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3-Aminopropyltriethoxysilane (APES): a new advance in section adhesion

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Various adhesives have been used to increase the adherence of tissue sections to glass slides and to prevent separation during staining. Traditionally glycerin albumin and gelatin solutions have been widely used in routine histology. More recently poly-L-lysine has been used to improve section adhesion, particularly in immunocytochemistry.

This method has been of great value but it is time consuming and slides cannot be stored. For the simultaneous immunostaining of T6 and S100 in tissue sections, we have found it necessary to use frozen sections of tissue fixed in formal calcium. With poly-L-lysine used as the adhesive, there was a high loss of sections after initial protease digestion. An alternative method for increasing adhesion was therefore sought.

For over 20 years alkoxy silanes have been used in an industrial context as coupling agents in coating and dyeing a variety of materials. Weetall et al described their use as coupling agents for insolubilising enzymes on inert surfaces, emphasising that the strong covalent bonding effect between the aminoalkyl groups and aldehyde or ketone functions of a reactive surface would withstand repeated washings with a variety of inorganic solvents. More recently aminoalkylsilane has been used as an adhesive for enhancing chromosome spreading on glass slides, and for in situ hybridisation of frozen sections.

In this paper we describe a new modification to the coating method for hybridisation originally introduced by Rentrop et al. The modified method is simple, cheap, and can be used in the routine laboratory, whenever improved adhesion of sections is necessary.

Method

3-AMINOPROPYLETRIETHOXYSILANE COATING OF GLASS SLIDES

1. Soak slides in 10% Decon at room overnight.
2. Wash in running tap water at 60°C for at least one hour.
3. Rinse in distilled water.
4. Dry overnight at 60°C. (At this stage the slides should be clean and sparkling, as any stain or foreign material remaining on the slides will affect the coating).
5. Immerse rack of slides for at least five seconds in a 2% solution of 3-aminopropyltriethoxysilane (Sigma Chemical Co, Poole, Dorset, England, No A-3648), in 99% industrial methylated spirits. The 2% solution should be freshly prepared.
6. Wash in 99% industrial methylated spirits.

7. Wash in distilled water.
8. Dry overnight at 37°C. A 2% solution of APES (200m) in 99% industrial methylated spirits can be used for 200-300 slides. No modifications to the usual laboratory methods of section drying is necessary.

Result

APES as an adhesive produces improved section bonding over other commonly used adhesives such as poly-L-lysine, glycerin albumin, and gelatin and withstands protease digestion. There is no background staining and it does not seem to interfere with the routine histological or immunostaining methods used in our laboratory. A large supply of coated slides can be stored in boxes, at room temperature until required. The bonding effect can be instantaneous, so that positioning of the section on the coated slide can be very difficult.

Discussion

An alcoholic solution of APES fulfils the requirements for an adhesive for the immunostaining of prefixed frozen sections. It also provides a useful method of stabilising histological and cytological preparations on glass slides for other purposes. It is a sufficiently simple, low cost technique (at 50 pence for 200 slides), making its use in routine histological practice feasible.

The APES concentrate is a toxic chemical that is moisture sensitive and requires storage at 0-5°C; it is not carcinogenic but is an irritant and can cause burns. Normal laboratory precautions, including the wearing of gloves and the use of a fume cupboard, should be taken when handling the concentrate solution. The method offers considerable benefit in saving laboratory time as it becomes possible to use routinely available spare sections for techniques such as immunocytochemistry and in situ hybridisation or other lengthy rigorous cytochemical techniques.

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