy and time consuming. Then we tried a dry fixative, the commonly available 'hair spray' (Freeman, 1969; Chandra and Jayaram, 1973). The slides with the smear were held about 2 to 3 inches distance and sprayed with just a single coat. These slides were then kept in a commonly used wooden slide filing box and sent to the central laboratory from time to time. When the slides are received in the laboratory they are put in ether-alcohol fixative for at least 6 hours to remove

completely the material of the spray.

Results

Smears from a total of 793 cases were collected from December 1973 to August 1975. These smears were taken mainly from the exocervical region though in some cases endocervical smears were also taken. The results of the cytological examination is given in Table 1.

TABLE I
Results of 793 Cases

Negative	Inflammatory	Inadequate	Mild dysplasia	Moderate dysplasia	Total
464	245	76	5	3	793

There were 41 cases of Trichomonas vaginalis infection and two cases of fungal infection were detected from these smears. Five cases of mild dysplasia and three cases of moderate dysplasia were also detected. These cases of dysplasia are being followed. In 76 (9.5%) of the cases it was not possible to give an opinion. In only 20 cases (2.5%) the inadequate report could be attributed to drying of the smears while in majority of the cases (56 i.e. 7%) the inadequate report was given as the smears contained only red blood cells, mucus and leucocytes. It must be mentioned here that these cases had continuous bleeding because of injection Depo Provera given to them for contraception, and these smears were taken during the bleeding period.

Discussion

Fixation is of paramount importance in any pathological examination and also in exfoliative cytology. Proper evaluation of the smear is possible only if the smears are properly fixed and stained. Other than the original Papanicolaou fixative, 95% ethyl alcohol and acetone (Sagi and Mackenzie, 1957); 95% ethyl alcohol followed by drying, 95% isopropyl-alcohol and at times air drying and rehydration have been tried (Nieburgs, 1956; Bonime, 1966). However, these methods are time consuming and not economical in our country. Commercially available synthetic resin preparations such as Diaphane, Cyto-spray, Cyto-fix, Spray-Cyte are being used as dry fixatives. These commercial fixatives are either sprayed or some drops of the fixative solutions are spread on the freshly made smears. These dry fixatives are not available in India, they are expensive and have been reported by some as inferior to the ether-alcohol fixative (Tweeddle and Dubilier, 1972).

Air dried smears would solve the problem of fixation and be very convenient to transport. But air drying produces such artifactual changes that it is very difficult to opine on such smears.

A locally available hair-spray was used in the present series. About 80 slides can be fixed from one container costing about 10 rupees. The smears are very satisfactorily fixed. Even Trichomonas vaginalis (Fig. 1) could be easily identified. Normal inflammatory, mild and moderate dysplastic changes could be clearly deciphered (Figs. 2, 3, 4 and 5).

Certain precautions must be observed to obtain the maximum cell clarity and good staining when using the hairspray. Firstly, the slides should be held 2 to 3 inches from the spray jet and only one coat of spray should be given. If the slides are held at a further distance it was found

that much of the spray failed to reach the smear and was wasted, thus requiring to be sprayed with more than one coat and many a times this results in the smear drying due to inadequate fixation. These slides should be kept in a closed box to prevent the dust from accumulating on the slides, for if the dust settles on the smear it cannot be removed. The sprayed slides should be kept in ether alcohol fixative for a minimum of 6 hours to remove all the spray material. Too thick coating of the spray material should not be given because this then becomes difficult to remove and results in not only bad staining, loss of cellular morphological clarity but the spray material contaminates and spoils the fixative and the staining solutions, leading to wastage of the alcohol.

Another very serious disadvantage of not removing the spray material has been brought to light by Rubio (1975). He sprayed blank slides with the spray fixative and found that 4 to 24% were contaminated with cells from other slides during staining. The contaminants ranged from leucocytes, normal epithelial cells to malignant cells. Hence it is of paramount importance that the spray material be removed from the smears before staining.

Another method adopted in the department to conserve alcohol was to collect all the coloured 95% and absolute alcohol used in staining instead of discarding them down the drain. This was then distilled in bulk and collected. This 'used distilled alcohol' as we call it, is routinely used in the laboratory for fixation. The department receives about 5000 to 6000 slides a year and all our slides are fixed in this 'used distilled alcohol' and ether, in equal parts. The fixation is adequate, there has never been any trouble in staining and above all clarity of the cell morphology is very good. We would like to emphasize here very emphatically that the 'used distilled alcohol' has good fixation properties but cannot and must not be used for staining. It will completely decolourise the smears.

Summary

Absolute ethyl alcohol is not easily available in India specially in the 'dry states' where it has to be 'imported' from the neighbouring states, and above all it is expensive. Two methods adopted by our laboratory limits the use of absolute ethyl alcohol. With the use of hair-spray and 'used distilled alcohol' a very large quantity of alcohol can be conserved. However, there are certain precautions to be taken for obtaining good results.

1. The smear should not be allowed to dry prior to spraying. The doctor has to be fast in taking and spraying of the smears.
2. The spray fixed slides should be kept in a slide box to prevent the dust from accumulating on them.
3. The coat of spray material should be removed by keeping the smears in ether-alcohol fixative for at least 6 hours. A thick coat of spray material is difficult to remove and this subsequently interferes with staining and interpretation of the smears. This also spoils the staining solutions. By removing the sprayed material contamination from one smear to the other is also avoided.
4. 'Used distilled alcohol' can be used as a replacement for the fixative solution of absolute ethyl alcohol, but must never be used for staining.

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See Figs. on Art Paper XV-XVI