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A new approach to minimise the problem of patient to patient contamination in histology

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Abstract

The histology laboratory at Monash Medical Centre undertook an extensive investigation of its work practices (from specimen receipt to reporting of the cut section) with an agenda to eliminate the presence of tissue contaminants on diagnostic slides. Fragments of tissue from other patients can contaminate test slides presenting the pathologists with a diagnostic dilemma. The investigation was conducted in conjunction with senior and principal scientists in the Anatomical Pathology department and the Southern Health Network quality team. The results of the investigation revealed that what is considered normal histological practices could be dramatically improved. In response to the report a number of changes were adopted and implemented to work practices. To evaluate the effectiveness of the changes we examined a total of 200 patient cases (572 slides) for the presence of tissue contaminants on slides for cases performed both before and after the implementation of changes.

Keywords: contaminant, decontamination, H&E

Introduction

Standard work practices in most histology laboratories were developed well over 50 years ago and many still remain with us today. An investigation was conducted by the Anatomical Pathology department of Monash Medical Centre (MMC) and members of the quality team from the governing board, Southern Health Care Network. The investigation revealed that what are considered normal histological practices could be dramatically improved. The aim of the investigation was to completely eliminate the possibility of tissue contaminants. It became evident during the investigation that a number of common histological practices dealing with patient biopsies are based on poor medical practices. A number of changes were subsequently made in the Anatomical Pathology department at MMC as a result of the investigation and these were a combination of better work practices and instrumentation. To test the effectiveness of the changes 100 cases from a period prior to the investigation and another 100 cases from a period one year after the implementation of changes (a total of 572 slides) were screened by two screeners for the presence of any possible contaminants. The results of the study revealed a reduction in the incidence of tissue contaminants both in frequency and size. In the past there have been many papers since which have tried to provide techniques such as polymerase chain reaction as ways of determining whether questionable tissue is indeed foreign (Berg and Murphy 2003, Christie et al 2006). There is however a lack of literature which looks at ways as to how we might (actually) be able to reduce the presence of contaminants. This study identifies possible sources of patient to patient contamination and provides some possible solutions for practical ways to reduce the incidence of tissue contaminants.

Methods

A team of scientists from within the pathology department at MMC undertook an exercise of critically evaluating every step of the processing of a histological sample. The basis of the critical evaluation was to try and identify at which steps the possibility existed for patient to patient tissue contamination. A group from outside pathology with no prior bias of normal histological practices were also recruited to provide an independent assessment; this group were part of the Southern Health organisation quality assurance department.

Initial observations and issues identified

Grossing

The grossing area of histology involves the dissection of tissue samples for the selection of areas of pathological interest. In most histology laboratories it is considered common practice for grossing instruments such as scalpels, forceps and implements for marking margins to be used for the duration of a normal working day. Instruments are cleaned at irregular intervals or when visibly soiled, but unlike other areas of a hospital the sharing of unsterilized instruments between patients is considered to be normal.
MMC had similar work practices but considered the overall cleanliness of the grossing area clean and tidy with instruments cleaned using soapy water and a brush when deemed necessary. At MMC we commonly used biopsy pads for dealing with smaller biopsies or friable tissue. Larger specimens were placed into cassettes with no biopsy pads which had multiple openings (holes) to allow for the transfer of fluids during processing. Observations to current grossing practices included:

- The use of instruments such as forceps and scalpel blades between patients.
- Swabs being placed into dye solutions and re-used.
- Up to 70% of tissue (larger biopsies) being processed in cassettes which contained holes.

The suggested changes for the grossing station included:

- The introduction of single use forceps per patient as is the practice in other areas of the hospital (i.e. operating theatres, clinics).
- Single use of scalpel blades and other disposable instruments such as swabs and gloves.
- The adherence to a clean work surface at all times.

**Processing**

The next step of the histological process is the processing of the biopsy material. This involves the tissue cassettes from multiple patients being placed into a processing instrument. These are taken through a series of graded alcohols and clearing solutions before being infiltrated with paraffin wax. The entire process routinely takes up to 12 hours in a retort containing the cassettes which have solutions pump in and out whilst being under pressure and vacuum. Observations for processing identified:

- Up to 300 individual patients are processed simultaneously in a single retort over a 12 hour cycle of which only 30% contained biopsy pads.

Due to the nature of tissue processing with fluids entering and leaving the retort and agitation during processing it provides the perfect opportunity for the unsupervised transfer of material between closely positioned patient biopsy cassettes. The suggested changes for processing included:

- Individual processing of cassettes.
- Not overfilling of cassettes with material during processing.
- A biopsy pad is used for all cassettes.

**Embedding**

During embedding the patient tissue is placed into a mould with liquid wax, orientated and allowed to harden by cooling on a cold plate. Common embedding practices include the use of one or more forceps which are warmed in heating wells on the embedding centres. A variety of specimen “stompers” are also used to assist in the flat embedding of specimens. It is common practice to reheat and reuse moulds that had been used earlier in the day to embed other patient material. Staff working at these stations are trained to be conscious of patient to patient contaminations and to clean their forceps and the specimen stomper regularly with tissue paper provided. Observations for embedding included:

- Embedding moulds, forceps and specimen stompers shared between different patients. Possibility of tissue sticking and transferring to another patient.
- Forceps placed into heating wells on embedding centres of which some contained fragmented biopsy material.

The suggested changes for embedding included:

- Single use forceps.
- Single use embedding moulds.
- Discontinue use of heating wells on embedding centres and specimen stompers.

**Microtomy**

The microtomy process involves the trimming and sectioning of the embedded biopsy material. Trimming exposes the biopsy tissue which is embedded in the wax, and this process can produce large volumes of wax shaving containing waste biopsy material. Trimmed blocks are then cooled on an ice bath or cold plate prior to sectioning. Sectioning of the cooled wax block produces a thin section of 3-5 microns which is floated out onto a warm water bath and collected onto a glass slide. Occasionally a cold water bath may be used to assist with poorly processed or fatty specimens. Observations for microtomy included:

- Multiple patients trimmed and sectioned at any one time.
- Wax trimmings on and around microtomes during sectioning.
- Residual debris on and around edges of water baths from previous patients.
- Some staff routinely used a cold water bath during sectioning.
- Glass slides placed face up on benches for labelling purposes.

The suggested changes for microtomy included:

- Only one case should be dealt with during any one episode of microtomy.
- The microtome should be cleaned upon the completion of trimming, including material which is likely to remain on the back of the knife holder.
- To limit the use of a cold water bath.
- Complete emptying and refilling of the warm water bath after each cassette is sectioned or to alternatively use a single use water bath for each patient.
- Slides should not be placed flat on the bench during sectioning.
Section staining

Slides are dried in a slide drying oven set to 70°C for a period of 12-15 mins before proceeding to a haematoxylin and eosin stain. All slides at MMC were stained on an automated stainer (Leica Microsystems Autostainer XL) on racks which held 30 patient slides each. The autostainer operates by transferring the racks through a series of 20 different stations each containing 200ml of fluid; this process is commonly referred to as a ‘dip and dunk’ staining method. Up to 500 slides were run through the autostainer daily, with all reagents changed on a daily basis except for the haematoxylin and eosin (H&E) which was changed weekly. Observations for staining of cut sections included:

• The potential for tissue fragments to transfer from one patient slide to another during the staining process.
• Fragments and debris on the bottom of staining baths.

