Methods for analysing AgNORs

In their interesting review on proliferation markers in tumours, van Diest and coworkers’ emphasise the importance of the assessment of AgNORs (nucleolar organizer regions), since AgNOR scores correlate well with other proliferation markers and can be used to estimate cell cycle time. The authors finalise the section with the statement “but at present these methods are difficult to apply in daily practice.” However, in the moment, indeed, the methods of the working groups determine the silver stained area in the nucleus, which requires morphometry of course, and is time consuming. Yet we would like to remind readers that there are alternative methods for AgNOR analysis that do not depend on image analysis systems and can easily be applied in daily routine work. In our own investigations we have always emphasised the different morphological appearances of silver precipitations within a common matrix. In the V region, several methods have always emphasised the differences in size of the nucleolar organizer regions (nucleoli) and dots (small singular precipitations without a matrix). AgNOR staining of cytological preparations from acute leukaemias allows the differentiation of clusters (aggregations of precipitations) with a common matrix in the nucleus and dots (small singular precipitations without a matrix).1-3 Our staining and counting procedures are standardised and the inter- and intraobserver variability is low. This alternative approach is justified because for acute leukaemias there is a good correlation between the BrdU index and the mean number of clusters (r = 0.60) or the percentage of cells with one cluster (r = -0.63), bearing in mind that in the correlation between the mean AgNOR size and BrdU shows a very similar value (r = -0.63).4 For chronic lymphocytic leukaemia (CLL) we also recommend making a differential count, separating cells with one or two compact nucleoli and cells with clusters.5 The percentage of cells with clusters correlates well with the tumour mass score (r = 0.72) and lymphocyte doubling time (r = -0.74) and permits one to differentiate between stable and progressive CLL. Furthermore the AgNOR pattern in CLL helps in the follow up monitoring of the patients and their response to chemotherapy.1,3

In summary, although we agree with others that measuring the AgNOR area provides very important information, especially with regard to the cell cycle time,6 we believe that there are alternative ways of analysing AgNORs which can be more easily applied in daily practice, but nevertheless are of equal pathophysiological and clinical relevance.

KONRADIN METZE
IRENE LORAND-METZE
Faculty of Medicine, State University of Campinas, BR 13081-970 Campinas-SP, Brazil

References

Cervical intraepithelial glandular neoplasia

Kurian and Al-Nafussi’s7,8 deserve our gratitude for shedding further light on the difficult subject of cervical intraepithelial glandular neoplasia. In particular, by establishing a ratio of 1:1.21 between the mean AgNOR size and BrdU shows a very similar value for low grade and high grade in situ lesions, they provide a means by which pathologists may monitor their diagnostic performance using the principles outlined by Wakely et al in 1998.9 This will be particularly informative in the case of low grade lesions which could be easily overlooked or passed off as reactive changes. The classification of in situ glandular lesions of the cervix is the subject of much controversy—for example, two methods are described in a standard British textbook of gynaecological pathology,2 each of which differs from the method used in the current study, which has also dispensed the term adenocarcinoma in situ. Variations in diagnostic criteria and terminology between papers may go some way to explaining why the conclusions in this paper, where progression from low grade to high grade disease is assumed to occur, differ from those of Goldstein et al,1 who suggested that there was no morphological evidence to support the existence of a spectrum of endocervical glandular changes culminating in what they recognised as adenocarcinoma in situ. Finally, Kurian and Al-Nafussi’s study highlights the importance of the cervical smear test in detecting these lesions when it is used as part of a screening programme.

M K HEATLEY
Department of Pathology, The Royal Liverpool Hospital, Liverpool L7 8XP, UK


Authors’ responses

Author’s response
It is a pleasure to read Dr Heatley’s response to our paper. The reason for abandoning the term adenocarcinoma in situ in our report is to conform with the new terminology for glandular lesions of the cervix. This is due to be released shortly in the Guidelines of Royal College of Pathology for reporting cervical biopsies.

A AL-NAFUSSI
Department of Pathology, University of Edinburgh Medical School

Manual of Diagnostic Antibodies for Immunohistology. By A S-Y Leong, K Cooper, and F J W-M Leong. (£45.00.)

