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Technical report

Use of alcohol fixed cytospins protected by 10% polyethylene glycol in immunocytology external quality assurance

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Abstract

***Aims*—To provide cytospins as a means of external quality assurance (EQA), while maintaining antigen expression integrity and achieving uniformity of material for all participating laboratories.**

***Methods*—Cells were collected from two adenocarcinoma and one reactive pleural effusion specimens. Lymphoid cells were also collected through aspiration of a resected tonsil specimen. All cells were collected in Hanks balanced salt solution (HBSS); cytospins were made and fixed in methanol or acetone alone or protected using a layer of 10% polyethylene glycol (PEG) in 50% methanol. Two laboratories participated (RGHT and UCL).**

***Results*—Cytokeratin expression detected using either CAM5.2 or AE1/AE3 antibodies was sensitive to temperature. Without PEG, unacceptable or negative staining was seen within six weeks of preparation. LCA was not sensitive to temperature, with good staining scores being achieved after eight weeks following preparation.**

***Conclusions*—It is possible to send alcohol fixed, air dried cytospins to laboratories participating as part of an EQA scheme for immunocytology. Some antigens will require protection from temperature variations during transit. A layer of 10% PEG in 50% methanol, allowed to air dry, is suitable for this purpose. Participating laboratories will only have to remove this layer using methanol before their localisation technique for assessment.**

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Keywords: immunocytochemistry; cytopathology; external quality assurance

Immunocytology on cytospins or smears has, to date, been used sparingly. Tissue sections made from cell blocks in a histopathology laboratory are often used in cytopathology for immunostaining. However, the increased use of cytological material for diagnostic purposes and the scarcity of cells in some specimens means that there is a need for this method of immunological localisation.[1–3] With this need comes the requirement for the laboratory to participate in an external quality assurance scheme. In this study, we describe a means by which cytospins may be used by such schemes to assess the localisation of antigens.

Methods

STORAGE AND TRANSPORT OF CYTOSPINS

The effect of storage at room temperature on antigen expression was tested by preparing cells collected into Hank's balanced salt solution (HBSS, Life Technologies). Cytospins were prepared on a Shandon Cytospin 2 and fixed in either methanol or acetone for 20 minutes. Cells were stained immediately and at weekly intervals over a period of six to eight weeks after storing at room temperature.

The effect of protecting the antigen from degradation was assessed by comparison with cytospins prepared at the same time as those described above and coated with 10% polyethylene glycol (PEG) in 50% methanol, air dried, and stained immediately and at weekly intervals for six weeks.

The remaining cells from each specimen were placed in Carbofix (Shandon), a fixative/transport medium. Cytospins were prepared at

Table 1 Assessment of cytokeratin localisation using CAM 5.2 antibody. Pleural effusion, first specimen of adenocarcinoma stained by both Royal Group of Hospitals Trust (RGHT) and University College, London (UCL)

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Acetone						
RGHT	6	7	8	ND	ND	ND
UCL	ND	ND	11	6	4	5
Methanol						
RGHT	7	4	6	ND	ND	ND
UCL	ND	ND	8	5	6	8
Carbofix, acetone						
RGHT	17	9	4	9	5	7
UCL	ND	ND	11	12	13	12
Carbofix, methanol						
RGHT	16	13	10	11	4	9
UCL	ND	ND	13	12	13	12
Acetone, PEG						
RGHT	15	17	16	17	17	16
UCL	ND	ND	ND	ND	16	16
Methanol, PEG						
RGHT	14	17	16	16	16	16
UCL	ND	ND	ND	ND	15	16

ND, not done.

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Table 2 Assessment of cytokeratin localisation using CAM 5.2 and AE1/AE3 antibodies. Pleural effusion, second specimen of adenocarcinoma, stained by the Royal Group of Hospitals Trust only

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Acetone						
CAM 5.2	15	16	15	12	15	14
AE1/AE3	15	16	10	8	12	12
Methanol						
CAM 5.2	11	17	16	15	14	14
AE1/AE3	18	13	12	12	11	12
Acetone, PEG						
CAM 5.2	19	19	15	15	16	17
AE1/AE3	16	16	12	12	12	9
Methanol, PEG						
CAM 5.2	13	15	15	19	19	20
AE1/AE3	19	19	19	9	16	16

Table 3 Assessment of cytokeratin localisation using CAM 5.2 and AE1/AE3 antibodies. Pleural effusion, diagnosed as reactive

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Acetone						
CAM 5.2	11	11	13	12	12	12
AE1/AE3	10	8	8	8	8	9
Methanol						
CAM 5.2	13	12	13	11	12	10
AE1/AE3	10	10	9	9	9	9
Acetone, PEG						
CAM 5.2	8	10	8	7	7	8
AE1/AE3	8	9	8	7	7	6
Methanol, PEG						
CAM 5.2	17	15	19	18	19	17
AE1/AE3	19	20	20	19	18	ND

weekly intervals, fixed in either acetone or methanol, and treated as for the protected cytospins before staining as described below.