The suggested changes for the staining of cut sections included:

• Increased vigilance surrounding contaminants in staining baths.
• Increased frequency for changing solutions.
• New technology for individual slide staining.

The implementation

The observations and suggested changes were reviewed by the senior staff in Anatomical Pathology. Multiple changes to current work practices were identified as being beneficial in reducing contaminants. Some improvements could be delivered through protocol changes; other improvements were going to require new equipment. With the support of pathology and hospital management new equipment was identified and purchased and protocols were changed.

Grossing

A single use policy was adopted in the grossing area; this required the purchasing of 300 pairs of forceps and an autoclave (Siltex™, Pratika S16, Reverberi. Italy). At the completion of each case the forceps are cleaned and autoclaved before returning to use. Studies within our department showed tissue remaining on the teeth of the forceps is sufficiently destroyed by autoclaving. This is demonstrated in Fig. 1 which shows a fragment of autoclaved tissue. The histology is extensively disrupted and the solid areas of the tissue have an amorphous eosinophilic appearance which would be readily identifiable at the microscopic level. Consumables such as blades, swabs and bench cover which may have come into contact with a patient specimen are disposed of at the completion of each case. Work areas in the grossing area were already considered to be maintained to a clean level, this was continued with an extra level of vigilance and care.

Processing

A consideration for implementing the use of liners on all cassettes had to be given to the tissue processing cycle in our laboratory. MMC uses a Peloris™ tissue processor (Leica Microsystems) incorporating an onboard reagent management system which degrades the solutions using an algorithm for the number of cassettes processed and time. Factors also considered by the algorithm is the amount of solution carry over between stations due to biopsy pads. The overnight program has a default setting of 30% for cassettes having a liner or biopsy pad; with 100% of our cassettes having a biopsy pad or liner the reagent management system had to be adjusted.

Embedding

At embedding we introduced single use of embedding moulds. After use the embedding moulds are cleaned with alcohol and histolene and then autoclaved. Any residual tissue remaining in the mould has the histology...
destroyed by the autoclaving process. We opted not to introduce single use forceps at the embedding area as per the recommendations due to work flow requirements for warm forceps. It was decided that all forceps were to be placed into a Bacti-Cinerator™ to destroy or cauterise any residual contaminants. The use of the warming wells on the embedding centres for forceps was discontinued; heating is now via the Bacti-Cinerator™ only. Tissue stompers used to flatten down larger pieces of tissue were completely removed from the embedding areas; this did not have any impact on the ability to embed tissue flat.

Microtomy

The changes introduced in microtomy were exclusively protocol driven. The technology available for microtomy simply does not address containment or reduction of tissue contaminants. Recommendations taken from the investigation suggested only dealing with a single patient and the complete cleaning of the microtome before starting the next patient. Although well in theory this is not a practical option for a busy histology laboratory. We decided to implement changes which could add a level of confidence to specimen integrity but also allow for few disruptions to normal workflow. Changes included the vacuuming of wax trimmings after batches of blocks have been trimmed and before any patient sections are cut. In addition staff performing sectioning are required to ensure no sections from a previous patient come into contact with the case they are working on and this includes the knife holder on the microtome and the water bath. Once sections are placed onto slides a quick visual inspection is also carried out to ensure the slide is free of any debris.

Slides are not permitted to be placed flat on the bench to combat the possibility of debris falling on or adhering to the back. Changes to work practices include slides remaining in their original packaging until required.

Cold water baths are not used for routine sectioning due to the difficulties in being able to sufficiently clean the surface; they are occasionally used for difficult cases which is generally limited to a single patient. The recommendations for single use or completely emptying and refilling water baths between patients were not considered as a practical option. Changes introduced at this point for reducing possible water bath contaminants included a more rigorous level of cleaning and surveillance for floaters between patient/specimens. Cleaning involves the use of a lint free tissue to clean the surface and surround of the water bath. Each bath is emptied and cleaned each day and they are bleached on a weekly basis.

Staining

The use of ‘dip and dunk’ H&E stainers are common place in histology and the evidence of contaminants associated with the use of them is well known with ‘tissue floaters and contaminants throughout the staining baths’ (Platt et al 2009, Dark Daily 2011). Literature also suggests that the molecular assessment of these floaters can be quite difficult as the “DNA may be altered by the staining process” (Platt et al 2009). The decision was made to implement new technology into our laboratory; our requirement was for a staining instrument which could stain patient slides without the use of communal reagents. The instrument we purchased was the Roche Ventana Symphony™ H&E staining instrument. The Symphony™ utilizes a single slide staining technology which applies fresh reagent to each patient slide for each step of the H&E staining process. This eliminates the traditional staining baths and the possibility of patient slides sharing common solutions during the staining process. The implementation of the Symphony™ involved a complete installation and validation of the instrument, but the assurance it offered for reducing contaminants could not be demonstrated by any other instrument on the market at the time.

Testing the effectiveness of changes

We reviewed a total of 200 cases to test the effectiveness of the combined changes in the histology department at Monash Medical Centre. The slides were screened by two screeners for fragments of tissue which were greater than five cells in size with a recognisable histology. The contaminants were recorded as being within 3mm or greater from the diagnostic tissue. The distance of the contaminant from the diagnostic tissue relates to likelihood that a contaminant is seen at the time of reporting. Slides which had a contaminant both within and greater than 3mm were counted as being within 3mm. Red blood cells and single scattered squamous cells were not included in this study. The initial slide series of 100 cases were from a period before any of the changes were implemented into our department and this series was stained on an automated staining instrument (Leica Autostainer XL). The second slide series of an additional 100 cases were from a period three months after the implementation of all changes. These were stained on the Ventana Symphony™ H&E staining instrument. Both slide series were processed on a Peloris (Leica, Microsystems), embedded using either a Medite TES-99™ or Tissue-Tek III™ embedding centre and sectioned on the same microtomes (Reichert-Jung 2040, Leica RM2245, Microm HM340).

Results

572 slides were screened for the presence of tissue contaminants comprising 282 slides prior to the protocol changes and new equipment and a further 290 slides post implementation of changes.

The two series screened show a comparable number of blocks and slides and are considered to be of similar complexity. The results for the pre-implementation slides demonstrated a presentation of foreign material to be present on 30 (10.6%) of the H&E slides within 3mm of the diagnostic tissue, with an additional 11 (3.9%) of
the slides having material greater than 3mm. The figures we recorded are higher than other similar reviews which show contaminants closer to 2.9% (Gephardt et al. 1996). A possible explanation might include that our survey included fragments which were folded or otherwise readily recognisable as contaminants. These were included to provide a real recording of the presence of contaminant material, not just potential sources of misdiagnosis.

The second series of slides screened showed a reduction in the frequency and presentation of contaminants. A total of 290 slides were screened of which only 11 (3.8%) had a contaminant within 3mm of the diagnostic tissue and a further 8 (2.75%) had a contaminant more than 3mm from the diagnostic tissue. The presentations of the contaminants in this series were notably smaller and more recognisable as foreign and less likely to be mistaken for patient biopsy material.

The category of ‘contaminants within 3mm of the diagnostic tissue’ has more potential for diagnostic errors leading to a possible false positive result as these contaminants are in the field of view for the patient biopsy. Results from this study show a reduction down from 10.6% to 3.8%, with the second series not presenting as sheets of tissue as seen in the first series.

Some examples of the contaminants from the first series can be seen in Figs 2 and 3.