Immunohistological staining has rapidly become accepted as an important, and in some cases indispensable, adjunct to histopathological examination and diagnosis. Histopathology laboratories have to be proficient in immunostaining procedures and fully conversant with the sensitivities and specificities of the primary antibodies used, the nature of the epitope demonstrated by each antibody, and its sensitivity to common fixatives. A thorough knowledge of tissue processing as well as methods of antigen retrieval is essential to routine practice.

This excellent manual provides a comprehensive list of diagnostic antibodies (151) presented in alphabetical order, and covers the background and applications of each reagent together with relevant references. Many of the antibodies described are immunoreactive in fixed paraffin embedded tissue sections as these remain the mainstay of routine diagnostic histopathology. There is much useful practical technical information in each of these brief descriptions—evidence of the authors’ extensive expertise—and as such this manual is much more than another antibody catalogue.

The authors rightly emphasise that immunohistological diagnosis is critically linked to an assessment of the morphological appearances and stress the need to employ a panel of antibodies to establish the immunoprofile of a tumour and thus reduce the risk of misinterpreting false positive and false negative staining. The text is cross referenced to an appendix that contains an extensive assembly of selected antibody panels (35) to help pathologists and their use. This book will become an important source of specific diagnostic situations, providing an easy to use practical guide. A further appendix addresses the value of heat induced epitope retrieval and provides a succinct and helpful protocol for antigen retrieval using microwaves. Surprisingly there is no section on proteolytic digestion techniques, though experience has shown that these techniques have advantages over heat induced unmasking methods for some antibodies—e.g. CD100, CD21, CD68, 34 E12, CMV, cytokeratins, and so on.

This manual provides a wealth of essential technical information for biomedical scientists and pathologists who regularly employ diagnostic antibodies in their work. There will, however, be a need for early revision as new antibodies are becoming available with increasing frequency and immunodiagnostic practices will be continually evolving with their use. This book will become an important practical reference source for all immunohistopathological laboratories and will be regularly consulted by both trainee and experienced biomedical scientists and pathologists as part of their routine work and research.

E L JONES
Instructions for Authors

Papers for publication should be sent to the Editor, Journal of Clinical Pathology, BMA House, Tavistock Square, London WC1H 9JR (tel: 0171 383 6209/6154; fax: 0171 383 6668; email: jclinpathol@compuserve.com). Receipt of manuscripts will be acknowledged by the editorial office.

Submissions of a paper will be held to imply that it contains original work not being offered elsewhere or published previously. Manuscripts should be prepared in accordance with the Vancouver style. The Editor retains the right to shorten the article or make changes to conform with style and to improve clarity. All authors must sign the copyright form after acceptance.

Failure to adhere to any of these instructions may result in delay in processing the manuscript and it may be returned to the authors for correction before being submitted to a referee.

General

* Authors must submit four copies of the original manuscript typed in double line spacing. The journal is now produced electronically and revised manuscripts should be submitted as an unedited copy and on disk. A guide to submitting an article on disk will be sent when requesting a revision or on notification of an acceptance. Authors should not submit the original paper on disk.

* The names of the authors, with initials, should be followed by the name of the institution where the work was carried out. An indication of the position held by each author should be given in an accompanying letter to the Editor, and manuscripts should bear the name of one author to whom correspondence should be addressed. If available, a fax number and an email address should be supplied.

* Identifying information should not be published in written descriptions, photographs, or pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent for publication; but patient data should never be altered or falsified in an attempt to attain anonymity. Informed consent should be obtained if there is any doubt. Masking the eyes in photographs of patients renders the patient's face indistinguishable and should be clearly stated. The consent for publication of any accompanying photograph or illustration must be signed by all authors.

* Tables should be presented separately in the text and must be typed, double spaced, with headings and footnotes. Each table should have a brief title and refer to the text. They should be numbered consecutively and each should have a brief title. The table should be designed to fit within the column width of the journal and should have a maximum of 15 columns. Each column should have a header and footnotes should be placed at the bottom of the table. Each table should be numbered and referenced in the text.

