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Technical notes

Improved section bonding using silanated glass slides – application protocol

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Summary. Silanated slides provide excellent tissue adhesion for routine histology, immunhistochemistry and in situ hybridization of frozen, paraffin embedded material or cell smears. An easy and inexpensive method of treating glass microscope slides with triethoxysilylpropylamine is reported.

Key words: Slide adhesive – Triethoxysilylpropylamine – Application protocol

Zusammenfassung. Objektträger, die mit 3-(triethoxysilyl)-Propylamin behandelt wurden, sind an der Oberfläche mit Aminoalkylgruppen bedeckt, die mit Keto- oder Aldehydgruppen in Paraffinschnitten, Gefrierschnitten oder Zellausstrichen Bindungen eingehen können. Es resultiert eine hervorragende Haftfestigkeit und das Ablösen des Präparates bei aufwendigen Verfahren wie der Immunhistochemie oder in situ Hybridisierung wird verhindert. Als weiterer Vorteil ist im Vergleich zu Albumin, Gelatine oder Poly-L-Lysin beschichteten Objektträgern die geringe Hintergrundfärbung sowie die einfache, schnelle und kostengünstige Herstellung zu nennen.

Schlüsselwörter: Objektträgerbeschichtung – Triethoxysilylpropylamin

Immunhistochemical methods and in situ hybridization require some kind of slide adhesive because due to proteolytic actions, temperature and extensive washing procedures the tissue tends to become detached from the glass slide.

The properties of aminoalkylsilane-treated glass slides for preparation of metaphase spreads following Robinson's procedure [3] and their staining quality have been studied by van Prooijen-Knegt et al. [4]. Using a shortened procedure Rentrop reported that aminoalkylsilane-treated slides provide ideal, irreversible adhesion of tissue for in situ hybridization [2] in most cases.

The protocol found to be most convenient for in situ hybridization and immunoenzyme techniques is as follows.

1. Slides are washed in 10% Extran neutral (Merck, Darmstadt) / aqua bidest (v/v) overnight
2. Rinse with hot running tap water for 2 h
3. Short wash in aqua bidest
4. Dry at 120°C
5. Dip slides in 2% Aminoalkylsilane: 5 min

Triethoxysilylpropylamine (Merck-Schuchardt, Darmstadt, Bestell-Nr.: 82 1619) 2 ml/98 ml dry acetone (Merck Darmstadt)

6. Dip in acetone: 3 min
7. Dip in acetone: 3 min
8. Dip in aqua bidest: 1 min
9. Dry overnight at 42°C
10. Store slides in a box at room temperature until use
11. Tissue sections or cell smears are incubated at 65°C overnight to increase adhesion before further use

The working solutions must always be made immediately prior to use, can be used only once and have to be discarded.

Concluding remarks

The bonding of the tissue to the aminoalkylgroups fixed on the glass surface is thought to be either an ionic or covalent mechanism [2, 3]. A precise description of a short and easy modification to prepare AAS (Aminoalkylsilane) slides is given in this note. In our opinion the extended precleaning procedure (steps 1–4) is required to avoid difficulties of positioning the tissue sections on the coated

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slides due to instantaneous bonding effects. In contrast to Rentrop [2], who reported a limit of 200–300 slides per batch of AAS, in our laboratory 600 slides could be treated in 300 ml 2% AAS with good results without exhausting the 2% working solution. It was also suggested that AAS slides may lose their favourable properties after prolonged storage [4]. No evidence of loss of adhesive properties or any lifting of tissues with slides aged 12 months was observed when the additional overnight incubation at 65°C (step 11) prior to lengthy rigorous techniques was performed. The AAS-procedure provides excellent adherence between slide and both paraffin and frozen tissue sections, or cytological preparations, a finding that is confirmed by others [1] and by routine histopathological laboratories.

In current forensic practice the use of AAS slides for vaginal smears offers the chance to separate male DNA and female DNA by differential lysis performed on the spermapositive microscopic slides [5]. Thus after PCR amplification additional genetic markers can be confirmed and a higher possibility of exclusion or individual identification in evidence of sexual assault is given.

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