Figure 2 is a breast core biopsy showing an invasive ductal cell carcinoma; directly adjacent to the breast tissue is a fragment of squamous material most likely to have originated from a skin biopsy. The skin is only present on one out of five histology sections used to diagnose this case which would suggest its origins stems from either the water bath or the H&E stainer. The staining intensity of the skin fragment is also darker than the breast core which might suggest a stain bath contaminant.

Figure 3 is a torted ovary which contained a simple cyst; adjacent to the serosal surface of the ovary is a contaminant which has cells with an epithelium with columnar differentiation. This presentation is obviously a contaminant due to the folded and poor presentation of the contaminant but if the columnar epithelium was better presented it could imply that the patient may have a perforated bowel.

![Figure 3. Simple ovarian cyst with contaminant cells showing columnar differentiation. Magnification x40](image)

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Number of cases</th>
<th>Number of cassettes</th>
<th>Number of slides</th>
<th>Contaminants within 3mm of diagnostic tissue</th>
<th>Contaminants greater than 3mm from diagnostic tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-changes</td>
<td>100</td>
<td>252</td>
<td>282</td>
<td>30</td>
<td>10.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Post-changes</td>
<td>100</td>
<td>248</td>
<td>290</td>
<td>3.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

Discussion

Contaminants on diagnostic H&E slides are a worldwide problem which has been recognised for many years. The main issue with contaminants is not the ability but the inability to identify what is foreign. Tissue which makes its way into another patient’s biopsy cassette before or during the embedding process is extremely hard to identify as foreign especially if from the same tissue origin. Embedded contaminants will appear on all sections and unless other warning signs are present, such as the result being an unexpected finding, then the pathology diagnosis can lead to an incorrect treatment for the patient potentially resulting in a life threatening situation or medico-legal action. The process which we undertook at MMC was aimed at directly investigating potential sources of contamination; to look at the way in which histology is traditionally performed and to then implement new methodologies which we were aware might require a different mindset to how we are trained to think. We wanted the new methodologies to be similar to best practices which might be employed in other areas of the hospital (such as the operating theatres) and it was after observing the practices in these areas, that we decided the sharing of instruments between cases was to be eliminated at all cost. Introducing single use forceps in the cut up area was and probably still is unheard of in other histology laboratories, but this practice adds certainty to cassettes before the embedding process. Some practices put
into use such as the lining of biopsy cassettes are relatively inexpensive but potentially have a dramatic impact on the ability for tissue to transfer between cassettes during processing. To introduce these changes took some time and convincing of the staff required at the grossing stations but ultimately became part of normal practice. Practices such as flaming of forceps at embedding and a high level of vigilance at microtomy are equally as inexpensive. The major changes introduced into the laboratory such as the acquisition of an autoclave and Symphony™ stainer provided us with the ability to have confidence in reusable equipment (forceps, embedding moulds) and the staining platform. The results of the retrospective screening of 200 cases for the presence of contaminants demonstrated an appreciable difference in both the frequency and presentation of contaminants on our diagnostic slides dropping from 10.6% to 3.8%. This result is a combination of all the noted changes from specimen receipt to H&E and unfortunately it cannot be ascertained as to which is the most effective component; however the aim of our investigation was not to identify the most effective but the aim of our investigation was not to identify the most effective but to minimise the potential for contamination.

References


Dark Daily Laboratory and Pathology News. Online at Dark-daily.com under White papers. Risk of misdiagnosis due to tissue contamination may be higher for certain specimen types. Accessed February 11th 2012.


The work in this article was originally submitted as an AIMS Fellowship Dissertation
The Immature Platelet Fraction: an assessment of its application to a routine clinical laboratory

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Abstract

The Sysmex XE-2100 and XE-5000 automated haematology analyzers (Sysmex, Kobe, Japan) offer an optional parameter called the Immature Platelet Fraction (IPF), which can be quickly and easily performed as a specifically ordered or reflex test for the investigation of thrombocytopenia. The purpose of this study was to assess its value for everyday use in a routine clinical laboratory servicing community collection centres, hospitals and specialist clinics. The reference interval was established on 114 normal patients as 0.7-5.5% with a mean of 2.0%, median of 1.6%, and standard deviation of 1.2%. Stability studies performed showed that specimens should be analyzed within 8h of collection and stored at room temperature, with no significant difference between storage at room temperature or 4°C within the 8h time frame. Reproducibility on patient samples was dependent on the IPF% range, with the highest coefficient of variation (CV) found for the lowest numerical values. The average CV was 19%. A review of 4060 routine samples showed that when tested within 8h of collection, 1.7% of patients with a normal platelet count had a raised IPF%, compared to 30.9% in the thrombocytopenic population. In patients undergoing bone marrow biopsy, the highest IPF% values were found in those with normal to increased megakaryocyte numbers or with dysplastic morphology. As a direct measure of peripheral platelet destruction, this study found it has suitable applications in a central haematology laboratory.

Keywords: immature platelet fraction, reticulated platelets, thrombocytopenia, immune thrombocytopenia, Sysmex

Introduction

Ingram and Coopersmith (1969) demonstrated that newly formed platelets released into the circulation after blood loss contained residual amounts of megakaryocyte-derived RNA. Several authors over the last decade have clearly shown that these circulating reticulated platelets correlate directly with thrombopoiesis (Ault et al. 1992, Rinder et al. 1993, Richards and Baglin 1995, O’Malley et al. 1996, Kurata et al. 2001).

More recently, the Sysmex XE-2100 and XE-5000 analyzers have offered the IPF as an automated parameter to quantitate reticulated platelets. The IPF is therefore a marker of the rate of thrombopoiesis analogous to the reticulocyte count for erythropoiesis and it comes as a standard test on the XE-5000 but requires loading of specific IPF Master Software on the XE-2100. Pons et al. (2010) reported good correlation between the IPF% and reticulated platelets (r=0.65) in thrombocytopenia caused by peripheral destruction.

The measurement of the IPF# or IPF% has been demonstrated by several groups to be of value in the assessment of thrombopoietic activity, aiding in the differential diagnosis of thrombocytopenia (Kurata et al. 2001, Briggs et al. 2004, Kickler 2006, Abe et al. 2006). The IPF% is a measure of platelet destruction, whereas the IPF# reflects platelet production. A high IPF% is indicative of platelet turnover arising from increased destruction, consumption or recovery from thrombocytopenia, such as in immune thrombocytopenia (ITP), thrombotic thrombocytopenic purpura (TTP) and disseminated intravascular coagulation (DIC). A low IPF% is seen in hypoproliferative states, such as marrow suppression or damage (Briggs et al. 2004, Abe et al. 2010). Conversely the mean absolute IPF (IPF#) was reported by Barsam et al. (2011) to be lower in ITP (3.2 x 10^9/L) than for healthy controls (7.8 x 10^9/L). Current applications of the IPF% are listed in Table 1.

The purpose of this study was to evaluate the application of the IPF % in a routine testing facility. This involved a four-fold approach: (i) the determination of reproducibility and stability (ii) the establishment of a reference interval (iii) a retrospective study of patients routinely tested for automated reticulocyte counts thereby also measuring the IPF parameters) (iv) a review of bone marrow episodes routinely tested for reticulocyte counts.