IMMUNOCYTOCHEMISTRY AND ITS ASSESSMENT

Anti-leucocyte common antigen (LCA, 1:50, Dako) and anti-cytokeratin (CAM 5.2, 1:20, Becton-Dickinson; AE1/AE3, 1:50, Dako) were localised using a Duet kit, peroxidase streptavidin–biotin complex method (StABC, Dako). Diaminobenzidene acted as the chromogen and cells were counterstained in Harris's haematoxylin. Endogenous peroxidase was blocked using 3% alcoholic hydrogen peroxide. All cytospins either prepared from cells in Carbofix or protected by 10% PEG in 50% methanol, were incubated in two baths of 95% alcohol for five minutes before staining. Results were confirmed at a second centre (UCL, University College Medical School, London) using a standard alkaline phosphatase anti-alkaline phosphatase method (APAAP, Dako).

The staining was assessed by a panel of four assessors, one of whom was a consultant cytopathologist (NA). Each assessor independently scored the slides using a 5 point scale. Two marks were available for antigen demonstration. Once antigen demonstration was seen, two marks were available for clarity of background or lack of non-specific staining. One mark was available for the quality of the counterstain. Collectively, therefore, each slide was scored out of 20. Acceptable staining was recorded when scoring exceeded 12 points.

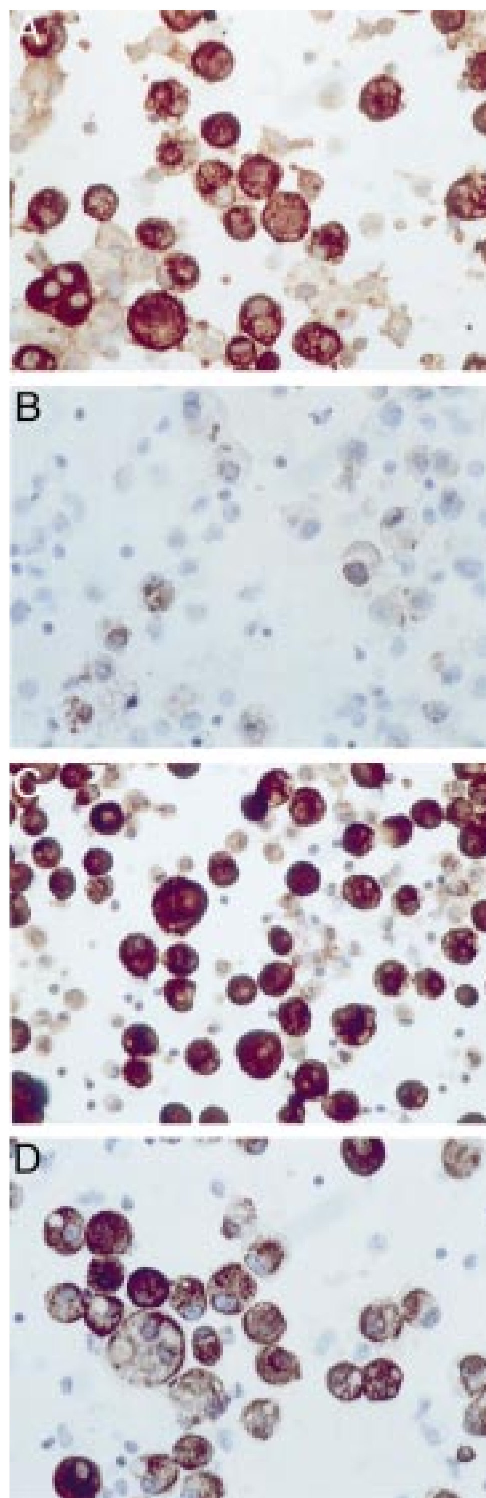

Figure 1 Pleural effusion, adenocarcinoma stained for low molecular weight cytokeratin (CAM 5.2). Methanol fixation after (A) one week and (B) six weeks. Methanol fixation with a layer of PEG after (C) one week and (D) six weeks.

Results

Both pleural specimens of adenocarcinoma showed an unacceptably low level of cytokeratin expression after six weeks when fixed in either methanol or acetone and compared with the results of staining immediately after

