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Three-dimensional reconstruction of free-floating thick whole mount sections displaying microvascular structures in human endometrium using Laser Scanning Confocal Microscopy (LSCM)

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Abstract

There is an increasing interest in studying the relationship between microvessels and the epithelial environment in human endometrium. However, microcirculatory networks have rarely been studied in three-dimensions. This study was designed to utilise thick (≤ 50µm) formalin fixed sections in order to visualize and measure the three-dimensional relationships of endometrial microvasculature structures. Specimens were carefully selected from women with conditions unlikely to affect endometrial structures. Monoclonal antibodies [mouse anti-human CD 34 and mouse anti-goat fluorescein (FITC)] were used to target and visualize the microvessels. Digital images were acquired using a Nikon Eclipse E800 microscope equipped with a Radiance 2000 confocal scanning laser attachment. Once the data sets had been collected third party visualisation software Imaris was utilised in preparation for qualitative and NeuronTracer for quantitative interpretation. Laser Scanning Confocal Microscopy (LSCM) is now affordable by individual research groups making multi-dimensional imaging more accessible. Recent developments have occurred in the area of computer hardware, primarily their ability to handle and process datasets in excess of 100Mb to produce complex digital images establishing itself as a valuable tool for the three-dimensional reconstruction of a variety of biological specimens.

Keywords: three-dimensional, 3-D, computer reconstruction, microvascular, endometrium, Laser Scanning Confocal Microscopy (LSCM), immunofluorescence

Introduction

The study of uterine microvascular structures spans several centuries using a variety of techniques, but mainly based on procedures for luminal injection of substances to outline the vessels (Manconi et al 2010). Most of the studies involved in the description of microvascular structures have relied on conventional histological tissue sections. In this type of study the detection of blood vessels has been greatly aided by the development of immunohistochemical staining using specific endothelial cell markers, most commonly these have been Ulex europaeus lectin (Yonezawa et al 1987), von Willebrand factor (Schlingemann et al 1991), CD31 and CD34 (Miettinen et al 1994).

In addition to the kind of immunohistochemical marker being used, the appearance of blood vessels in histological sections is greatly influenced by the thickness of the section. In thinner sections only short segments of blood vessels are visible, such as those acquired using bright field or fluorescence microscopy (Fig. 1a). Using thicker sections in combination with either laser scanning confocal (Fig. 1b) or multiphoton emission microscopy (Manconi et al 2003), the three-dimensional patterning of the vascular network can clearly be observed.

![Figure 1](image_url)

Fluorescence microscopic imaging showing the effect of section thickness on the appearance of endometrial microvasculature in histological sections a. 5 µm section: b 50 µm section, labeled for CD34 immunoreactivity, fluorescence label: fluorescein isothiocyanate (FITC), false coloured red to highlight microvasculature. (Scale: 50 µm). The interrelationships of much longer segments of micro-vessels are clearly visible in section b.
The quantification of blood vessels in endometrial sections has normally been investigated by measuring microvascular density. This has provided an index for the number of blood vessels per unit area of a thin histological section (Goodger and Rogers 1994; Song et al 1995; Gargett et al 1999).

These conventional methods for the identification of blood vessels in histological sections, even when labelled by reliable immunohistochemical markers, have at best recorded only a two-dimensional projection of a three-dimensional environment.

Biological systems exist and operate in a three-dimensional environment; therefore it makes sound sense to access three-dimensional quantitative information, based upon observations made on three-dimensional projections instead of the traditional approach of using stacks of two-dimensional sections derived from thin sections mounted individually on microscope slides.

Materials and Methods

Collection of human uterine tissue

This study was approved by the Human Ethics Committee the University of Sydney.

Longitudinal uterine slices containing endometrial tissue were collected from 32 hysterectomy operations from inpatients of reproductive age that underwent a hysterectomy at the Sri Jayewardenapura General Hospital, Sri Lanka.

Each case met the following criteria: aged between 25 – 45, no usage of exogenous contraceptives in the three months prior to hysterectomy, undergoing hysterectomy for prolapse or preinvasive cervical disease, no history of local radiotherapy, no clinical evidence of endometrial dysfunction and no uterine pathology on hysteroscopic examination, when performed.

Endometrial dating

Serial sections were stained with haematoxylin and eosin and the endometrium was dated from the women’s menstrual history and blindly assessed by a specialist gynaecological pathologist using the criteria of Noyes, Hertig and Rock (Noyes et al 1950). Subjects were from the early proliferative (days 5 – 7, n = 5), mid proliferative (days 8 – 11, n = 5), late proliferative (days 12 – 15, n = 5), early secretory (days 16 – 19, n = 4), mid secretory (days 20 – 23, n = 4), late secretory (days 24 – 27, n = 5), menstrual (days 28 – 4, n = 4) stages of the cycle.

Uterine strip pretreatment

Uterine slices were initially pretreated for 72h at room temperature with aqueous 4% phenol solution (BDH Ltd, Poole, England). After five rinses with distilled water and then 3 x 10 min washes in 0.05 M Tris Buffer Solutions (TBS) (pH 7.4), the samples were ready for sectioning.

Endometrial section pretreatment

Uterine slices were sectioned at 50µm using a vibratome and pretreated for 4h at room temperature with aqueous 3% sodium deoxycholate (D6750-25G, Sigma-Aldrich, St Louis, MO, USA). The sections were then rinsed three times with distilled water and a further 3 x 10 min washes in 0.05 M TBS (pH 7.4). The sections were further pretreated with 0.1% pepsin (P-7012, Sigma-Aldrich) in 0.36 mol / L HCl (BDH Chemicals, Australia, Pty, Ltd, NSW, Australia) for 1.5h at room temperature (Hashimoto et al. 1999). The samples were rinsed three times with distilled water and then 3 x 10 min washes in 0.05 M TBS (pH 7.4).

Immunofluorescence

Non-specific (background) staining was reduced by pre-incubating for 24h at 4° C with TBS + 0.3% Triton X-100 + 3% Bovine Serum Albumin (BSA) (A-7888, Sigma-Aldrich).

Monoclonal antibody mouse anti-human haematopoietic progenitor cell, CD 34 (M7165, DAKO, Corporation, Carpinteria, USA), was diluted 1:800 and incubated for 72h at 4° C. Fluorescein labeled goat anti-mouse (F-2761, Molecular Probes, Inc, Eugene, OR, USA) (FITC), was diluted 1:800 and incubated for 24h at 4° C. Each incubation step was followed by two 15 min rinses in TBS pH 7.6, unless otherwise stated.

Polyclonal antibodies rabbit anti-human keratin (A0575, DAKO), was diluted 1:800 and incubated for 72h at 4° C. Tetramethylrhodamine labeled goat anti-rabbit (T-2769, Molecular Probes, Inc, Eugene, OR, USA) (TRITC), diluted 1:800 and incubated for 24h at 4° C. After immunofluorescence all slides were mounted using fluorescent mounting medium (S3023, DAKO). Clear nail polish was used to seal the coverslip to the slide, to prevent the evaporation of the mounting medium and drying out of the specimen during storage.

Effect of mounting on three-dimensional structures

When using whole mounts or thick sections, mounting onto microscope slides can introduce deformations in the three-dimensional structure of a specimen. This can occur if the coverslip touches the surface of the specimen, or it may be due to the chemical properties of the mounting medium used.

To alleviate the former of these potential problems, care was taken to mount the specimen on a slide and construct spacers using acrylic support made with non-
aqueous mounting media such as Ultramount (Fronine Pty, Ltd, Riverstone, NSW, Australia) or the use of nail polish streaks in order to build a gasket to separate the specimen from the coverslip. The use of double sided adhesive tape (0.1 mm thick) and then applying nail polish streaks on the inside edge of the tape to form a liquid proof seal was also useful.

The selection of the mounting medium used was not only important to preserve the structure of the specimen, but with the addition of certain chemicals, it served to reduce bleaching/fading of fluorescence. Caution was taken when choosing the mounting media to ensure that it did not contain polyvinyl alcohol, as this can cause shrinkage of the specimen.

**Laser scanning confocal microscopy (LSCM)**

A total of 32 longitudinal uterine slices containing endometrial tissue were divided into two regions (fundus, n=32 and isthmus n=32) providing a total of 64 samples.

The three layers of the endometrium: subepithelial capillary plexus, functionalis and basalis, were mapped by bright-field microscopy on a Nikon Eclipse E800 microscope (Nikon, Shinagawa-Ku, Tokyo, Japan). Low magnification sections were viewed through a x10 eyepiece using a x4 (NA = 0.13, Nikon) objective.

The subepithelial layer, containing the subepithelial capillary plexus, was defined as being ≤ 200 µm below the surface epithelium. The basalis was defined as being ≤ 300 µm above the myometrium. The functionalis was selected from the area in between the subepithelial capillary plexus and basalis areas. The functionalis depth varied greatly depending on cycle stage.

The datasets to be utilized for quantitative estimations a magnification of x20 Plan Fluor (NA = 0.30, Nikon) objective was utilised and acquired using a Nikon Eclipse E800 microscope (Nikon) equipped with a Radiance 2000 confocal scanning laser attachment (Bio-Rad Microscience, Hemel Hempstead, UK).

A total of 20 confocal volumetric fields of view (ranging between 40 – 50 µm depending on endometrial tissue thickness) were selected from each endometrial layer (subepithelial capillary plexus, functionalis and basalis) for both uterine regions, resulting in a total of 60 confocal volumetric fields of view per uterine region per subject. Thus, samples from 32 subjects generated a total of 3,840 confocal volumetric fields of view containing a total of 70,691 microvascular segments (i.e. multiple microvascular segments were contained within each confocal volumetric field of view). Thus, there were 70,691 measurements for vessel length density, branch point density, mean vessel segment length and capillary radial diffusion distance. Once each of the microvascular segments within a confocal volumetric field of view was summed, there were a total of 1,613 measurements (Manconi et al 2011).