Literature values for reference intervals

Table 2 lists the variation in reported values for the IPF% reference interval (RI). The mean IPF in normal neonates was 4.1+/- 1.3% (Cremer et al. 2009). This study...
Materials and Methods

Measurement of IPF

In this study, XE IPF MASTER Software was loaded onto a Sysmex XE-2100 in our central haematology laboratory for specimen analysis. The IPF% is measured simultaneously with the optical platelet count (PLT-O) utilizing fluorescent flow cytometry with a semi-conductor diode laser system. Platelets are stained by two fluorescent dyes (polymethine and oxazine) present in the RET–SEARCH (II) reagent in the RETIC channel during a fixed temperature-controlled incubation. As the stained cells pass through a semi-conductor diode laser beam, the resulting forward light scatter (cell volume) and fluorescent intensity (RNA content) are measured. A platelet scattergram is produced by ACAS (advanced cluster analysis system) and discriminates the mature platelets from the immature fraction by the intensity of forward light scatter (cell size) and fluorescence (RNA content). Mature platelets are represented as blue dots and immature platelets which have larger volume and higher fluorescent intensity appear as green dots. The IPF MASTER Software differentiates the immature from the mature platelet fraction, and expresses the IPF as a percentage of reticulated platelets of the total optical platelet count. An absolute value (IPF#) can also be reported (IPF% x optical platelet count).

Table 1. Current applications of the IPF%

<table>
<thead>
<tr>
<th>Applications of the IPF%</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prediction of DIC mortality and morbidity</td>
<td>Hong et al (2009)</td>
</tr>
</tbody>
</table>

Table 2. Examples of normal reference intervals for IPF% reported by various authors

<table>
<thead>
<tr>
<th>RI</th>
<th>Mean (male)</th>
<th>Population size</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 - 3.2% (male)</td>
<td>1.3 (male)</td>
<td>2039</td>
<td>Jung et al (2010)</td>
</tr>
<tr>
<td>0.4 - 3.0% (female)</td>
<td>1.1% (female)</td>
<td>14</td>
<td>Pons et al (2010)</td>
</tr>
<tr>
<td>1.7 - 3.4%</td>
<td>2.6%</td>
<td>50</td>
<td>Briggs et al (2004)</td>
</tr>
<tr>
<td>1.1 - 6.1%</td>
<td>3.4%</td>
<td>80</td>
<td>Kickler et al (2006)</td>
</tr>
<tr>
<td>2.8 - 3.5%</td>
<td>3.1%</td>
<td>82</td>
<td>Abe et al (2006)</td>
</tr>
<tr>
<td>1.0 - 7.7%</td>
<td>3.3%</td>
<td>100</td>
<td>Yamaoka et al (2010)</td>
</tr>
<tr>
<td>0.5 - 5.7%</td>
<td>2.0+/- 1.1%</td>
<td>142</td>
<td>Yong Gon Cho et al (2007)</td>
</tr>
<tr>
<td>0.7 - 7.3</td>
<td>3.0+/- 1.4%</td>
<td>52</td>
<td>Cannavo et al (2010)</td>
</tr>
<tr>
<td>0.4 - 5.4%</td>
<td>1.7%</td>
<td>114</td>
<td>Sinclair (2011)</td>
</tr>
<tr>
<td>1.0 - 4.5%</td>
<td>2.2%</td>
<td>80</td>
<td>8+/- 1.5%</td>
</tr>
<tr>
<td>0.7 - 5.5%</td>
<td>2.0%</td>
<td>100</td>
<td>142</td>
</tr>
</tbody>
</table>

IPF% immature platelet fraction, percentage of the total optical platelet count.
Briggs et al (2006) reported that the value of the IPF# as opposed to the percentage count is limited by the low values that are inevitable when the platelet count is low, even if the IPF% is high. The Sysmex XE-2100 has a switching algorithm to determine the most reliable platelet count by either optical or impedance technology, for example in conditions with large platelets or small or fragmented red cells. This study used the IPF% rather than the IPF#.

Quality control (QC)

Sysmex supply three levels of internal QC material which were run daily. However there are significant limitations with these controls because the levels do not have a wide range of values across the reportable range. They are essentially the same target means (21.5, 20.0, and 20.7%) and therefore there is no control with values within the reference interval, the low or extremely high values.

Reproducibility

Between run imprecision was determined by performing 10 repeat analyses of each of 10 samples over a range of different IPF% and platelet counts. Within run imprecision was calculated by running two normal and two thrombocytopenic patients each 10 times.

Stability studies

Temperature and time of storage affect the stability of the IPF%. The IPF was initially reported to be stable over two days at room temperature (Briggs et al 2004) but following studies used 2-4h within collection as criteria (Briggs et al 2006). Other authors have reported between 3-24h at room temperature (RT) as the cut-off (Pons et al 2010, Ruisi et al 2010, Yamaoka et al 2010). A significant rise in the mean IPF% was found after 12 h with a steady increase up until 24h (Osei-Bimpong 2009) and the use of an algorithm was proposed to correct the IPF % for specimens stored at 4 °C up to 24h (Osei-Bimpong 2009) and the use of an algorithm was proposed to correct the IPF % for specimens stored at 4 °C up to 24h to extend the utility of this parameter. The IPF% minus 1.34 times the number of hours of storage was proposed to correct the IPF % for specimens stored at 4 °C up to 24h (Osei-Bimpong 2009) and the use of an algorithm was proposed to correct the IPF % for specimens stored at 4 °C up to 24h to extend the utility of this parameter.

For this study, 16 peripheral blood samples with platelet counts ranging from 24-320 x 10⁹/L were collected into K₂EDTA (dipotassium ethylene diamine tetra acetate acid) manufactured by Becton Dickinson. The effect of storage temperature was evaluated by storing duplicate samples at room temperature (RT) and 4 °C and measuring the IPF% on each aliquot. Time of storage was evaluated for short term stability at T0 to T8, where T0 samples were analyzed less than 4h after collection, and T8 at 8h after collection. Longer stability was assessed by analyzing samples at T0, (baseline) T8 (8h), T24 (24h) and T48 (48h). A comparison of the two groups of data obtained for storage at RT vs 4 °C was conducted using one way paired ANOVA and F statistic. Values of p <0.05 was considered statistically different (95% confidence). The Tukey result was within 95% confidence interval range of -1.51 to 1.10.

Reference interval studies

The determination of the RI may be a costly and onerous task, and encompasses rigorous steps from identification and selection of reference individuals, specimen collection, exact analytical methods, data collection and analysis. Another approach is to use transference, where one may adapt a previously established RI to a new location. The CLSI guidelines state that transference of the RI between laboratories is acceptable provided there is comparability between the analytical system and the reference population (CLSI 2008, Section 10). However given the spread of RIs published and the variation in acceptable storage times, it was decided that transference was not applicable for this parameter.

An indirect sampling approach was used in this study for the determination of the RI based on the assumption as stated in CLSI 2008 (Section 7.4.2) that results for most individuals presenting for routine screening tests in the general population appear normal. Important considerations include an adequate sample size, the selection of “reference individuals” and the avoidance of pre-analytical variables.

K₂EDTA specimens were collected from 114 apparently healthy male and female patients over 18 years of age, presenting to our community based collection rooms for routine testing. Platelet counts for this reference group ranged from 159-398 x 10⁹/L. A normality test was conducted to determine if the RI data had a Gaussian distribution, then a non-parametric and a parametric log transformation method was applied, using the software program, Analyse-it® (Analyse-it Software, Ltd). All specimens were run within 8 h of collection. All individual sets of results considered for the RI showed normal haematological parameters, (haemoglobin, white cell count, platelet count) and relatively normal chemistry profiles. There was no evidence of platelet clumping or cold agglutinins.