* Figures should be submitted in the form of high-quality black-and-white prints. All figures should be numbered consecutively and each should have a brief title. The figure should be designed to fit within the column width of the journal and should have a maximum of 10 columns. Each figure should be numbered and referenced in the text.

* Authors should provide up to fourkeywords/phrases for the index.

* All measurements must be in SI units apart from blood pressure measurements, which should be in mm Hg, and drugs in metric units.

* Abbreviations should be used rarely and should be preceded by the words full form in full before the first appearance.

* In the statistical analysis of data 95% confidence intervals should be used wherever appropriate.

* Any article may be submitted to outside peer review and for statistical assessment.

* No free offprints will be printed; reprints may be ordered when the proof is returned.

Original articles

* Papers should be no more than 2000 words long and should report original research of relevance and standing and practice of clinical pathology. They should be written in the standard form: abstract; introduction; methods; results; and discussion.

* The journal uses a structured form of abstract in the interests of clarity. This should be short (no more than 250 words) and include four headings: Aims—the main purpose of the study; Methods—what was done, and with what material; Results—the most important results illustrated by numerical data not p values; and Conclusions—the implications and relevance of the results.

Leaders/Editorials

* Leaders and Editorials are published by editorial invitation, and reviews or commentaries are unlikely to be accepted, though the Editor is always pleased to receive suggestions.

Short reports

* Single case reports of outstanding interest or clinical relevance, short technical notes, and brief investigations are welcomed and usually published in the form of a Short/Technical report.

* Length must not exceed 1500 words, including an unstructured abstract of less than 150 words, up to two figures or tables (or one of each) and up to 10 references. If more illustrations are required the text must be reduced accordingly.

Letters

* Letters must be typed in double line spacing, should normally be no more than 500 words, have no more than five references, and must be signed by all authors. Two copies should be provided.

Tables and illustrations

* Tables should be presented separately in the text and must be typed, double spaced, with headings and footnotes. Each table should have a brief title and refer to the text. They should be numbered consecutively and each should have a brief title. The table should be designed to fit within the column width of the journal and should have a maximum of 15 columns. Each table should be numbered and referenced in the text. They should be numbered consecutively and each should have a brief title. The table should be designed to fit within the column width of the journal and should have a maximum of 15 columns. Each table should be numbered and referenced in the text.

* Figures should be submitted in the form of high-quality black-and-white prints. All figures should be numbered consecutively and each should have a brief title. The figure should be designed to fit within the column width of the journal and should have a maximum of 10 columns. Each figure should be numbered and referenced in the text.

* Authors should provide up to four keywords/phrases for the index.

* All measurements must be in SI units apart from blood pressure measurements, which should be in mm Hg, and drugs in metric units.

* Clarification of reproduction of figures in papers is encouraged and is heavily subsidised by the Journal. Advice on costs and material to be submitted for colour work should be sought from the editorial office. The journal can accept colour images as the TIFF files in the following media: zipped or unzipped files on floppy disks, compact disks, or optical disks. A hard copy of the image should be provided.

* If any tables or illustrations submitted have been published elsewhere, written consent to republication should be obtained from the author (or copyright holder) and the authors. A copy of the letter giving consent must be included.

Descriptions of laboratory methods

* When a manufacturer's method is used in a study with a particular item of equipment or kit of reagents, the source of this method and reference to the scientific literature on which it was based should be given. Authors might consider it courteous to inform manufacturers that an article assessing their product has been submitted for publication.

* For quantitative methods, information on the sensitivity, precision, and accuracy in the hands of the authors should always be provided. When a well recognised method is used, these requirements could be met simply by providing the manufacturers with the methodology and discussing the performance in a recognised current quality assurance scheme. Modifications to methods that have not been previously published should be described in the text and supported by evidence of their efficacy.

* It is useful to indicate, either from personal observations or by reference, the working range of an assay and the normal reference range when it is used on samples from humans. When information is expressed as mean ± 2 SD, the distribution of the range (normal, skew, or logarithmic) should be stated.

References

* References must be numbered in the order they appear in the text and include all information (Vancouver style; references with more than three authors should give only the first three followed by et al).