For LSCM analysis the sections were viewed using the confocal system’s HQ 500LP or E 570LP filter sets that are normally used for either FITC or TRITC detection respectively. Using the HQ 500LP filter set the specimens were illuminated with the 488 nm excitation line of a 25 mW argon ion laser and any fluorescence emitted at wavelengths greater that 500 nm then passed to a photomultiplier detector. With the E 570LP filter set, the specimens were illuminated with the laser’s 543 nm excitation line and fluorescence emitted at wavelengths greater than 570 nm passed to the photomultiplier.

The 50 µm sections were imaged without a neutral density filter (NDF) to improve imaging of regions furthest away from the coverslip and the pinhole was closed to 1.2 mm to minimize collection of signals from out of focus planes. To reduce photobleaching of both the FITC and TRITC fluorophores, time spent observing the endometrium using fluorescence was kept to a minimum. A Kalman filter of factor 5 was used when taking images at each layer, which resulted in each image being an average of five successive micrographs. The images became darker for layers further away from the surface of the endometrium, so a computer normalization algorithm was used to adjust the background to a more uniform brightness. The confocal fluorescence signals detected from the surface of each section were displayed on a monitor and stored as a PICT graphic file. Using the ability of confocal microscopy to optically section intact or thick specimens, confocal fluorescence images of approximately 0.5 µm optical section thickness were collected with the x20 objective through a depth of 50 µm section. Sections analysed by bright-field microscopy were captured using a NCB11 filter through a CCD video camera connected to a frame grabber card within a Pentium III personal computer, images were then saved and archived. Sections analyzed by LSCM were photographed using the confocal scanning laser attachment then saved and archived. The images were then observed on the computer monitor using the LSCM integrated software LaserSharp (Bio-Rad Microscience, Hemel Hempstead, UK). LaserSharp was used either to play through the collected series of images as a movie file or to render them as a projection.

Once the datasets had been collected it was necessary to process them using a method that was appropriate to the type of information to be visually extracted. In order to do this third party visualization software Imaris (Bitplane, AG Scientific Solutions, Zürich, Switzerland) was utilized, to prepare the datasets for quantitative measurements in NeuronTracer 1.0 (Bitplane) (Manconi et al 2011).
Three-dimensional visualization using Imaris

Imaris is a commercially available volume rendering program which accepts stacks of registered two-dimensional images and creates three-dimensional projections from any viewpoint. The tools included in the suite are for two-dimensional and three-dimensional measurements of individual isosurfaces and groups, and they allow for two-dimensional slice viewing and movie loop generation. The three-dimensional images can be displayed as either solid or transparent structures.

Results

The following images have been acquired using Laser Scanning Confocal Microscopy (LSCM). The use of Imaris three-dimensional visualisation software has enabled the addition of false colouring to further illustrate the endometrial structures.

The reconstructed three-dimensional renderings of the endometrial microvessels have been displayed as orthogonal projections. A ‘false’ colour (red) has been added to highlight the microvascular structures and (green) to highlight the glandular structures.

Types of three-dimensional visualization

There are several ways in which to visualize the data in LSCM.

Side-by-side stereo

Side-by-side stereo means that the normal display is divided into two halves, a left view and a right view, each occupying one-half of the original display area. Each view displays the original image from a slightly different perspective, corresponding to the left and right eye of the viewer. The images are separated, however, so to actually see a 3-D object you must direct your eyes until the two images are on top of each other, and then focus on the resulting image until you can see it as threedimensional.

The way in which the images are presented in (Fig. 2) is called a wall-eyed stereo, the left eye’s image is located on the left side of the display, and the right eye’s image is on the right. This is the standard method for displaying stereo images in publication as it works well when the display (in this case, the piece of paper) is close to the eyes. It is called wall-eyed because the eyes are directed the same way they would be if looking at a distant wall.

Figure 2
Stereo pair projection, illustrating microvascular and glandular structures in endometrium.

Traditional anaglyph

Viewing anaglyphs, two images are taken from two slightly different vantage points. In a red-blue anaglyph, the red lens is covering the left eye which sees the red parts of the image as ‘white’ and the blue parts as ‘black’. Whilst the blue lens is covering the right eye which perceives the opposite effect. True white or black regions are perceived the same by each eye. By restricting the proper views to the appropriate eye the brain interprets the differences as being the result of different distances producing a three-dimensional illusion without requiring the viewer to cross their eyes (Fig. 3).

Figure 3
Anaglyph projection, illustrating endometrial microvascular and glandular structures.
Perspective projection

Most three-dimensional rendering software adds perspective: the closer items appear to be larger than most distant items. Perspective projection was found to be a strong monocular cue for depth, so this technique created a powerful illusion of depth (Fig. 4). In some instances it was helpful to have perspective free images (‘orthographic rendering’).

Orthogonal projection

An orthogonal (also known as orthographic) projection was a way of providing a two-dimensional graphic view of an object in which the projecting lines are drawn at right angles to the plan of projection (Fig. 5). It had a couple of advantages; it was quicker, and allowed a region to remain a constant size in different views.

Figure 4
A perspective projection of human endometrium, the closer microvessels appear to be larger than the most distant microvessels. A false colour has been added to highlight the microvascular segments (red).

Figure 5
A perspective projection of human endometrium, the closer microvessels appear to be larger than the most distant microvessels. A false colour has been added to highlight the microvascular segments (red).

Figure 6
The top two diagrams represent potential distortion during the sectioning and reconstruction process when performed on thin sections. The bottom two diagrams illustrate how three-dimensional information is better preserved in thick sections.
Discussion

The employment of LSCM has provided for the first time a three-dimensional visualization of well developed endometrial microvascular networks and their relationship with endometrial glandular structures and the provision of quantitative indices to include: (a) length density (Lv), (b) numerical density (Nv), (c) vessel segment length (SLv) and (d) diffusion distance (r(diff)) (Manconi et al 2011) for the endometrial tissue across the stages of the menstrual cycle.

This was a study conducted to combine whole-mount/thick sections and laser scanning confocal microscopy (LSCM) to scan endometrial tissue sections to a depth of 50 µm. This produced a series of high resolution, low background images, similarly to another study which used Multiphoton Excitation microscopy (MPE) (Manconi et al 2003). Both LSCM and MPE provided distinct advantages in comparison to light microscopy studies (Manconi et al 2001; Simbar et al 2004).

The use of thick sections ≥ 40µm has a theoretical advantage over all thin section techniques ≤5µm in that they contain edge-gradient information, rather than edge-position information. Optical sections collected through the use of either an MPE or LSCM are perfectly aligned. Correct shapes can be retrieved without resorting to external sources of alignment information (Fig. 6).

The non-invasive nature of optical sectioning enables for the observation of living cells (Patel and McGhee 2007) as well as fixed cells to be made with greater clarity. Scanning in the z direction, as well as in the x and y directions, gives an overall effect of viewing the focal plane from the side. Confocal microscopes have a shallow depth of field (between 0.5 – 1.5 µm), this enables the information to be collected from a well-defined optical section.

In conventional light or fluorescence microscopy the information is collected from most of the specimen depth. LSCM allows for the recording of true three-dimensional data sets and the stacks of optical sections obtained from successive focal planes are known as a z series and can be reconstructed to produce a three dimensional view of the specimen. Stray light is minimized, and this is due to the small dimension of the illuminating light spot in the focal plane. By image processing, many slices can be superimposed, giving an extended focus image, when using conventional light or fluorescence microscopy this can only be achieved by reducing the condenser aperture and thus sacrificing specimen resolution.

Precise scientific imaging of the human body has progressed dramatically, aided by the improvements in bright-field microscopy; the development and refinement of optical sectioning microscopes (i.e. laser scanning confocal, multiphoton emission microscopy), computer software engineering.

Imaging technologies have a vital function in aiding the understanding of the mechanisms and functional implications of endometrial vascular morphology. Computer-generated three-dimensional reconstruction of the endometrial microvasculature and glandular structures has proved to be a powerful tool for increasing our comprehension of their relationships.

Conclusion

Imaging technologies have a vital function in aiding the understanding of the mechanisms and functional implications of endometrial vascular morphology. Computer-generated three-dimensional reconstruction of the endometrial microvasculature and glandular structures has proved to be a powerful tool for increasing our comprehension of their relationships. The three-dimensional quantitative analysis and adding to our knowledge of the vascular micro-architecture by estimating the vessel length density, numerical density, vessel segment length and capillary radial diffusion distance. The utilization of LSCM produced a stack of two-dimensional images that were then rendered to produce a three-dimensional image that could be rotated 360° in any direction, and direct observations and estimations were made as the structures exist in-vivo and now can be applied to understanding other endometrial pathologies.

References


their specificity and sensitivity in the diagnosis of vascular tumors and comparison with von Willebrand factor. Modern Pathol 7: 82-90.


ERRATUM

Australian Journal of Medical Science February 2011 Vol. 32
Cancer stem cells and the side population
Yen Siew Loh and Daniel Man-yuen Sze

Due to a typesetting error, the figure from the previous journal, Cancer stem cells theory on page 3 is incorrect. It should read as follows:

**Figure 1**

Cancer stem cells theory
Cancer cells are heterogeneous and seem to be maintained by a hierarchical organisation. Only the cancer stem cell subset (CSC; yellow) has the ability to self-renew, proliferate extensively and form new tumours.

Australian Journal of Medical Science February 2011 Vol. 32
Performance of the Liatest D-dimer assay on Stago Coagulation Analysers using EDTA plasma

Averil Drummond

PALMS Pathology, Royal North Shore Hospital, Sydney

Abstract

D-dimer measurement is widely used for the diagnosis of venous thromboembolism (VTE) and of disseminated intravascular coagulation (DIC). There is some uncertainty as to the stability of D-dimer in plasma, with great variability in acceptable storage times and conditions quoted in package inserts of D-dimer kits, even when the same assay principle is used. Almost always these time limits fall short of D-dimer stability that has been demonstrated in the literature. In Australia D-dimer is usually measured in citrated plasma, however, the D-dimer antigen has been shown to be stable in plasma from other anticoagulants and in serum. In this study D-dimer measurement using ethylenediaminetetraacetic acid (EDTA) plasma on a Stago analyser with the Liatest D-dimer assay is shown to be directly comparable to results obtained on citrated plasma provided that a correctional factor of 0.9 is applied to the EDTA result to correct for the dilution effect of the citrate anticoagulant. Samples were compared for up to 18h after collection with no loss of sensitivity for the assay.