Patient studies

The first group studied consisted of 4060 patient samples for routine full blood count (FBC) and reticulocyte counts. This data was collected on three separate two week intervals over a period of three months. The clinical condition was reviewed from clinicians’ notes and patient history. In the second group, the IPF% was measured on 50 patients undergoing bone marrow biopsy, and correlations between their IPF%, diagnosis, platelet count, megakaryocyte numbers and megakaryocyte numbers were performed.
Results

Reproducibility/imprecision of IPF%

The CV of all samples tested ranged between 9-36% (average CV=19%). Between run and within run reproducibility were 11-36% and 9-23% respectively (Table 3). In general the highest CVs were found for the lowest values of IPF% (CV=22% within the RI, CV=13% for elevated IPF%). The standard deviation (SD) ranged from 0.3 – 1.5 (between run) and 0.2-0.3 (within run). The three levels of internal QC (L1, L2, L3) showed SDs between 1.09-1.14 and CVs ranging from 5.0-5.6%.

Stability

Effect of temperature

Figure 1 shows there was no significant difference in IPF% values for samples stored for up to 4h at RT (p=0.2835) and no significant difference between storage at RT or 4 °C up to 8h (p = 0.694, Tukey contrast difference -0.25).

Effect of storage time

Short term stability was determined over an 8 h period (Fig. 2) and the data showed there was no significant difference in the IPF% value between 0 and 8h (p=0.249). This validated the findings of Jung et al (2010) in that the IPF% remains stable when blood samples are stored at RT over an 8h period. Long term stability over a 48h period showed significant increase in the IPF% over time. There was a significant difference between results obtained at 8h compared with 24 and 48h (p= 0.0038 and p=0.0028 respectively). With an IPF% cut off point of 5.5%, approximately half of the patients tested would have been incorrectly interpreted as abnormally high if samples were tested between 24 and 48h. The recommendation from this
Data is that specimens should be tested within 8h of collection and stored at room temperature.

**Table 3. Reproducibility of the IPF%* (patient testing)**

<table>
<thead>
<tr>
<th></th>
<th>Mean IPF%</th>
<th>SD</th>
<th>CV%</th>
<th>Platelet count</th>
</tr>
</thead>
<tbody>
<tr>
<td>3(a) Between run imprecision (n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>0.3</td>
<td>23</td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>1.5</td>
<td>0.4</td>
<td>31</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>0.6</td>
<td>36</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>1.9</td>
<td>0.3</td>
<td>15</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>2.9</td>
<td>0.4</td>
<td>15</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>0.4</td>
<td>12</td>
<td>304</td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td>1.5</td>
<td>28</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>6.6</td>
<td>0.7</td>
<td>11</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>7.8</td>
<td>1.1</td>
<td>14</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.2</td>
<td>14</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean IPF%</th>
<th>SD</th>
<th>CV%</th>
<th>Platelet count</th>
</tr>
</thead>
<tbody>
<tr>
<td>3(b) within run imprecision (n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>0.3</td>
<td>23</td>
<td></td>
<td>270</td>
</tr>
<tr>
<td>1.7</td>
<td>0.2</td>
<td>9</td>
<td>217</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>0.3</td>
<td>23</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>2.9</td>
<td>0.3</td>
<td>15</td>
<td>94</td>
<td></td>
</tr>
</tbody>
</table>

*IPF% immature platelet fraction, percentage of the total optical platelet count.

**Figure 2. Effect of storage time on the IPF%* A. IPF% of 8 individual specimens at RT, 24h B. IPF% of 8 individual specimens at RT, 48h C. IPF% of 8 individual samples at RT, 8h D. IPF% of 8 individual samples at 4 °C, 8h *immature platelet fraction percentage

Reference interval (healthy individuals)

114 specimens were analyzed and converted into a histogram which suggested a non-Gaussian distribution. When analyzed non-parametrically with the Kolmogorov-Smirnov test, the IPF% RI was 0.7 – 5.5 %, with a mean of 2.0% and median of 1.6%. The data is skewed towards values of <2.75%, therefore the exclusion of outliers needs to be performed with caution.

Patient studies

Routine patient samples

IPF% results were generated from the 4060 routine samples (Table 4). Under ideal storage conditions of <8h from collection, 4% of the total population showed an elevated IPF%, and thrombocytopenic patients were more likely to have a higher IPF% than normal patients (30.9% vs. 1.7% respectively). Platelet clumping and cold agglutinins were shown to cause an increase in IPF%; these were excluded from the data.
Table 4. IPF% *results in the screening population.

<table>
<thead>
<tr>
<th>Platelet count</th>
<th>Storage</th>
<th>IPF%</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>&lt;24 h</td>
<td>normal</td>
<td>93.1%</td>
</tr>
<tr>
<td></td>
<td>&lt;8 h</td>
<td>high</td>
<td>1.7%</td>
</tr>
<tr>
<td></td>
<td>&gt;8 h</td>
<td>high</td>
<td>5.2%</td>
</tr>
<tr>
<td>low</td>
<td>&lt;24 h</td>
<td>normal</td>
<td>27.8%</td>
</tr>
<tr>
<td></td>
<td>&lt;8 h</td>
<td>high</td>
<td>30.9%</td>
</tr>
<tr>
<td></td>
<td>&gt;8 h</td>
<td>high</td>
<td>41.2%</td>
</tr>
</tbody>
</table>

Summary of the pattern of results in the screening population (4060 patients), comparing the IPF% with platelet count and storage. *immature platelet fraction percentage

Thrombocytopenic patients who had a specific diagnosis documented in their clinical history were further reviewed. Small numbers for each disease category were available, and more studies would be required for results to be statistically significant. However a number of interesting observations were made. The highest average IPF% (33.0%) was found in the two patients diagnosed with primary myelofibrosis. The presence of large dysplastic platelets or megakaryocyte fragments may contribute to the raised IPF% in this condition. A group of ITP patients (n=6, comprising 15 total episodes) had the second highest average IPF% of 14.9%. In the myelodysplastic syndrome (MDS), the average IPF% was 12.5% (n=10, comprising 27 total episodes). The lowest results values were seen in patients with renal disease (n=7) and pancytopenia (n=10) with values of 2.6% and 2.7% respectively. In the acute leukaemia group (n=13), the three highest IPF% values were seen in the presence of myelodysplasia-related changes, an aberrant T cell population or a bi-lineage phenotype.

Several patients were tracked periodically and showed a corresponding increase in IPF% as the PLT-O fell (Fig. 3). Patient A was diagnosed with myelofibrosis with no evidence of transformation to acute leukaemia and showed a high average IPF% (44%) which rose in response to a gradual fall in PLT-O. Patient B diagnosed with acute myeloid leukaemia with maturation with a complex karyotype initially showed a high IPF% of 18% which fell to 6.3% over 17 days, indicating a decrease in platelet turnover accompanying the rise in platelet count.
Patient C was diagnosed with aplastic anaemia by bone marrow examination, was pancytopenic and had no evidence of paroxysmal nocturnal haemoglobinuria by flow cytometry. Over a 48 day period, the PLT-O fell by 86% with a rise in IPF of 70% (mean IPF% was 9.3%). Patient D had a splenectomy performed for chronic ITP and was followed for 11 days, with a 72% fall in PLT-O and a corresponding 43% increase in IPF%. The mean IPF% was 17% with a peak of 23.5% at the lowest PLT-O level. Patients E and F were diagnosed with MDS and both showed similar results, with the IPF% at its highest at the lowest platelet count. The mean IPF% values were 17.4% and 19.2% tracked over 40 days and 26 days respectively.