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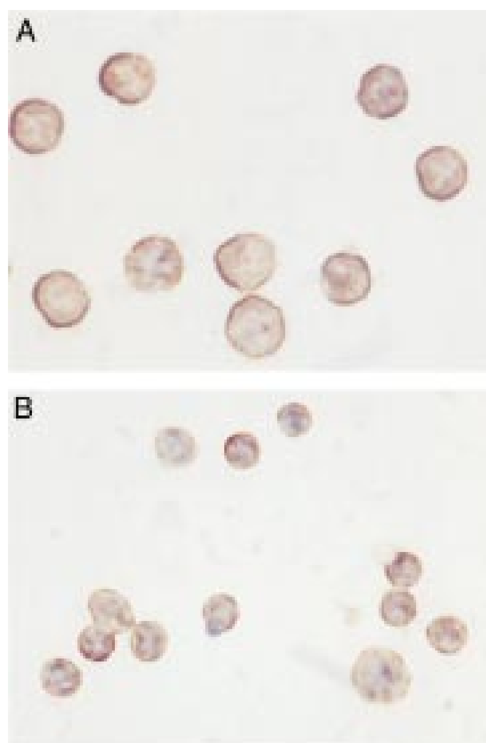

Figure 2 Aspirated tonsil cells stained for anti-leucocyte common antigen (LCA). Methanol fixation after (A) one week and (B) eight weeks.

Table 4 Assessment of LCA localisation. Cells collected from specimen of resected tonsil and stained by both Royal Group of Hospitals Trust (RGHT) and University College, London (UCL)

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Acetone								
RGHT	13	13	12	15	12	12	15	16
UCL	ND	ND	ND	ND	12	14	13	16
Methanol								
RGHT	16	16	13	15	16	16	15	16
UCL	ND	ND	ND	ND	13	15	13	12
Carbofix, acetone								
RGHT	16	15	16	15	17	16	13	ND
UCL	ND	ND	ND	ND	15	14	15	13
Carbofix, methanol								
RGHT	17	16	15	16	15	16	14	ND
UCL	ND	ND	ND	ND	15	14	15	14

preparation. When protected using PEG, however, cytokeratin expression was maintained and demonstration was scored as being excellent (tables 1–3). During week 1, higher scores for cytokeratin expression were noted in one case of adenocarcinoma and in reactive cells when placed in Carbofix or when PEG was used as a protective layer.

Overall, there was a similar reaction to all handling protocols when cytokeratin expression was demonstrated using either CAM5.2 (low molecular weight cytokeratin, fig 1) or a combined AE1/AE3 (pan-cytokeratin) antibody.

The demonstration of LCA in tonsil cells was unaffected by storage at room temperature after eight weeks (fig 2). There was also little difference in expression when fixed in acetone, methanol or when cells were placed in Carbofix (table 4).

The results were confirmed by the second laboratory (UCL) which received cytospins made from the tonsil and one adenocarcinoma pleural specimen by post at week 3 of the study.

Discussion

Due to minimum invasiveness of the procedure and the potential speed of diagnosis, techniques such as fine needle aspiration (FNA) cytology are becoming increasingly popular in diagnostic pathology.[1] Provided adequate cells are available, it is possible to process the sample to paraffin wax to make a cell block which may be used for immunocytochemistry.[2 3] Small numbers of cells, however, often mean that cytospin preparations are all that are available for immunocytochemistry. The maximum amount of information therefore needs to be obtained from a minimum number of slides. Immunocytochemistry has become a standard procedure in most histopathology laboratories, but to date it has been used sparingly in cytopathology laboratories. The nature of the cytology material, however, does not exclude the use of immunocytochemistry and the slides are often passed on to the histopathology laboratory to stain cells obtained on sampling.

The need for a controlled immunocytochemical procedure on these samples is evident. Recently, 12 laboratories in The Netherlands participated in a limited external quality control study on material obtained from effusions.[4] In this study, laboratories were sent cells to prepare their own cytospins or unstained control specimens. In our experience, sending cells through the post to participating laboratories is restricted by Health and Safety practices and postal regulations.

The present study has shown that sending air dried cytospins in the post requires fixation and a layer of protection in the form of PEG. The effect of degrading antigen expression was dependent upon the antigen. LCA appears to require no protection, whereas cytokeratin expression, irrespective of molecular weight, seems to be degraded at room temperature. Interestingly, methanol fixation gave consistently better results than acetone fixation, perhaps because of the removal of PEG by the methanol. This wash in methanol may also have a secondary fixation effect, contributing towards an enhanced staining reaction.

We have found, therefore, that it is possible to send alcohol fixed, air dried cytospins in the post to other laboratories and expect the material to be robust enough to resist reasonable temperature changes. Other applications, such as using the procedure in storing cytospins at room temperature for control material, can also be considered. For cytokeratin expression, the cytospins require a further layer of protection. The application of 10% PEG in 50% methanol, appears to protect the cytokeratin from degradation.

This material should be suitable for use in EQA schemes in immunocytology on cytological material. Some laboratories already perform immunocytochemistry either on cytospins or cell blocks.[2 3] In the latter instance,

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these laboratories participating in EQA schemes for immunohistochemistry still require adequate monitoring. For those cytopathology laboratories performing occasional immunocytology, the proposed scheme is the first to offer cytospins which should be suitable for the production of uniform demonstration of antigen. This means that all participating laboratories will be able to assess equally.

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