Keywords: D-dimer, stability, correlation, EDTA plasma

Introduction

The development of rapid sensitive assays for the measurement of D-dimer in recent years has lead to an increase in the demand for this useful test mainly driven by emergency clinicians wishing to exclude venous thromboembolism (VTE) in suitable patient populations. D-dimer levels are also commonly used to help diagnose and monitor the coagulation activation process in disseminated intravascular coagulation (DIC). Recently further prognostic applications are being developed in a variety of clinical situations such as risk stratification for VTE recurrence and risk assessment for thrombosis during the administration of haematopoietic growth factors and in thalassaemia and sickle cell disease (Adam et al 2009). D-dimer levels have also been shown to be prognostic in many different malignancies (Dirix et al 2002; Drummond and Coyle 2009). The status of D-dimer as one part of a coagulation testing protocol means that it is almost universally performed on a citrated sample. However, the principle of its measurement is based on the antigenicity of D-dimer and the D-dimer antigen should remain intact whether the medium being tested is plasma or serum provided any anticoagulant used is not antagonistic to D-dimer stability and EDTA and lithium heparin specimens have been shown to be suitable for testing (Favaloro et al 2008; Lippi et al 2007). The focus of this study is firstly to compare the performance of a D-dimer assay using two different types of plasma: plasma from whole blood diluted 9:1 in sodium citrate and plasma from undiluted whole blood collected into the dry anti-coagulant EDTA, and secondly to assess the stability of the D-dimer antigen in EDTA plasma. The EDTA specimen is collected for a full blood count on almost all patients presenting for pathology tests and its potential use may spare the patient from the need to collect an additional tube of blood and, over time, also prevent the need for many recollections when the citrate tube is either not collected in error, or is unsuitable for any reason. The use of an alternative specimen and an increased time window for testing will be particularly attractive to pathology laboratories who are under constant pressure to accommodate doctor’s requests for “add on” testing, and will reduce turn around times for results on specimens that have often already been transported for several hours. D-dimer testing is already affected by a number of variables that make assay standardisation difficult. Some of these are discussed below. It is essential in all laboratory testing, that all methods be thoroughly validated and that no additional sources of error be added to the testing procedure. For

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this reason this paper considers modifying the D-dimer procedure for only one additional specimen (EDTA), and tested with one commonly used coagulation analyser/test combination (Stago/Liatest), in an attempt to present sufficient data for method validation.

Materials and Methods

Patients

One hundred and twenty two patient samples were tested over a period of six months.

Patient samples tested were selected in a random manner subject only to the availability of suitable specimens. Sixty of the patients were male and 62 female. The age distribution was from 19 to 99 years. The patients suffered from a variety of clinical conditions including haematological disorders, pulmonary embolism, pulmonary oedema, sepsis, postpartum and post surgical complications. Three patients had elevations of serum bilirubin but these were below the level of 200mg/L shown to cause interference in this assay. In all cases specimen turbidity was minimal and considered insufficient to affect the analysis. In appropriate patients Rheumatoid factor was excluded as an artefact causing overestimation of D-dimer. Twenty patients from the cohort whose citrate D-dimer levels fell in the range 0.4µg/ml to 0.6µg/ml fibrinogen equivalent units (FEU) (or +/- 0.1 of this laboratory's negative predictive cut off value for VTE which is 0.5µg/ml FEU) where also selected and compared as a separate group.

Specimens

All routine coagulation testing is performed on whole blood collected in 0.106M (3.2%) sodium citrate tubes. Specimens are accepted for testing if they are filled from 90% to 110% capacity. The EDTA specimens used for comparison purposes were not subject to any requirements for fill capacity. The only requirement was that sufficient plasma (0.5ml) was available, after centrifugation, for testing. In order to make the comparison more useful for the situation of potential “add on” testing, any EDTA specimen collected within the same working day period i.e. 6am till 12pm, a period of 18h, was deemed suitable for inclusion in this study.

Sample preparation and testing

For the D-dimer assay by the standard laboratory method, venous blood was collected into 0.106M (3.2%) sodium citrate, in a ratio of 1 volume citrate to 9 volumes blood (see above). Plasma was separated within 4h of collection. Platelet poor plasma was obtained by centrifuging for 7 mins at 4000 rpm at 16-18°C. D-dimer was measured using the immuno-turbidometric method (Liatest D-dimer), on STAR analysers (Diagnostica Stago). The Liatest D-dimer reagents have a lot specific calibration curve accurate from 0.22 to 4.0 µg/ml FEU validated daily with two levels of controls. These controls are lyophilised human citrated plasma with assigned values of D-dimer. The laboratory protocol is that requests for D-dimer for VTE testing are performed on undiluted plasma and D-dimer for DIC testing with an automated plasma pre-dilution of 1:5. EDTA specimens used for comparison purposes where stored uncentrifuged at 2-6°C after processing for the full blood count. For D-dimer testing they were subject to the same centrifugation and testing procedure as the original citrate sample.

Statistical analysis

Statistical analysis was performed using Microsoft Excel and Analyze-it software with Passing and Bablok fit of linear regression analysis and Bland Altman difference plots of ranked data.

Results

A direct comparison of D-dimer results between EDTA and citrate plasma cannot be made as plasma used for coagulation testing is collected into the liquid anticoagulant 3.2% sodium citrate in a ratio of nine parts whole blood to one part anticoagulant. To compensate for this dilution of the citrated plasma a factor of 0.9 was applied to every D-dimer value obtained on EDTA plasma so that a direct comparison of the data could be performed. Regression analysis of data showed a correlation co-efficient (Pearson's) r statistic of 1.0 for D-dimer quantitation using the two types of plasma specimens (citrated plasma and EDTA plasma with a correction factor of 0.9 applied) with a 95% CI of 0.99-1.0. For the Passing & Bablok fit, bias was 0.01 with a 95% CI of –0.03 to 0.05 indicating no significant bias for the method over the range of values tested (Fig. 1).

The difference between values (Fig. 2) did increase with absolute values measured however with the exception of one outlier all differences were less than 0.5 up to a D-dimer value of 3.5 µg/ml FEU and less than 1.0 up to a D-dimer value of 11.5 g/ml FEU.

Occasional out of tolerance pairs in a difference plot may result from imprecision in either methodology due to unknown causes. There was no relationship between increase in difference values and time between testing although specimens tested between 12 and 18h did show a slightly increased overall bias of 0.2 compared to the value of 0.1 obtained from specimens tested at less than 12h. Figures 3,4 and 5 show difference values for specimens compared within 6h (Fig. 3), from 6 to 12h (Fig. 4) and from 12 to 18h (Fig. 5).
Figure 1
Passing & Bablok method comparison of D-DIMER results obtained on citrated plasma samples and EDTA plasma samples with the correction factor of 0.9 applied.

<table>
<thead>
<tr>
<th></th>
<th>Intercept</th>
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<tr>
<td>n = 122</td>
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<td>-0.03 to 0.05</td>
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<td>Proportional bias</td>
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Figure 2
Bland – Altman Bias plot of samples tested using citrated plasma compared to EDTA plasma (with 0.9 correction factor for dilution applied).
Figure 3
Bland–Altman Bias plot of samples using citrated plasma compared to EDTA plasma tested from zero to six hours post collection (with 0.9 correction factor for dilution applied).

Figure 4
Bland–Altman Bias plot of samples tested using citrated plasma compared to EDTA plasma tested from six to twelve hours post collection (with 0.9 correction factor for dilution applied).
Table 1
Tabulated results of 20 patients whose D-dimer results were +/- 0.1 mg/ml of the 0.5 mg/ml VTE cut off value. Discrepancies are shown in red type.

<table>
<thead>
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<th>0.9 EDTA</th>
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Discussion

Fibrinogen is formed as an end product of the coagulation cascade. It is an essentially symmetrical molecule composed of a central E domain flanked by two identical D domains. As fibrinogen is forming it is cleaved by thrombin allowing polymerisation and the formation of fibrils of fibrin. These are stabilized when FXIIIa, which is also activated by thrombin, cross-links adjacent D domains. The end product of this process is the insoluble, cross-linked fibrin clot.

Fibrinolysis occurs when plasminogen is converted to plasmin on the fibrin surface. A number of fragments of variable molecular weight known as fibrin degradation products (FDPs) are formed. These consist of various sized combinations of the D and E fragments. The terminal product of fibrin degradation is the D-dimer/E complex (DD/E). (Adam et al 2009). D-dimer measurement has extensive and increasing clinical use in the diagnosis and prognosis of patients with VTE and pulmonary embolism (PE) in outpatients and hospitalised patients. It has an important role in the diagnosis and monitoring of patients with DIC, prediction of thrombotic risk in certain situations and in prognosis in many types of malignancy.

This study was designed to answer two specific questions: firstly, is it acceptable to perform the D-dimer assay on a Stago analyser using EDTA plasma as a replacement for citrated plasma? secondly, is the D-dimer
antigen sufficiently stable in EDTA plasma to allow for "add-on" testing during the course of a normal working day, (in this context taken as 18h)?