**Bone marrow patients**

Of the 50 patients undergoing bone marrow biopsy, the 12 patients found to have an elevated IPF% and/or thrombocytopenia were evaluated for their diagnosis, karyotype, megakaryocyte number and morphology. There was no direct correlation between IPF% and megakaryocytic parameters but those with normal to increased megakaryocytes and/or dysplastic morphology tended to have the highest IPF% values (8.7-41.2%) (Table 5).

**Table 5.** Results of bone marrow biopsies of patients with thrombocytopenia and/or elevated IPF%.

<table>
<thead>
<tr>
<th>IPF%</th>
<th>Platelet count</th>
<th>Megakaryocyte number</th>
<th>Megakaryocyte morphology</th>
<th>Diagnosis*</th>
<th>Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>111</td>
<td>Normal</td>
<td>some dysplastic forms</td>
<td>RCMD</td>
<td>NK†</td>
</tr>
<tr>
<td>4.5</td>
<td>80</td>
<td>Moderate decrease</td>
<td>Normal</td>
<td>RCMD , B-CLL</td>
<td>NK</td>
</tr>
<tr>
<td>6.7</td>
<td>35</td>
<td>Normal</td>
<td>Normal</td>
<td>AML post allograft</td>
<td>NK</td>
</tr>
<tr>
<td>7.6</td>
<td>155</td>
<td>Mild decrease</td>
<td>Normal</td>
<td>Myeloma - post stem cell collection</td>
<td>NK</td>
</tr>
<tr>
<td>8.1</td>
<td>77</td>
<td>Moderate decrease</td>
<td>Normal</td>
<td>CML</td>
<td>NK, previous BCR/ABL1</td>
</tr>
<tr>
<td>8.3</td>
<td>57</td>
<td>Mild-moderate decrease</td>
<td>Normal</td>
<td>AML with MDS related changes, monoclonal plasma cell dyscrasia</td>
<td>NK</td>
</tr>
<tr>
<td>8.4</td>
<td>47</td>
<td>Moderate increase</td>
<td>Normal</td>
<td>B-NHL, adequate mega consistent with peripheral destruction, sequestration</td>
<td>NK</td>
</tr>
<tr>
<td>8.7</td>
<td>112</td>
<td>Moderate increase</td>
<td>Marked atypia</td>
<td>PMF</td>
<td>Trisomy 9</td>
</tr>
<tr>
<td>8.9</td>
<td>115</td>
<td>Mild increase</td>
<td>Normal</td>
<td>MDS-U , MGUS</td>
<td>NK</td>
</tr>
<tr>
<td>10.7</td>
<td>67</td>
<td>Mild increase</td>
<td>Large dysplastic forms</td>
<td>MDS - RCMD</td>
<td>NK</td>
</tr>
<tr>
<td>13.2</td>
<td>11</td>
<td>Mild increase</td>
<td>Normal</td>
<td>ITP</td>
<td>NK</td>
</tr>
<tr>
<td>41.2</td>
<td>72</td>
<td>Normal</td>
<td>Dysplastic, micro megakaryocytes</td>
<td>t-AML, aberrant T cell pop,</td>
<td>Monosomy 7</td>
</tr>
</tbody>
</table>

*R CMD: Refractory anaemia with multi-lineage dysplasia; B-CLL: B-Chronic lymphatic leukaemia; CML: Chronic myeloid leukaemia; AML: Acute myeloid leukemia; B-NHL: B-Non Hodgkin lymphoma; PMF: Primary myelofibrosis; MDS-U: Myelodysplastic syndrome-unclassified; MGUS: Monoclonal gammopathy of undetermined significance. † NK: normal karyotype

The diagnosis of thrombocytopenia is often one of exclusion and it is clinically useful to know whether hyperdestruction or hypoproduction is more dominant. The tests available have shortcomings and are indirect markers of platelet lifespan and production. The differential diagnosis of ITP offers particular challenges, as there is no reliable gold standard test to diagnose ITP from other thrombocytopenias.

The establishment of a stability cut-off point was a significant aspect of this study, as all specimens analyzed in our main haematology department have been collected off site, transported, received and processed through our specimen reception area before distribution and testing. A test that requires priority transport and handling is
logistically unattractive. An 8h window is feasible; however elevated results when the specimen is older than this should be interpreted with caution. In general, the IPF% increases with time up to 48h. Presumably the balance between platelet swelling and RNA degradation with time is weighted towards the former.

Several laboratories performing this test appear to have adopted the IPF% RI of 1.1-6.1% as originally reported by Briggs et al (2004). The values for the normal reference interval established in this study (0.7-5.5%) and median IPF% of 2.0% were in keeping with other authors’ published data, in particular with Takami et al (2007). However the upper limit of 5.5% was higher than the cut-off of 3.0-3.2% (male vs female) reported by Jung et al (2010), using a similar stability criteria of less than 8h post-collection. Our study did not evaluate RIs by gender.

A review of 4060 routine samples showed that a raised IPF% when collected within 8h was more commonly found in thrombocytopenic patients than those with a normal PLT-O. Further studies with larger patient numbers would be required to establish defined sensitivity and specificity levels for various clinical conditions. The tracking of specific patients during their course of treatment has the potential application for monitoring the effectiveness of their treatment, and could benefit referring doctors if the laboratory information system could report this parameter graphically.

In this study of 50 bone marrow specimens, the IPF% did not show significant correlation with megakaryocyte parameters, therefore its value in this setting appears to be supportive rather than discriminatory. This may also be related to sampling and distribution variations in bone marrow examination and the subjective nature of reporting, rather than a reflection on the IPF%. Larger numbers would need to be studied to establish the value of the IPF% in routine reporting of bone marrow biopsies.

The IPF% is only offered by one manufacturer as an extended haematology parameter and requires specialized technology and software. The Sysmex IPF software cannot be adapted to the smaller XT-1800i or XT-2000i analyzers in our hospital laboratories where a large population of haematology and oncology specimens are tested.

Internal quality control (IQC) material is available but no external quality assurance (EQA) program exists, and given the limited availability of this test, it is unlikely that this will be given priority unless other manufacturers offer this test. The IQC could be improved by extending the range to include normal results and around the decision points of the 5-10% range. In the meantime, the establishment of an exchange program to produce inter-laboratory comparisons would be beneficial.

The lack of standardization and the stability of this parameter may also cause limitations for its clinical application. All of these factors necessitate the establishment of an individual RI for each laboratory. A recommendation from this study is that all results be reported with a comment to support clinicians in the interpretation and relevance of the result, including both artefactual and clinical causes of an elevated IPF%.

**Conclusion**

The purpose of this study was to assess the suitability of the IPF% as a routine test. The reticulocyte count is a recognized routine measure of erythropoiesis, and it would seem logical that the IPF% is also valuable as a marker of thrombopoiesis, yet it has not become a mainstream test. The restricted availability of the IPF% is its greatest limitation. To be a useful parameter, the stability needs to be robust enough to allow for collection and transport to the central testing laboratory within a reasonable time period. This study has shown it can easily be incorporated into routine testing, either as a pre-ordered test, or as a reflex test for investigation of thrombocytopenia within the recommended testing time of within 8h of collection. Any laboratory with this capability should consider offering the IPF%; however it would be most beneficial to haematology and oncology specialists.

