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A Highly Efficient Method for Staining Ultrathin Sections

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Summary: A simple and efficient staining method to handle simultaneously thirty (or even more) electron microscope grids with ultrathin sections is described. The device applied is easily constructed from inexpensive and readily available materials but can bring easily high quality staining results, and the amount of stain and washing water required is very small.

Key words: staining method, staining apparatus, uranyl acetate, lead citrate, ultrathin section

The simplest and most commonly used method for staining ultrathin sections is the droplet technique described by Kay[1], by which grids are individually floated on droplets of staining solutions, followed by washing in distilled water. But in practice it is difficult to produce high quality well-stained grids for several reasons. Firstly, it is time-consuming to handle each grid individually, and the staining and washing times within a batch of grids may vary and produce differences in the results; secondly, processing a number of grids can easily result in overstaining and contamination; thirdly, the staining process usually involves the grids being plucked from the droplets by fine forceps and carried on to the next step, this procedure obviously increases the risk of section damage. In an attempt to overcome these problems many electron microscopists have made much effort to improve the staining method and device[2-10], and some of the problems have been efficiently solved. However, some of these methods require large volumes of staining solutions and increase the risk of contaminating the environment when using toxic stains; some of the devices are difficult to construct, or the number of the grids stained is somewhat limited.

This paper presents an improved and simple piece of apparatus for staining EM grids, which overcomes the

problems described above. This device is easy and cheap to construct, convenient to use and it can accomodate thirty grids (and more if desired) in a single loading and effectively avoid contamination, thus improving the quality of the stained sections. Moreover, the amount of staining solution used is at a minimum, thereby decreasing the possibility of contaminating the environment when uranyl acetate and lead citrate are used.

MATERIALS AND CONSTRUCTION

The design of the apparatus is shown in fig.1.

Staining tube (A) One of the two ends of a glass tube of 130 mm length x 6 mm I.D. is fused until the inside diameter is 1 mm. At a distance of 20 mm from the other end of the tube a hole is made by using a blowing-flame, there a smaller glass tube of 20 mm length is fused on at an angle of 30 – 45°, the opening (H) of which is used for adding staining solutions.

Grid holder (B) A strip of a white silicone rubber tube (90 mm long, 1.5–2 mm wide) is cut off longitudinally to be used as the grid holder. Along the cut edges of the tube 30 nicks (approximately 1 mm deep, 3 mm wide) evenly distributed to hold the grids are cut out with a sharp narrow-tipped blade.

Reservoir (C) A 100 ml funnel is used as reservoir to hold double distilled water.

ASSEMBLY

The connection between A and C is made by using a silicone rubber tube (E). The other end of A is bushed with a silicone rubber tube of 30 mm length (F), and is closed with a clamp (G). The whole apparatus is mounted in a vertical position on a test tube stand or some other suitable stand.

METHOD

A straight ophthalmic forceps is held with the left hand to open the nicks of the grid holder. The edges of the grids are inserted with section side outwards into the nicks. On removal of the forceps, the grids are held firmly in place due to the elasticity of the rubber. The loaded grid holder is inserted into the staining tube. To ensure even filling, the stopcock (D) is opened slowly to allow the water to wet all surfaces before introducing staining solution. The pipette for adding stain should be rinsed with double distilled water beforehand. The solutions should be drawn from an intermediate level in the stock bottle to avoid introducing surface contaminants of precipitates. The first and the last drop of the stain are dispersed. The staining tube should be covered with black paper to inhibit light when staining with uranyl acetate. It is found that since only a very small surface area of stain is in contact with air in the staining tube, precipitation of lead carbonate tends to become less problematic. After staining, the grids are washed free of stain by opening the stopcock slowly to avoid washing the sections off the grid and allowing the water in the re-

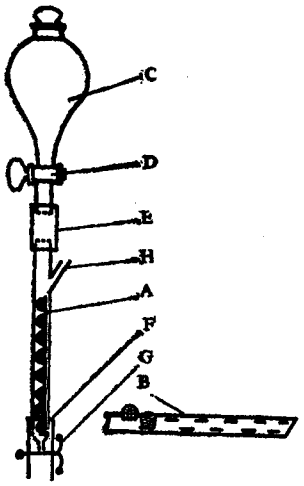

Fig.1. Design of staining apparatus
A staining tube; B grid holder; C reservoir; D stopcock; EF silicon rubber tube; G clamp; H opening used for adding staining solutions.

servoir to flow gently through the staining tube. In order to prevent the environment from being contaminated, the used stain should be collected with a container and treated properly afterwards. The grids are dried immediately after washing by blotting their edges at the point where they meet the holder with a triangle of filter paper. 1 milliliter of stain suffices for staining 20 grids. The time of the total process is only 30 minutes.

The water for preparing the staining solutions should be newly double distilled, or it can be boiled for 3–5 min before use. It is also desirable to use newly prepared 1 N NaOH, since NaOH can react with the silicone dioxide in glass bottle, thus affecting the quality of staining. The brown bottles for stocking uranyl acetate and lead citrate should be kept from light, and they should not be moved to avoid stirring of the precipitates when the stains are being used. The stain can not be used when small white contaminants occur on the surface of lead stain.

This method has been in use in our laboratory for more than one year. The sections turned out to be well and evenly stained without contaminations. The stained sections showed good contrast and clear structures. Thirty (or more) grids could be stained simultaneously and efficiently, thus ensuring high quality and identical staining conditions.

Goodman [3] and Brown [4] reported that the staining is improved when the grids are kept damp prior to staining, a conclusion we have also found to be true. We recommend that staining be carried out as soon as the sections are cut.

REFERENCES

1. Kay DH. Techniques for Electron Microscopy. 2nd ed. Oxford, Blackwell Scientific Publications, 1965.
2. Baratz RS. Grid staining – a useful device. Stain Technol 1976;51:193–5.
3. Goodman SL. A pipetting device for staining electron microscope grids. J Microscop 1981;123:329–31.
4. Brown JN. An improved apparatus for staining large numbers of electron microscope ultrathin sections simultaneously. Micron 1983;14:69–71.
5. Fisher DG. A holder for simultaneous fluid processing or carbon coating for electron microscope grids in lots of 10 or more. Stain Technol 1972;47:235–8.
6. Fordsyke JB. A simple apparatus for staining and washing a batch of grids. J Microscop 1979;117:437–40.
7. Gorycki MA. An efficient staining method for ultrathin sections. Stain Technol 1978;53:11–5.
8. Hiraoka JI. A holder for mass treatment of grids, adapted especially for electron staining and autoradiography. Stain Technol 1972;47:297–301.
9. Sjostrom M, et al. An efficient standardized method of staining thin sections for electron microscopy. J Ultrastruct Res 1973;42:180–5.
10. Springer M. A simple holder for efficient mass staining of thin sections for electron microscopy. Stain Technol 1974;49: 43–6.