To answer the first question it is important to examine the results that were obtained in this study in the larger context of D-dimer analysis. Discrepancies revealed by inter-laboratory quality assurance programs for D-dimer have many causes. Among these are: variation in methodologies incorporated into the large number of commercial assays available, the lack of a reproducible standard, variability in the type of calibrators available and in reporting units, and negative cut off values (Gardiner et al 2005; Adam et al 2009; De Maat et al 2000). A large body of literature exists comparing the performance of commercial D-dimer assays. Many of these studies take the BioMerieux Vidas D-dimer assay as the standard to which other methods are compared. This is the automated version of the sensitive enzyme linked immunoabsorbant assay (ELISA) technique first used in D-dimer research (Heim et al 2004; Adam et al 2009). Stago Liatest D-dimer, used in this study, has been found to have comparable sensitivity to the Vidas assay for diagnosis of VTE (Schrecengost et al 2003; Fancher et al 2004). These assays with high sensitivity have a relatively low specificity of approximately 40%. That is, they detect more false positive cases than less sensitive assays. This, however, makes them more suitable for diagnosis of patients at moderate risk. Less sensitive assays such as SimplyRed (Agen Biomedical) are suitable for the testing of low risk patients only (Fancher et al 2004). To achieve acceptable clinical utility the D-dimer assay should provide for a negative predictive value (NPV) for DVT of >98%, that is equivalent to optimum results obtained by radiological techniques such as compression ultrasound.

To achieve this, it is recommended by most experts that all diagnostic techniques be used with a verified pre-test clinical probability algorithm. The most commonly used is that developed by Wells (Wells et al 2003), but several other algorithms have extensive validation in the literature (Kelly & Hunt 2003; Adam et al 2009). Unfortunately, some D-dimer assays still in use lack sufficient sensitivity or specificity for VTE diagnosis even when used with the above algorithms (Gardiner et al 2005).

One major reason for discrepancies between assays is the large number of monoclonal antibodies used which react with different epitopes on the cross-linked D-dimer domains of fibrin (Adam et al 2009). It has been shown that the binding behaviour of these antigens and antibodies shows significant differences (Gaffney et al 1995). The antibodies will also react with any FDP containing one or more D-dimer motif, of which there are many in plasma. High molecular weight fragments being most plentiful, for example, in DIC. (De Maat et al 2000; Dempfle et al 2001). The search for a suitable D-dimer standard was begun when the test was developed and has yet to be successful. This lack of standardization results in the production of calibrators that at the present time are usually kit specific. The most recent proposal (2010), from the Fibrinolysis Subcommittee of the International Society of Thrombosis and Haemostasis (ISTH) is for a calibrator composed of plasma samples from a large number of patients with high D-dimer levels. A suitable pool has been collected and lyophilised for trial studies to begin. A pooled plasma calibrator appears to increase conformity between assays, as was discussed at the Scientific and Standardization Committee meeting of the ISTH in 2007. Between method coefficient of variation (CVs) from participating laboratories in the United Kingdom National External Quality Assessment Service (UK NEQAS) surveys decreased significantly when results were recalculated using consensus median values for three plasmas used as calibrators. Disadvantages of pooled plasma calibration are infection risk and matrix problems due to the presence of unknown compounds (Dempfle et al 2001). Of note, the External Quality Assessment Program in Haemostasis and Thrombosis (ECAT) Foundation QAP program uses a variety of quality control material for D-dimer testing, either normal plasma, normal plasma spiked with varying amounts of DIC plasma, commercial lyophylised plasma whose composition is not disclosed, and more recently lysed fibrin clots. It is beyond the scope of this article to speculate on any changes this may have made to the performance of participating laboratories. At the 2007 ISTH meeting, a calibrator for D-dimer formed from terminal plasmin digestion of a cross-linked fibrin clot prepared from a known amount of fibrinogen was also proposed.

Another cause for lack of conformity is the choice of units for reporting. These vary widely between different countries and institutions, both in the molecular weight of the unit chosen, (the fibrinogen equivalent unit – FEU being approximately twice the molecular weight of the D-dimer unit – DDU) and in the magnitude of the units chosen e.g ug/ml, ug/L, mg/L or others. This may not present problems in the individual institution but is an undesirable situation for international standardization. The Clinical and Laboratory Standards Institute (CLSI) document H59-P is, at the time of writing, awaiting finalisation, after being available for international comment with a view to obtaining consensus in this area. The value for negative VTE prediction (NPV) chosen by the laboratory is an area of high clinical relevance also amenable to standardization. The majority of laboratories use the value recommended in the manufacturer’s package insert, but a proportion use local validation or literature information, or a combination of both (Spannagl et al 2005; De Maat et al 2000; Heim et al 2004).

In light of the above comments it is not surprising that external quality assessment of laboratories has revealed
some alarming failures of performance. In one case, when
duplicate citrate specimens from the same specimen where
presented blindly to laboratories both intra-laboratory
and inter-laboratory CVs were unacceptably high,
exceeding 23% and 57% respectively for some methods,
although CVs were much lower for the better performing
methodologies such as Vidas, and to a lesser extent, Liatest
(Spannagl et al 2005). Some of these discrepancies were
attributable to laboratory uncertainty as to appropriate
reporting units. In an ECAT study in 2000, however,
when only a qualitative assessment i.e. normal or elevated,
was required agreement was found to be good for negative
or strongly positive samples but 60% of laboratories
misclassified a sample with slightly elevated D-dimer
levels as normal (DeMaat et al 2000). At the most recent
meeting of the Fibrinolysis Subcommittee of the ISTH in
2010 it was noted that inter-laboratory variation, while
improving, was still sub-optimal. This improvement is
reflected in the most recent ECAT Foundation results
in which 92% of laboratories using D-dimer units and
89% using FEU had intra-laboratory CVs of less than
10% for a duplicate citrate sample (ECAT Foundation
D-dimer exercise 2010-2). In Australian laboratories the
Stago Liatest D-dimer assay is the most commonly used
among laboratories participating in the Royal College of
Pathologists of Australia (RCPA) QAP program. Results
on QC material using this assay are substantially superior
to those reported above. For one random cycle the method
CV was 7.7% and for the authors’ laboratory the average
of two coagulation analysers was 3.5% (RCPA end of
cycle report – D-dimer Cycle 9). The relatively high CVs
produced by some methods has lead to discussion on the
use of a cut off range, as opposed to one value, (De Maat
et al 2000) and/or the use of repeat testing for results close
to the VTE negative predictive cut off value (ISTH 2007).
However the use of a range may merely result in the value
at the upper limit of the range becoming the default NPV
and repeat testing will have no value in assays known to
show high CVs.

The results obtained in this study show a correlation
between the two samples used approaching 1.0 with
confidence intervals showing a small non-significant
bias over the range of values tested. This indicates that
no increased inaccuracy of any significance is introduced
by the use of EDTA plasma compared to citrate plasma
provided that a correction factor of 0.9 is applied to the
EDTA result obtained. The 20 values compared which
are close to the decision value of 0.5mg/L FEU do show
two discrepancies (allowing for rounding to one decimal
place) should the cut off limit be applied with no other
consideration, however the maximum difference between
the two results is 0.09, well within the allowable limits of
performance for the RCPA surveys which is +/- 1.0 mg/L
FEU. The Clinical Laboratory Improvement Amendments
(CLIA) has not allocated a total allowable error for the
d-dimer analysis so no comparison can be made on this
basis. In both cases the discrepancy would favour further
testing i.e. the EDTA value obtained is the higher of the
two. It is unlikely in these circumstances that the diagnosis
of thromboembolism would be missed with substitution
of EDTA plasma. The use of the crystalline EDTA plasma
also removes one possible source of error, the dilution
error that may occur when under filled citrate tubes are
used in error for testing.

The second objective of this study was to investigate
the stability of D-dimer in EDTA plasma. The stability
of D-dimer in clinical specimens has been established
for a large variety of samples and storage conditions. In
citrate plasma long-term storage is usually carried out
at -70°C. It has been shown that storage at -20°C does
not significantly alter D-dimer values over a period of 6
months (Garcia et al 2005), or over 36 months at -60°C.
The same authors found only a small positive bias of
average 2.7% when centrifuged citrated samples were
allowed to stand in the original tube for 4h at room
temperature and an additional 24h at 2-8°C, when tested
using the Innovance D-dimer method (Bohm-Weigert et
al 2010). In another case where citrated and heparinized
plasma were compared, no significant difference in
D-dimer results was found for either specimen type
after storage at room temperature for 24h (Schutgens et
al 2002). Uncentrifuged citrate samples transported at
ambient temperature for 24 hours showed no significant
difference in results to samples centrifuged and tested
in approximately one hour, although a slight increase in
D-dimer levels, not considered clinically significant by the
authors, was found if the transportation time was extended
to 52h (Zurcher et al 2008). D-dimer levels also remain
relatively stable in vivo even though subject to the body’s
clearance mechanisms. Post abdominal surgery they were
shown to peak at day 7 and then decrease at 6% per day
with a half-life of 11 days, only returning to pre-operative
levels after 38 days (Dindo et al 2009).

The package insert provided with the Stago Liatest
D-dimer kit suggests that analysis may be performed
on samples stored at room temperature for up to 8h.
No indication for time limits for testing for refrigerated
samples is given. Inserts for other kits give a variety
of storage times and conditions including up to one
week at 4°C for K-Assay D-dimer (Kamiya Biomedical
Company), an immuno-turbidometric assay, although this
is the exception with most times being considerably shorter.
Approximately half the EDTA samples over the whole
range of D-dimer values in this study were tested at
greater than 8h post collection time. Due to time and cost
restraints no attempt was made in this study to establish
stability in the citrated samples.
Conclusion

There seems to be some dichotomy between the reliance that may be placed by clinicians on the D-dimer assay as a negative predictor for VTE, and the performance capability of the assay in some laboratories. It could perhaps be suggested that clinicians make themselves aware of the measurement of uncertainty (MU) for D-dimer in their own institution and incorporate this information into their decision making when deciding whether to proceed to further testing. However it can be said with confidence that in this context the use of EDTA plasma did not decrease assay sensitivity and therefore it is a suitable specimen for performance of the Liatest D-dimer assay, provided that a correction factor of 0.9 is applied for lack of citrate dilution. Any potential for staff error due to lack of application of the correction can easily be overcome using a suitable computer algorithm when an EDTA specimen is used. Use of EDTA plasma also removes an important source of dilution error occurring from the testing of incorrectly filled citrate specimens. The ability to add-on a request for D-dimer testing within 18h to a commonly collected sample such as EDTA will greatly improve diagnostic turn around times with associated savings for the public health budget.