**Acknowledgements**

Craig Williams, Haematology Department, Sullivan Nicolaides Pathology, for assistance with the testing and provision of data.

Dr Bev Rowbotham, Haematology Department, Sullivan Nicolaides Pathology, for mentorship.

Robert Flatman, Biochemistry Departmenr, Sullivan Nicolaides Pathology, for assistance with statistical analysis.

**References**


of an immature platelet fraction (IPF) in peripheral thrombocyto-


Garibaldi B, Malani R, Yeh HC, Lipson E, Michell D, Bennett M, Moliterno A, McDevitt MA, Kickler TS. 2009. Estimating plate-

Goncalo AP, Barbosa IL, Campilho F, Campos A, Mendes C. 2011. Predictive value of immature reticulocyte and platelet frac-


Jung H, Jeon HK, Kim HJ, Kim SH. 2010. Immature platelet frac-

Karpattkin S. 1978. Heterogeneity of human platelets. VI. Correla-


Kraytman M. 1973. Platelet size in thrombocytopenias and throm-
bocytosis of various origin. Blood 41: 587-598.

lets, plasma glycoplacin, and thrombopoietin levels for discriminat-


Pons I, Monteagudo M, Lucchetti G, Munoz L, Perea G, Colomina I, Guir J, Obiols J. 2010. Correlation between immature platelet fraction and reticulated platelets. Usefulness in the etiology diagno-


An 82-year-old male presented at the Haematology Clinic for investigation of erythematous cutaneous lesions over his trunk and legs. A full blood count was performed.

The results were as follows:

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>122 g/L</td>
<td>130 - 180 g/L</td>
</tr>
<tr>
<td>Hct</td>
<td>0.353</td>
<td>0.40 - 0.54</td>
</tr>
<tr>
<td>MCV</td>
<td>81.5 fl</td>
<td>80 - 100 fl</td>
</tr>
<tr>
<td>MCH</td>
<td>28.2 pg</td>
<td>26.5 - 33.0 pg</td>
</tr>
<tr>
<td>WBC</td>
<td>2.4 x 10^9/L</td>
<td>3.5 - 11.0 x 10^9/L</td>
</tr>
<tr>
<td>Plats</td>
<td>170 x 10^9/L</td>
<td>150 - 400 x 10^9/L</td>
</tr>
</tbody>
</table>

The WBC showed a leucopenia with a neutropenia of 1.3 x 10^9/L and lymphopenia of 1.0 x 10^9/L.

A bone marrow aspirate and trephine were performed. The bone marrow was hypercellular with a large population (77%) of abnormal, primitive cells resembling blast cells. These cells had a variable amount of cytoplasm, mostly scanty, with elongated irregularly shaped nuclei; many of the nuclei contained small nucleoli. Erythropoiesis and megakaryopoiesis were mildly dysplastic; granulopoiesis was normal. The trephine was also hypercellular, especially for age. There was an infiltrate of monomorphic medium sized abnormal cells with irregular nuclei, fine chromatin pattern and one to several small nucleoli. The cytoplasm was scanty, grey-blue and agranular. Immunophenotyping was performed on the marrow aspirate with the following results:

CD45+/HLA-DR+/CD4+/CD11c+/CD38+/CD56+/CD123+

Immunohochemistry was performed on the trephine with the following results:

CD4+ and CD56+ were both strongly positive. CD3/CD20/CD68 and MPO were all negative.

Cytogenetics revealed a normal male karyotype with no abnormality detected. A diagnosis of blastic plasmacytoid dendritic cell neoplasm (BPDC) was made on this patient.
BPDC is a highly aggressive neoplasm. It occurs more commonly in males with a male to female ratio of 3.3:1. The patients are usually elderly, between the age of 61 and 70 years however it can also occur in children. It is characterised clinically by a single or multiple skin lesions together with a lymphadenopathy. The peripheral blood and bone marrow may be involved at diagnosis. The peripheral blood is characterised by cytopenias, especially thrombocytopenia.

The bone marrow is characterised by a monomorphic population of blast cells with scanty, grey-blue, agranular cytoplasm and irregularly shaped nuclei with several small nucleoli. The bone marrow can sometimes be associated with a myelomonocytic or acute myeloid leukaemia secondary to an underlying myelodysplasia. Immunophenotyping and cytochemistry will distinguish BPDC from what appears to be an associated leukaemia. BPDC can relapse and transform to a myeloid leukaemia.

Cytochemistry shows that the blast cells of BPDC are α-naphthyl acetate esterase and myeloperoxidase negative while immunophenotyping demonstrates that they express CD4, CD43, CD45RA and CD56. In some cases CD56 can be negative however this does not rule out a diagnosis of PBDC especially if CD4, CD123 and TCL1 are present. As acute myeloid leukaemia for example can express CD56 together with or without CD4, a full panel of CD markers must be examined to make a positive diagnosis of BPDC. In approximately two thirds of patients with BPDC genetic studies have shown that complex karyotypes are common. The most common chromosomal abnormalities include 5q21, 5q34, 12p13, 13q13-21, 15q and loss of chromosome 9.

BPDC is a very aggressive neoplasm with a median survival of 12-14 months. Younger patients, who are treated with leukaemia induction chemotherapy followed by an allogeneic stem cell transplant have been shown to have long lasting remissions.
**Case Study**

*Anaerobiospirillum succiniciproducens* bacteraemia in a patient with abdominal pain and vomiting

Anup Patel

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**Abstract**

An 89-year-old lady presented to the Emergency Department with abdominal pain, vomiting, confusion and fever. A spiral pleomorphic anaerobic Gram-negative bacillus was isolated from blood, confirmed as *Anaerobiospirillum succiniciproducens* by the bioMérieux API Rapid ID 32A bacterial identification system. As a commensal of dogs and cats, *Anaerobiospirillum spp.* are rare causes of gastrointestinal illness and bacteraemia in humans. There is no consensus on antibiotic therapy. However, accurate laboratory diagnosis of *Anaerobiospirillum spp.* will aid in understanding of its epidemiology, treatment and ultimately lead to a decrease in patient morbidity and mortality.

**Introduction**

*Anaerobiospirillum spp.* was first reported by Davis et al. in 1976 as a new genus of spiral shaped Gram-negative anaerobic bacteria, isolated from the throat and faeces of beagle dogs, with the first case of sepsicaemia reported by Rifkin and Opdyke in 1981. Gastrointestinal symptoms are a common sequelae of bacteraemia, and this organism has since been implicated as an occasional cause of bacteraemia in the USA, Hong Kong, New Zealand, Australia, the UK, Germany, Spain, South Africa and Israel (Pinaar et al. 2003), suggesting a global distribution. This report describes a case and laboratory diagnosis of *Anaerobiospirillum succiniciproducens* in an elderly lady with gastrointestinal symptoms and confusion, but with no apparent underlying illness.