* References in the text should be identified by arabic numerals in brackets—for example [1] [2].

* Information from manuscripts not yet accepted, or personal communications may be cited only in the text and not included in the references. References are not checked by us; authors must verify references against the original documents before submitting the article.


Manuscript checklist:

* Is there an abstract?

* Are the abbreviations spelt out?

* Are the measurements in SI units?

* Are the references in Vancouver style?

Revised January 1999
Best Practice articles (formerly “Broadsheets”) prepared by the Association of Clinical Pathologists

Just published

154 Helicobacter pylori 1999 CAM MCNULTY, JJ WYATT (with correction in June issue)

Recent Publications

153 The laboratory investigation of vaginal discharge 1998 KF MACSWEEN, GL RIDGWAY
152 Clinical implications of plasma homocysteine measurement in cardiovascular disease 1998 RA STILL, IFW MCDOWELL

Other Best Practice articles are still available for purchase

151 Investigation of dyslipidaemias 1997 AF WINDER, W RICHMOND, DT VALLANCE
150 Antenatal serological testing and prevention of haemolytic disease of the newborn 1997 JRM DUGUID
149 Serological diagnosis of gluten sensitive enteropathy 1996 DJ UNSWORTH
148 Laboratory diagnosis of malaria 1996 DC WARHURST, JE WILLIAMS
147 Mycological techniques 1996 KG DAVEY, CK CAMPBELL, DW WARNOCK
146 Macroscopic examination of prostatic specimens 1995 P HANNDEN, MC PARKINSON
145 Investigation of patients with autoimmune haemolytic anaemia and provision of blood for transfusion 1995 RJ SOKOL, DJ BOOKER, R STAMPS
144 The investigation of hypercalcaemia 1994 PL SELBY, PH ADAMS
143 Detection of autoantibodies to neutrophil cytoplasmic antigens 1994 RJ LOCK
142 Measurement of carbon monoxide and cyanide in blood 1993 RW MAYES
141 Role of endocrine biochemistry laboratories in the investigation of infertility 1993 GH BEASTALL
140 Techniques in pulmonary cytology 1993 JA YOUNG
139 Post mortem techniques in the evaluation of neck injury 1993 P VANEZIS
138 Gross examination of uterine specimens 1993 JS CURRY, K PATEL, M WELLS
137 Obtaining samples at post mortem examination for toxicological and biochemical analyses 1993 ARW FORREST
136 Detection and importance of anticardiolipin antibodies 1993 MA KHAMASHTA, GKV HUGHES

Earlier Broadsheets may still be available from the author. A full list can be obtained from the Publications Secretary, Association of Clinical Pathologists, 109 Dyke Road, Hove, East Sussex BN3 1TL.

Prices

INLAND One copy, £2.50; 2–10 copies (of any one broadsheet or reprint), £2.00 each; 11–100 copies (of any one), £1.75 each; 101 plus copies (of any one), price to be agreed; authors (over 50 free copies), £1.25 each.

OVERSEAS One copy, $6.75; 2–10 copies (of any one broadsheet or reprint), $5.25; 11–100 copies (of any one), $3.75; 101 plus copies (of any one), price to be agreed. Authors $2.25. Prices include postage but air mail will be charged extra. Trade discount 10%. All orders (and all changes of address of regular subscribers) should be sent to the Publishing Manager, Journal of Clinical Pathology, BMJ Publishing Group, BMA House, Tavistock Square, London WC1H 9JR.
Methods for analysing AgNORs.

K Metze and I Lorand-Metze

*J Clin Pathol* 1999 52: 550
doi: 10.1136/jcp.52.7.550a

Updated information and services can be found at:
[http://jcp.bmj.com/content/52/7/550.1.citation](http://jcp.bmj.com/content/52/7/550.1.citation)

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
[http://group.bmj.com/group/rights-licensing/permissions](http://group.bmj.com/group/rights-licensing/permissions)

To order reprints go to:
[http://journals.bmj.com/cgi/reprintform](http://journals.bmj.com/cgi/reprintform)

To subscribe to BMJ go to:
[http://group.bmj.com/subscribe/](http://group.bmj.com/subscribe/)