Acknowledgements

Lynne Connelly for proof reading and help with statistical analysis, Margaret Aboud and Luke Coyle for proof reading and suggestions.

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Dempfle C-E, Zips s, Ergul H, Heene D, and the FACT study group. 2001. The Fibrix Assay

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The platelet section follows on and adheres to the same format. There is a section on haematology relating to paediatrics which describes red cell, white cell and platelet disorders occurring in cord blood, the neonate and childhood.

The last section is on Blood Parasites and describes the four species of human malaria. A description of the characteristic features of each species as it occurs in the red cell is accompanied by images clearly depicting the various stages of maturation of each species of malaria.

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Severe anaemia in a 22-year-old female

Gillian Rozenberg

South Eastern Sydney and Illawarra Area Health Services, Prince of Wales Hospital, Sydney, NSW

A 22-year-old female, found to have severe anaemia by her general practitioner, presented at the Haematology Clinic for further investigative studies. A full blood count and haemoglobin electrophoresis (EPG) were performed. The results were as follows:

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<th>Parameter</th>
<th>Value</th>
<th>Reference range</th>
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<tr>
<td>Hb</td>
<td>76 g/L</td>
<td>115-165 g/L</td>
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<tr>
<td>Hct</td>
<td>0.244</td>
<td>0.37-0.47</td>
</tr>
<tr>
<td>MCV</td>
<td>83.3 fl</td>
<td>80-100 fl</td>
</tr>
<tr>
<td>MCH</td>
<td>25.9 pg</td>
<td>26.5-33.0 pg</td>
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</table>

The blood film was mildly microcytic and hypochromic. There was an occasional nucleated red cell present while many of the red cells showed coarse basophilic stippling.

The Hb EPG results were as follows:

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</thead>
<tbody>
<tr>
<td>Hb EPG Cellulose Acetate (pH 8.6)</td>
<td>No abnormal bands detected.</td>
<td></td>
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<tr>
<td>Hb A2</td>
<td>2.6 %</td>
<td>2.0-3.5 %</td>
</tr>
<tr>
<td>Hb F</td>
<td>&lt;1.0 %</td>
<td>&lt;1 %</td>
</tr>
</tbody>
</table>

Hb H inclusions: not detected

No abnormality detected.

Iron studies were normal in this case.

A bone marrow aspirate was performed. The bone marrow was hypercellular with increased erythropoiesis and mild dyserythropoiesis. No abnormal sideroblasts were seen.

As coarse basophilic stippling in the red cells is a feature of lead poisoning, a lead level was performed. The lead was found to be 5.0 umol/L with a normal range of 0-0.5 umol/L. The level of mercury was also examined and found to be <1 nmol/L with a normal range of <=50 nmol/L.
After questioning the patient in this case study it became evident that the patient had been taking a medication supplied to her by her Indian doctor. The medicine was in a powder form. A sample of this medication was sent for analysis which showed that the powder contained 12% lead.

Patients with lead poisoning clinically present with abdominal colic and constipation, peripheral neuropathy and anaemia. The anaemia is invariably a hypochromic microcytic anaemia as the ingestion of lead interferes with haem synthesis. It does so by inhibiting several enzymes directly involved with haem synthesis. Pyrimidine 5′-nucleotidase is one such enzyme. In its absence, pyrimidine nucleotides accumulate in the red cells, preventing iron from being incorporated into haem at a normal rate. This leads to a shortened red cell life span resulting in a mild haemolytic anaemia. The characteristic feature on the blood film in lead poisoning is coarse basophilic stippling in the red cells. The hypochromic microcytosis is not necessarily associated with iron deficiency.

The patient in this case study was treated with Meso-2,3-dimercaptosuccinic acid (DMSA) commonly known as succimer, a compound approved by the FDA in the 1960’s for the chelation or removal of heavy metals, particularly lead and mercury. She was initially given a three week course of succimer, 400 mg in the morning and 600 mg in the evening. Her haemoglobin began to rise while the level of lead began to fall. Two months later a similar course of succimer was administered. By the end of the second course the lead level was 0.8 umol/L, still significantly raised. The haemoglobin had returned to normal.

The Public Health Act (1991) states that a lead level of >=0.72 umol/L must be notified and that repeat testing should occur after 3 months to assess the effectiveness of treatment.
YEAR 2011

JULY 4–8: ASM Annual Scientific Meeting & Exhibition
ASM 2011 Hobart - Microbiology on the Edge
Hotel Grand Chancellor - Hobart
http://www.theasm.org.au

JULY 23–28: XXIII Congress of the International Society on Thrombosis and Haemostasis. Kyoto, Japan
http://www.isth2011.com/

JULY 24–28: AACC Annual Meeting. Atlanta, USA

AUGUST 2-5: 28th Annual NRL Workshop on Infectious Diseases (formerly NRL Workshop on Serology) Hotel Realm, Canberra.
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AUGUST 5–6: RCRA QAP / AIMS Morphology Workshop

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http://www.alloccasionsgroup.com/aimsnzimls11/

AUGUST 23–26: Australasian Flow Cytometry Group Annual Conference
Wrest Point, Hobart

AUGUST 28–31: Endocrine Society Meeting
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OCTOBER 2-6: 12th International Congress of Therapeutic Drug Monitoring and Clinical Toxicology
Stuttgart, Germany

YEAR 2012

SEPTEMBER 24-27: NSM 2012
AIMS National Scientific Meeting
Darwin, NT

http://aacb.asn.au/

OCTOBER 19-23: XXVI World Congress of the World Association of Societies of Pathology and Laboratory Medicine (WASPaLM), Las Vegas. Hosted by the American Society for Clinical Pathology.
http://www.waspalm.org

OCTOBER 30: NOVEMBER 2:
HAA Meeting
Sydney

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KEY TIME FRAMES

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<td>27 May 2011</td>
</tr>
<tr>
<td>Early bird registration date closes</td>
<td>10 June 2011</td>
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BOOK REVIEWS

Microscopic Haematology 3rd Edition
A practical guide for the laboratory
Gillian Rozenberg
Churchill Livingstone
Soft cover, 256 pages, over 400 colour illustrations
ISBN: 978-0-7295-4072-8
RRP: $135.00

This is the third edition of this most professionally and expertly presented atlas of haematology. The first edition was published in 1996 followed by the second in 2003. Once again a standout feature of the book is the exceptionally high quality illustrations. The supporting text is thorough yet concise and always relevant.

The third edition includes 92 additional images detailing cell morphology and ultra-structure resulting in over 400 photomicrographs of slides in total. There is also online access for students to free learning resources and activities to supplement the learning material in the book (Evolve). For instructors there is online access to case studies relating to the illustrations contained in the book that would be very helpful as a teaching tool.

Gillian Rozenberg FAIMS is an acknowledged authority on blood cell morphology and has once again utilised her knowledge and expertise to produce an invaluable volume for reference in the medical laboratory and for students in the field of haematology.

This book contains four sections. The first section includes erythropoiesis, anaemias, haemoglobin disorders, membrane disorders and miscellaneous. The second section, dealing with leucocytes and platelets covers maturation, abnormal cells, neoplasms, and includes special stains where warranted. The third section on paediatric haematology covers cord blood, red cell disorders, bone marrow failure, benign disorders of leucocytes, myeloproliferative neoplasms, non-haemopoietic malignancies, storage disorders and platelet abnormalities. The fourth section deals with blood parasites, including the four generally recognised species of human malaria and also Plasmodium knowlesi, now recognised as a fifth species infecting humans, as well as non-malarial blood parasites.

The text in all sections includes accurate and succinct descriptions and the illustrations are always relevant and have been reproduced to represent faithfully what would be seen when viewed microscopically. In the main, the detail of the slides is excellent, and the cell structure/inclusions very clear, however some of the illustrations for the fourth section dealing with the malarial parasites are a little too small for enough detail to be seen. The slides that have been selected as illustrations are eminently illustrative of the condition under discussion. Appropriate special stains and diagnostic techniques are also incorporated and illustrated when relevant.

The descriptions of neoplasms in this edition are classified according to the generally accepted fourth edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Cytogenetic and cytochemical/ immunophenotypic details are included where applicable.

Having spent much of my career in the Haematology Department of a large metropolitan children’s hospital, I know too well that paediatric haematology, both normal and abnormal, is significantly different from that seen in the adult. The inclusion of a chapter dedicated to the detailed description of paediatric haematology is most welcome.

The book does not purport to be a haematology text book, and nor is it. The minimalist texts assist the student of haematology in identifying and recognising the morphological features, however there is little or no explanation of the underlying cellular and physiological mechanisms which give rise to those morphological abnormalities, for example the shortened red cell life span in lead poisoning is mentioned but not elucidated. Perhaps references to suitable texts could be incorporated. Labelling of the features in the photomicrographs would also be a welcome improvement. In addition, a glossary of terms would add value, for example burr cells appear in figure A3-17 with no previous mention or description.

I would recommend this practical guide as an absolute must for all laboratories and teaching institutions. It provides an exceptional resource for remote laboratories and will prove invaluable for training students who will appreciate the e-learning feature and the book’s very competitive price. The compact size makes the book easy to use next to the microscope, especially when compared to other atlases.

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Australian Journal of Medical Science  May 2011  Vol. 32  No. 2
Calcium and Bone Disorders in Children and Adolescents
Endocrine Development: Volume 16
Editors: J Allgrove, NJ Shaw
Karger 2009
Hard cover, x+300 pages
ISBN: 978-3-8055-9161-4
USD$196.00

This book is a unique compilation dedicated to paediatric and adolescent bone disease. It fills a gap in the medical literature, left by a scarcity of text books dedicated to metabolic bone diseases in this age group.

The first chapter, Voyages of Discovery is aptly named and placed, and takes the reader through the history of discoveries related to calcium and bone metabolism and diseases. It certainly re-ignited my passion in this area. Much of our current knowledge stems back to early descriptions made on growing children and an opening chapter on this topic is eminently suitable.

The next two chapters are dedicated to the normal physiology of calcium, phosphate, magnesium and bone. The inclusion of magnesium together with calcium and phosphate in describing the physiological regulation is a rare treat. Standard text books treat these elements as separate entities, but in a biological system they clearly are interrelated and should be considered together.

Chapters on bone biopsies and bone density measurement are more relevant to clinicians but other chapters such as the Practical Approach to Hypocalcaemia, Hypercalcaemia and Rickets in particular are of value to diagnostic laboratory scientists or biochemists. Biochemists are often called upon to comment or provide advice on test result interpretation and further investigations. The clinical background provided here allows the rational decision making in terms of further investigation and assists in the differential diagnosis.

Disorders of calcium metabolism are sufficiently common in the community to ensure these detailed chapters are a welcome source of information for general practitioners who often are the first to encounter such situations in children. Medical students will equally benefit from this book. Although primary osteoporosis is rare in children secondary osteoporosis is common and should be identified early in order to prevent more severe disease, fractures and deformity. Children who suffer from chronic illnesses are susceptible to secondary osteoporosis and this potential outcome should be considered at the time of diagnosis of the primary disease. Disorders such as cystic fibrosis, celiac disease and eating disorders as well as chronic medication (anti convulsants, glucocorticoids) deserve to be mentioned and are discussed in sufficient detail. The chapter on miscellaneous bone disorders is interesting and provides an excellent list of gene mutations which can be used as a reference guide. Although the chapter on medication used in skeletal disorders may not be directly relevant to biochemists, a basic knowledge of the type of drug and the mechanism of action may help in the interpretation of biochemical results of these patients.

What I enjoyed most are the case histories. They follow the traditional format of presentation followed by biochemical findings and other relevant history. Cases on Paediatric and Adolescent Skeletal Disorders are very hard to come by, and these cover a wide range of conditions, age groups and presentations. Each case is followed by Key Learning Points which are clear and succinct ‘pearls’ that can be read out as a quick revision on their own right.

The references used in each chapter are up to date and a useful resource for those who wish to pursue specific areas further. Being a hard cover book, it is more suitable as a reference or text book on the shelf. The book achieves its aims of enabling the understanding of bone mineral metabolism and ‘makes calcium interesting’.

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Rapid Review Microbiology and Immunology
3rd Edition
By KS Rosenthal, MJ Tan
Moby Elsevier 2010
Soft cover, 221 pages
ISBN: 978-0-323-06938-0
AUD$57.00

Rapid Review Microbiology And Immunology is part of a series of books aimed at assisting students prepare for the United States Medical Licensing Examination (USMLE) step 1. The Book features an outline format that is headings and bullet points, there are numerous diagrams, tables and boxes to highlight the text. The book also features margin notes to further highlight the text. The authors Professor Ken S. Rosenthal PhD, Department of Microbiology and Immunology, Northeastern Ohio Universities Colleges of Medicine and Pharmacy has contributed other publications in the field of medical microbiology.

Rapid Review Microbiology And Immunology is divided into four main sections. Section I Immunology consisting of five chapters, Section II Bacteriology consists
12 chapters, Section III Virology consists of 10 chapters and Section IV has three chapters covering Mycology, Parasitology and Infectious disease. Each section has introductory chapters, the immunology section has one chapter giving an overview of the topic. The bacteriology and virology sections are the largest sections and these have three chapters each.

The immunology section covers components of the immune system, the role of T cells Immunoglobulins and their production by B cells, normal and abnormal immune responses and the laboratory investigation of the immune response. Section II chapters 6 to 17 deals with bacteriology the first three chapters in this section deal with introductory topics bacterial structure, bacterial growth, genetics and virulence and diagnosis, therapy and prevention. Each chapter is only four to five pages long thus there is only space for the major pathogens. This also forces unusual groupings of organisms thus chapter 12 which covers Gram Negative Cocci and Coccobacilli deals with Neisseria species, Bordetella sp, Haemophilus sp and Legionella sp. Organisms are grouped according to gram reaction, morphology and oxidase reaction. Thus chapter 9 the gram positive cocci covers Staphylococci, Streptococci and Enterococci. The Enterobacteriaceae and Mycobacteria have a chapter each.

This book is focused on medical students preparing for the USMLE exams and is designed as study guide and due to the limited space it is not possible to cover the range of organisms described in the more comprehensive text books. There are I believe some notable exceptions which I think present with sufficient frequency to warrant mention. These include Stenotrophomonas maltophilia, Fusobacterium species, Moraxella catarrhalis, Pasteurella multocida to name a few.

Section III deals with Virology. There are ten chapters, as with the bacteriology section the first three chapters deal with introductory topics: viral structure, classification and replication, viral pathogenesis and diagnosis, therapy and prevention. The remaining seven chapters deal with the viruses. This book uses a classification based upon the composition of the viral genome (DNA or RNA) and the presence or absence of a capsule. There is a separate chapter each for Retroviruses and Hepatitis viruses. In contrast to the bacteriology section all the major viral families of clinical significance are represented in section III, although there is more detail provided on the more predominant viral pathogens such as Human Papilloma Virus, Herpes simples 1 and 2, Varicella Zoster, EBV, CMV, Enteroviruses, Influenza and so on. As with the previous sections there are numerous tables, and diagrams to illustrate the text.

Section IV Covers mycology, parasitology and infectious diseases. There is a chapter of eight pages for each. It is difficult to believe that the authors could do these subjects justice in the space available. The chapter on mycology has a brief introduction with a few sentences covering morphological forms, reproduction and the types of fungal disease i.e. superficial, cutaneous, subcutaneous, systemic and opportunistic. There is a brief section on laboratory diagnosis, and a table illustrating antifungal drugs. The remainder of the chapter expands further on the introduction. The agents of cutaneous mycoses are identified as Trichophyton, Microsporum and Epidermophyton there is no attempt of mention species of these three genera. They are discussed under the headings of cause, tissue infected, clinical manifestation e.g. Tinea Capitis, etc., and laboratory diagnosis and treatment. The categories of subcutaneous fungi are mentioned including Sporotrichosis, Chromoblastomycosis and Eumycotic mycetoma Sporothrix schenckii is named as a dimorphic fungus and the cause of sporotrichosis, however the authors do not define what is meant by dimorphic. The causes of Chromoblastomycosis and Mycetoma are simply given as dematiaceous fungi without actually naming any, although they do define dematiaceous. They also do not mention Pheohyphomycosis also caused by a variety of fungi including dematiaceous fungi. Chapter 29 deals with parasitology and covers the protozoan parasites such as cryptosporidium, E. Histolytica Giardia lamblia., and the blood and tissue parasites e.g. Leishmania, Plasmodium, Toxoplasm, Trypanosoma and the Helminth parasites. Only the significant pathogens are covered. Chapter 30 deals with infectious diseases: clinical correlations. Infectious diseases are grouped in seven sections 1. STD’s, 2. Urinary tract infections. 3 Infectious Diarrhoeas 4. Pneumonia, 5. Meningitis and Encephalitis, 6. Arthropod borne disease and 7. Others. There are several quite useful tables in this chapter. The final section of this book are three appendices which provide summary tables related to the chapters in the book these contain quite a lot of useful information.

In summary this book is designed as a study guide for students attempting the USMLE exams. I suspect it is designed for use in conjunction with other materials in fact the front cover provides information relating to online access to an online study resource www.studentconsult.com As a study guide for the USMLE the book probably achieves it’s aim. I am a little concerned that the format of the book seems to foster rote learning and the ability to sprout facts possibly without true understanding. Would I recommend the book medical scientists? It certainly has a lot of useful tables and diagrams and illustrations which may be useful in preparing case studies or a resource for exam questions, however there is very little information relating to laboratory investigations and even though the book is inexpensive other texts may be more useful.

David Condie FAIMS Mackay Base Hospital Pathology Dept
David Penington’s memoirs make interesting reading, moving from his studies in Melbourne and Oxford, through his career in medicine, academia and public health. Occasionally pompous, Prof Penington writes well, and the description of his years at St Vincent’s Hospital will be interesting to many Melbourne medical scientists, filled as it is with famous Melbourne medical names. At St Vincent’s, Penington built up the medical research capacity, and he also worked in clinical medicine.

Prof Penington originally trained as a haematologist and he became Dean of Medicine at the University of Melbourne before becoming Vice Chancellor in 1988. He worked with the Australian Red Cross Society National Blood Transfusion Committee and participated in the support of international blood transfusion programs in Nepal and China.

Penington’s forays into public health included chairing the National AIDS Task Force working with Neal Blewett in the 1980s, and chairing the Premier’s Drug Advisory Council in 1995. He also worked on a national Committee of Inquiry that was instrumental in the introduction of Medicare. In Penington’s time as Vice Chancellor at the University of Melbourne he reformed education, research and management practices, making waves amongst academics. He also resisted damaging government intrusion into the operation of universities.

I found the descriptions of the early days of the AIDS epidemic particularly fascinating. Australia was quite advanced in early policy decisions in this area and Penington obviously contributed to these important decisions. In his work with the Drug Advisory Council and in other work with government Peninton was not shy of controversy and he made some brave suggestions. Penington also describes his work on the establishment of the Melbourne Museum and the establishment of Bio 21.

‘Making Waves’ is more than 340 pages of interesting reading and I recommend it to anyone who is interested in biography, medical history and public health.

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Molecular techniques based on the polymerase chain reaction (PCR) are now common to medical laboratories particularly in the disciplines of microbiology, genetics and cell pathology. These techniques are amenable to in-house development and laboratory scientists need to be skilled in the principles of the technology. Scientists involved in the development or conduct of conventional and real time PCR analytic work would value this compendium of international erudite contributions.

Commencing on the foundations of PCR technology, subsequent chapters elaborate on technical aspects such as PCR inhibition; sample extraction; in silico design primer-probe systems; website molecular tools; mathematical formulas for real-time analysis; detection systems; amplification of difficult templates; optimization of reverse transcriptase-PCR reactions; improving sensitivity and specificity; and use of controls and standard curves. Other chapters focus on issues of development and applications including instrumentation, high-resolution melting analysis, microfluidic emulsion and epigenetic research. A chapter on the guidelines of the minimum information for publication of quantitative real-time PCR experiments (MIQE) serves as a checklist and a validation protocol for novel PCR assays.

The processes from extraction to amplification, probe hybridization, detection and melting point analysis are explained and strategies to improve performance or circumvent pitfalls in each are elaborated. Commonly encountered problems that compromise PCR performance and appropriate corrective strategies are discussed such as: sample inhibition, primer dimer formation, GC-rich templates, secondary structures at the 3’ end of a primer, and variation of DNA melting points.

The format of each chapter is standardized throughout with sub-headings, tables and figures. The information is wholesome and appears to target both students and scientists knowledgeable in molecular applications. The comprehensive and comprehensible content indeed qualifies the text as an essential guide to the development, optimization and troubleshooting of PCR assays.

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Transfusion - Think About It
Edited by TJ Cobain
Nova Biomedical
Hard cover, 201 pages
ISBN: 978-1-61668-969-8
USD$116.10

This book, Transfusion – Think About It, had its origins in the aftermath of the 2000 Bali bombing where major burns victims, who were unable to receive comprehensive treatment for the first 24h, were subsequently transfused with fresh unrefrigerated whole blood. For many this approach would be considered unconventional and yet the successful outcome provided a basis to consider the scientific virtues of this product. So this book has been designed to stimulate, question and present some alternatives when considering blood product production and clinical transfusion.

While burns may have been the genesis for the book, and there are two chapters dealing with that experience, the remaining chapters cover the broader aspects of clinical decision making in transfusion. There are eleven chapters in this book, dealing with a diverse but complementary range of topics including clinical decision making and evidence based blood production, safety and supply. Each of the chapters has been written by different authors with a blend of Australian and international experts.

Transfusion has become an increasingly complex, highly regulated, emotive and expensive area of medicine. As pointed out, the priorities of those that produce the blood products often contrast with those that transfuse these products.

The guiding principles of clinical decision making are summarized by the author of the first chapter with the use of the three E’s: Evidence (or scientific basis for decisions), Ethics (or moral responsibilities toward patients, donors and the community) and Economics (or cost effectiveness and value for money). These three E’s also encapsulate the essence of the book. Each of the chapters incorporates the latest clinical and scientific evidence. Current platelet products and their efficacy are discussed along with the findings of in vitro platelet and coagulation analysis of fresh unrefrigerated whole blood which was utilized in the debridement of the burns victims. The value of data in decision making and the utility of information technology, including clinical decision support systems, are also reviewed. The complexities and challenges in balancing expectations of “zero risk” and moral responsibilities against finite and competing financial resources are highlighted in the chapters utilizing mathematical modeling for national production planning and estimation of risk associated with blood safety decisions.

The area of plasma products and future and new products is deliberately absent from this book with the editor recognizing that a separate group of experts be required to deal with this substantial field.

The criticism of this book is very minor with a few grammatical errors and poor reproduction of some of the figure diagrams.

This book uniquely offers insight for those involved in the transfusion of blood products or in their production. It successfully encourages the reader to think about the complexities and challenges of transfusion in its entirety. This book is a commendable addition to the field of transfusion medicine.

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Mosby’s Diagnostic and Laboratory Test Reference, 10th Edition.
K. D. Pagana and T. J. Pagana
Elsevier Mosby
Hard cover, 1152 pages
ISBN: 978-0-323-07405-6
RRP: USD $48.95

The range of diagnostic and laboratory tests is forever increasing. An up-to-date compendium of such tests inclusive of acronyms, descriptions and reference ranges would be valued by clinical and laboratory professionals.

This book is a compilation of test descriptions organised in alphabetical order. Each test is headed with their complete name inclusive of abbreviations and alternate names. The type of test is then defined - indicating whether it is a blood test, x-ray assessment, ultrasound, microscopy, or bacteriologic examination etc. Where applicable, normal findings are given in both conventional and the International System of Units. Possible critical values requiring physician notification and medical intervention are discussed. A concise and detailed explanation of each test and related physiology is given including indications for requesting, how a test is performed, association with pathology, and related pathophysiology. A section relevant to clinical professionals describes the contraindications; potential complications; interfering factors; procedure and patient care before, during after test. Finally, a section listing the diseases or conditions associated with abnormal findings is listed. This is followed a by a blank space headed “notes” for the recording of additional information.

In the inside of front and back covers is a comprehensive list of abbreviations for diagnostic and laboratory tests. Appendices include a list of tests by body
system (i.e. pulmonary, gastrointestinal, haematologic, renal/urologic and immunologic systems); a list of tests by types (i.e. blood, endoscopy, bacteriologic, ultrasound, and x-ray); a section on disease and organ panels commonly used for screening or assess diseases; and a list of symbols and units of measurements.

The book is prepared in a resilient waterproof cover appropriate for a frequently accessed reference in the workplace. Further, the text is organised with thumb tabs for quick reference, and has a consistent format augmented with tables and illustrations.

Although the book targets nursing and medical professionals it would be a useful resource for medical science students and laboratory scientists in employed diagnostic laboratories.

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The Mystery of Yawning in Physiology and Disease
Frontiers of Neurology and Neuroscience Vol 28
Edited by O Walusinski
Karger
Hard cover, XIV + 160 pages
ISBN: 978-3-8055-9404-2
USD$141.50

I was attracted to this text for 2 reasons: Firstly, as someone who suffers from bouts of excessive yawning (presumably as a result of over tiredness); and secondly because the title of the text implies that the “science” of yawning may be far more mysterious than is often perceived.

Indeed, I had always thought that yawning was an involuntary reflex initiated by tiredness, often associated with boredom and intended to re-oxygenate. Several years ago I worked on clinical research trials for a new antidepressant and noted that many subjects reported a significant increase in yawning activity. This peeked my interest as to the pharmacological or neurophysiological interactions that might be at play.

This text is indeed, quite fascinating. The book opens with a chapter devoted to the historical perspectives of yawning dating back to Hippocrates and the Middle Ages. This chapter is rather difficult to read, largely because the text in this chapter is interspersed with direct quotations and early medical descriptions of yawning behaviour. Herman Boerhaave (1668-1738) is considered the founder of clinical medicine. In 1680 he provided the first novel explanation of yawning: “Yawning and pandiculation favour the equitable distribution of spirits in all the muscles and unblock the vessels of which sleep may have slowed the functions...yawning and pandiculation favour blood flow and re-establish the nervous influx...”

Having been associated with forced ventilation for centuries, now with the neurohormal mechanisms well established, yawning has become in the 21st century, rather “an emotional sterotypy exteriorizing homeostatic phenomena in the systems controlling arousal, hunger and sexuality”.

This chapter certainly illustrates that the science behind the behaviour of yawning through the ages has remained uncertain. The second chapter looks at the popular beliefs and myths associated with yawning in Arabic, Western and Indian cultures.

The book is subsequently divided into a further 16 chapters followed by a comprehensive author and subject index. The text represents the best thinking on the role and function of yawning by researchers from general practice, psychology, neurologists, pharmacologists and biologists.

Some of the topics covered in this text include:

- Yawning throughout life (starting in foetuses as early as 12-14 weeks gestation)
- Sleepiness and yawning—the relationship between yawning, sleep onset, awakening and sleepiness
- The experimental evidence for and against the role of yawning in vigilance/arousal
- The hidden sexuality of the yawn and the future of Chasmology (the scientific study of yawning)
- Yawning in non-human primates—a mechanism of communication during times of threat
- Contagious yawning-social, evolutionary and neuroscientific basis
- The Brain Cooling Hypothesis- yawning as a thermoregulatory behaviour
- Associated diseases

Overall the subject matter throughout this text is often very complex and requires a prerequisite knowledge of medicine, biology, pharmacology and some psychology. Each chapter is well supplemented with a comprehensive list of references.

There is no doubt that this is a very specialised area of science and for this reason the text should be targeted to scientists and academics undertaking research in this area, or for general readers who are interested in the topic. The information presented is topical and reflects current thought and research. I found the book interesting and thought provoking if not a little “mysterious”. Indeed the subject is fascinating—exploring more about the physiological, emotional and neuropharmacological aspects of yawning is highly recommended. I shall now look at my own yawning in a very different light!

Paul Cohen MAIMS, MASM, FARCS
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Latest Additions:
Hepatitis C Antiviral Drug Discovery and Development
Viruses and Interferon
Diagnostics of Endocrine Function in Children and Adolescents

1. Anaphylaxis edited by J Ring Karger (Series title: Chemical Immunology and Allergy) 2010 xxvi+228 pages.


27. Insulin a Voice for Choice by A Teuscher, Karger. XIV + 82 pages.


29. Laboratory Diagnosis in Neurology edited by B Wildemann, P Ochsmann and H Reiber. Thieme (available through Elsevier Australia) 296 pages.


Journal-based CPD No.28
Page 1 of 1

Questions relating to *Performance of the Liatest D-dimer assay on Stago Coagulation Analysers using EDTA plasma*, page 49 of this issue.

1. The premise of this paper is that as the Liatest D-dimer assay is based on the principle of antigenic detection, then the type of anticoagulant the specimen is collected into should not affect the assay result?  
   **True/False**

2. This study compared the performance of the D-dimer assay using citrated and EDTA plasma?  
   **True/False**

3. The study population contained three samples that had bilirubin levels greater than 200 mg/L, these samples were excluded from the study.  
   **True/False**

4. The Liatest D-dimer assay is an immuno-turbidometric method?  
   **True/False**

5. In this study a direct comparison was made between the results obtained from the EDTA and plasma samples?  
   **True/False**

6. Fibrinogen is composed of a central E domain flanked by two identical D domains and during formation is cleaved by plasmin allowing polymerisation and the formation of fibrils of fibrin?  
   **True/False**

7. D-dimer measurement has extensive and increasing clinical use in the diagnosis and prognosis of patients with VTE and PE in both outpatients and hospitalised patients?  
   **True/False**

8. For the D-dimer assay to be clinically acceptable it should achieve a negative predictive value of greater than 98 percent?  
   **True/False**

9. D-dimer assays exhibit differences in assay results due to the use of different monoclonal antibodies that react with different epitopes on the crosslinked D-dimer molecule?  
   **True/False**

10. The half life of D-dimers in post abdominal surgery patients was found to be 11 days?  
    **True/False**

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The Australian Journal of Medical Science (AJMS) will consider for publication any paper relevant to the field of Medical Science. Disciplines include Blood Banking, Clinical Biochemistry, Haematology, Histopathology, Immunology, Microbiology and Molecular Biology. Areas of general interest to medical laboratory scientists, including toxicology, epidemiology, public and community health, and professional and management issues will also be considered.

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- Letters to the Editor
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Requirements and preparation of manuscripts

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Number pages consecutively commencing with the title page.

Arrange the article in the following sequence:

- Title page
- Abstract and key words
- Main Text
- Acknowledgements
- References
- Tables - each table, complete with title and footnotes, on a separate page
- Legends for illustrations.

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The title of the article should not exceed three lines (40 characters per line), including punctuation and spacing. All authors must be identified on the title page (e.g. William Smith, Susan Yeo, ....”). Where applicable, the title page should also include the name of the institution with which each author is affiliated and to which the work should be attributed. In the case of multiple authors, the name, postal address, email address, telephone and facsimile number of the author responsible for correspondence relating to the manuscript should be indicated.
Abstract and Keywords

The abstract should be approximately 150 words and should make sense when read alone or in conjunction with the article. The abstract should be a concise overview that describes the important details of the article including the purpose of the study/investigation, basic procedures (study subjects/experimental animals/observational and analytic methods) and the results and principal conclusions. New and important aspects of the work and its implications may also be included. References should not be included.

Three to ten keywords may be listed. Authors are advised to comply with the terms from the Medical Subject Headings (MeSH) list from Index Medicus. (see: http://www.nlm.nih.gov/mesh/meshhome.html). Keywords should be given below the Abstract.

Text

The style of writing should conform to acceptable English usage. Do not use slang, medical jargon or unnecessary abbreviations. Accepted spelling is the first choice given in the latest edition of the Macquarie Dictionary.

Wherever possible observational or experimental articles should be divided into sections headed:

- Introduction
- Materials and methods
- Results
- Discussion
- References

For other types of articles such as commentaries, reports and reviews, use an appropriate format or consult the Editors for guidance.

Introduction

Clearly state the purpose of the article; lead the reader from the known to the unknown. Summarise the rationale for the study and state the question to be answered as appropriate. Give only strictly pertinent references, and do not review the subject extensively.

Materials and methods

Present the materials and methods in a logical sequence. Describe the selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Notification of ethics approval must be given where relevant. Identify the methods, apparatus and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Adequately describe new or substantially modified methods. Identify precisely all drugs and chemicals used, including generic name(s), dosage(s), and route(s) of administration. Do not identify patients or hospitals without consent.

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Present the results in the same sequence as given in the Materials and methods; use tables and illustrations where these will help the reader understand the work being presented. Do not repeat in the text all the data in the tables or illustrations.

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Indicate the new and important aspects of the study and emphasise the conclusions that follow. Do not repeat in detail data given in the Results section and do not add new data. Include in the Discussion the implications of the findings and their limitations and compare the observations to other relevant studies. Recommendations may be included if appropriate. Link the conclusions with the goals of the study and answer the experimental question stated in the Introduction. However avoid unqualified statements and conclusions not completely supported by your data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

Acknowledgements

Acknowledge individuals who have made substantial contributions to the study including technical work and financial support. Authors are responsible for obtaining consent from all the individuals acknowledged by name as inclusion may be interpreted as an endorsement of the article’s contents.

References

The AJMS uses a modified Harvard System (author-date system).

Throughout the body of the manuscript cite the author/s name and the publication year in parentheses as in the following examples:
(i) Research in this area (Jones 1999)…

(ii) It has been successfully demonstrated that ...(Smith and Brown 1981; Auteur 1995; Scienziato et al 2007).

(iii) Following further investigation, Wetenschapper (2002) highlighted the difficulties inherent in...

Where there are three or more authors, acknowledge only the first author, e.g. (Smith et al 2007). For two authors the following style should be used (Smith and Brown 2007).

The reference list should be in the format described below journal titles should be abbreviated in Index Medicus format (see: ftp://nlmpubs.nlm.nih.gov/online/journals/ljwweb.pdf) using standard abbreviations from the ISSN List of Title Word Abbreviations (see: http://www.issn.org/en/node/344) All authors should be given in the reference list.

Do not use abstracts as references. “Unpublished observations” and “personal communications” may not be used as references, although references to written, not verbal, communications may be cited (in parentheses) in the text. Include in the references manuscripts accepted but not yet published, designate the journal followed by “in press” (in parentheses). Information from manuscripts submitted but not yet accepted should be cited in the text as “unpublished observations” (in parentheses).

Examples of the correct form for references are given below:

i) Journal Reference:


ii) Personal Author(s) of a book:


iii) Editor, Compiler, Chairman as Author:


iv) Chapter in Book:


v) Online documents:


Tables

Number tables consecutively with Arabic numerals and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in headings. Explain in footnotes all non-standard abbreviations used in each table.

For footnotes, use the following symbols in this sequence:

* † ‡ § ¶ ** ††

In preparing tables, consideration should be given to the page width of the Australian Journal of Medical Science. All tables should be prepared for publication vertically. In the text, cite each table in consecutive order, and mark in the margin of the text its approximate location.

If data from another published or unpublished source is used, written permission must be obtained and a copy must accompany the manuscript.

Illustrations

Colour illustrations may be submitted on a CD. Images should be scanned at a minimum of 300 dpi.

When plotting points, the following symbols are preferred:

In most instances, figures will be reduced to one
column in width. All letters and numbers should be
drawn to be at least 1.5 mm high after reduction,
symbols at least 1.0 mm. Titles for illustrations
belong in the legends for illustrations and not on the
illustrations themselves.

Photomicrographs must have internal scale markers
and the magnification must be stated. Symbols, arrows, or
letters used in the photomicrographs should contrast with
the background.

Cite each figure in the text in consecutive
order, e.g., “Figure 1 illustrates …” or “… as shown
(fig. 2)”. If a figure has been published, acknowledge the
original source and submit with the manuscript written
permission from the copyright holder to reproduce
the material. Permission is required, regardless of
authorship or publisher, except for documents in the
public domain.

Legends for Illustrations

When symbols, arrows, numbers, or letters are used
to identify parts of illustrations, identify and explain each
one in the legends. The figure legend must contain a
boldface (a) name (“Figure” + arabic figure number) and
(b) substantive title.

Abbreviations

Use only standard abbreviations (see list of
commonly used abbreviations, above right).

Avoid abbreviations in the title. The full term for
which an abbreviation stands must precede its first use
in the text unless it is a standard abbreviation for a unit
of measurement.

Report measurements in the units in which
the measurements were made. In most countries the
International System of Units (SI) is standard.

<table>
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</table>

Additional Information

The following are useful sources of information. The
first two publications are used by the AJMS as standard
references.

   Editors. *Scientific style and format: the CBE manual
   for authors, editors, and publishers.* 6th ed. Cambridge

2. Style manual for authors, editors and printers. 6th ed.

3. O’Connor M, Woodford FP. *Writing scientific papers
   in English: an ELSE-Ciba Foundation guide for
   authors.* Amsterdam, Oxford, New York: Elsevier-
   Excerpta Medica, 1975.

4. Day RA. *How to write and publish a scientific paper.*
   Philadelphia, Institute for Scientific Information

5. Zeiger M. *Essentials of writing biomedical research

6. Matthews JR, Matthews RW. *Successful scientific
   writing: a step-by-step guide for the biological and
   medical sciences.* 3rd ed. Cambridge, Cambridge
   University Press, 2007 [Also available in eBook
   format.]
AIMS NZIMLS South Pacific Congress
8 - 12 August 2011
Gold Coast Convention Centre
Queensland

The Australian Institute of Medical Scientists and the New Zealand Institute of Medical Laboratory Science is proud to host and invite you to the South Pacific Congress, 8-12 August 2011. The Congress will bring to the Gold Coast Convention Centre a top level forum of leading national and international speakers to address topical issues in the medical science industry.

Keynote speakers include:
• Dr Barbara Bain (UK), Haematology
• Associate Professor Mark Shephard, PoCT
• Dr Robert Webb, Director, Hyperbaric Medicine Service
• Carol Turnbull (UK), Anatomical Pathology
• Professor Peter Rathjen, Stem Cell Research

The Congress theme ‘Lights! Camera! Action!’ has been chosen as a call to action for delegates to spend a focussed 4½ days in the vibrant Gold Coast at a Congress filled with topical and relevant presentations. Daily sub-themes will logically group presentations and have a little fun based on the Congress theme!
• ‘Waterworld’ – water trauma/diseases
• ‘Basic Instinct’ – back to basics
• ‘Aliens’ – the immune system
• ‘Back to the future’ – new technology
• ‘That’s all folks!’ – closing day

In the spirit of the joint AIMS and NZIMLS South Pacific Congress, the Inaugural Bloodisloe Cup held on the final day of the Congress, will be an event not to be missed!

For further information about the Congress please contact All Occasions Management
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W. www.allocasionsgroup.com/AIMSNZIMLS11
Severe anaemia in a 22-year-old female

ORIGINAL ARTICLE

Three-dimensional reconstruction of free-floating thick whole mount sections displaying microvascular structures in human endometrium using Laser Scanning Confocal Microscopy (LSCM)

ORIGINAL ARTICLE

Performance of Liatest D-dimer assay on Stago Coagulation Analysers using EDTA plasma