**Case Report**

An 89-year-old lady presented to the Emergency Department with symptoms of abdominal pain and vomiting. Upon examination she was febrile and showed signs of confusion. Two sets of blood cultures were collected five hours apart on the day she was admitted, and a further third and fourth set collected six hours apart the day after with one blood culture set consisting of one aerobic and one anaerobic bottle. She was given a course of ceftriaxone and gentamicin after the fourth set of blood cultures were collected and subsequently discharged.

All blood cultures were analysed using the BD Bactec automated system (Becton Dickinson Diagnostic Instrument Systems, USA). The instrument analyses blood cultures over a seven day period and flags cultures where microbial growth is detected. All flagged positive blood cultures are vented and sub-cultured onto Chocolate agar, Columbia horse blood agar, MacConkey agar and Anaerobe agar (Thermo Fisher Scientific, Adelaide, Australia). All culture plates are incubated at 37°C, with the Chocolate and Columbia horse blood agar being in 5% carbon dioxide, the

<table>
<thead>
<tr>
<th>Blood culture set number</th>
<th>Aerobic culture (days incubation)</th>
<th>Anaerobic culture (days incubation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No Growth (7)</td>
<td>No Growth (7)</td>
</tr>
<tr>
<td>2</td>
<td>No Growth (7)</td>
<td>No Growth (7)</td>
</tr>
<tr>
<td>3</td>
<td>No Growth (7)</td>
<td>Growth detected (1)</td>
</tr>
<tr>
<td>4</td>
<td>No Growth (7)</td>
<td>No Growth (7)</td>
</tr>
</tbody>
</table>

The anaerobic bottle from set 3 flagged positive and after Gram-staining showed a spiral 6-8μm pleomorphic Gram-negative bacillus, (x100 magnification, oil immersion objective) (Figs. 1 and 2). Phase-contrast microscopy (x40 magnification, phase contrast objective) showed the organism was actively motile in a corkscrew fashion.

Figure 1. Gram stained blood smear showing spiral shaped Gram-negative bacilli.
After observing the characteristic colony morphology of the organism, and after performing supplementary oxidase, catalase and indole tests, which were all negative, a presumptive identification of *Anaerobiospirillum spp.* was made. This was identification was based on the medical scientist's previous knowledge and experience with the morphology of this organism. At this point it was decided that the best possible confirmatory identification method would be the bioMérieux API Rapid ID 32A (bioMérieux, Marcy l’Etoile, France). This allows identification of a wide variety of anaerobic bacteria utilising 32 cupules, (three of which are empty) each containing different dehydrated substrates and is based on the detection of preformed enzymes by use of chromogenic substrates.

The organism was harvested from a culture plate and a suspension prepared for inoculation of the test cupules in accordance with the manufacturer's instructions. The test strip was then incubated in air at 37°C for four hours. After addition of reagents, test reactions were then examined and colour changes recorded as either positive or negative. Table 2 shows the complete list of biochemical tests in the bioMérieux API Rapid ID 32A, and the corresponding positive and negative reactions that resulted in the organism identification being confirmed as *Anaerobiospirillum succiniciproducens*.

**Table 2.** API (biochemical) test codes and reactions for the bioMérieux API Rapid ID 32A, with the resulting positive or negative reactions for *Anaerobiospirillum succiniciproducens*.

<table>
<thead>
<tr>
<th>API Test Code</th>
<th>Biochemical Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>URE</td>
<td>Urease</td>
<td>-</td>
</tr>
<tr>
<td>ADH</td>
<td>Arginine DiHydrolase</td>
<td>-</td>
</tr>
<tr>
<td>αGAL</td>
<td>α-Galactosidase</td>
<td>-</td>
</tr>
<tr>
<td>βGAL</td>
<td>β-Galactosidase</td>
<td>+</td>
</tr>
<tr>
<td>βGP</td>
<td>β-Galactosidase 6 Phosphate</td>
<td>-</td>
</tr>
<tr>
<td>αGLU</td>
<td>α-Glucosidase</td>
<td>-</td>
</tr>
<tr>
<td>βGLU</td>
<td>β-Glucosidase</td>
<td>+</td>
</tr>
<tr>
<td>αARA</td>
<td>α-Arabinose</td>
<td>-</td>
</tr>
<tr>
<td>βGUR</td>
<td>β-Glucuronidase</td>
<td>-</td>
</tr>
<tr>
<td>βNAG</td>
<td>β-N-Acetyl-β-Glucosaminidase</td>
<td>+</td>
</tr>
<tr>
<td>MNE</td>
<td>Manose</td>
<td>-</td>
</tr>
<tr>
<td>RAF</td>
<td>Raffinose</td>
<td>+</td>
</tr>
<tr>
<td>GDC</td>
<td>Glucamic acid DeCarboxylase</td>
<td>-</td>
</tr>
<tr>
<td>αFUC</td>
<td>α-Fucosidase</td>
<td>-</td>
</tr>
<tr>
<td>NIT</td>
<td>Reduction of Nitrates</td>
<td>-</td>
</tr>
<tr>
<td>IND</td>
<td>Indole</td>
<td>-</td>
</tr>
<tr>
<td>PAL</td>
<td>Alkaline Phosphatase</td>
<td>-</td>
</tr>
<tr>
<td>ArgA</td>
<td>Arginine Arylamidase</td>
<td>+</td>
</tr>
<tr>
<td>ProA</td>
<td>Proline Arylamidase</td>
<td>-</td>
</tr>
<tr>
<td>LGA</td>
<td>Lecyl Glycine Arylamidase</td>
<td>-</td>
</tr>
<tr>
<td>PheA</td>
<td>Phenylalanine Arylamidase</td>
<td>-</td>
</tr>
<tr>
<td>LeuA</td>
<td>Leucine Arylamidase</td>
<td>-</td>
</tr>
<tr>
<td>PyrA</td>
<td>Pyroglutamic acid Arylamidase</td>
<td>-</td>
</tr>
<tr>
<td>TyrA</td>
<td>Tyrosine Arylamidase</td>
<td>-</td>
</tr>
<tr>
<td>AlaA</td>
<td>Alanine Arylamidase</td>
<td>-</td>
</tr>
<tr>
<td>GlyA</td>
<td>Glycine Arylamidase</td>
<td>+</td>
</tr>
<tr>
<td>HisA</td>
<td>Histidine Arylamidase</td>
<td>-</td>
</tr>
<tr>
<td>GGA</td>
<td>Glutamyl Glutamic acid Arylamidase</td>
<td>-</td>
</tr>
<tr>
<td>SerA</td>
<td>Serine Arylamidase</td>
<td>-</td>
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</table>

A 10-digit numerical profile value was then generated by recording test reaction results, and entering the profile value into the bioMérieux API Web Online based microorganism database. The numerical value generated was 0505010400, that resulted in an identification of *Anaerobiospirillum succiniciproducens* (99.9% ID). API Rapid ID 32A strip reactions are shown in Figure 5 along with positive (Fig. 6) and negative (Fig. 7) strips for comparison purposes.
Discussion

Anaerobiospirillum spp. are uncommon causes of gastrointestinal illness, diarrhoea and bacteraemia in humans (Tee et al. 1998 and Pinaar et al. 2003), with two species: Anaerobiospirillum succiniciproducens and Anaerobiospirillum thomasi known to infect humans (Malnick 1997). Anaerobiospirillum spp. are considered a commensal in the intestine of dogs and cats. However, this patient did not report having any pets, or having recent contact with any animals, thus zoonotic transmission as suggested by Fadzilah et al. (2009) is uncertain.

Past reports have showed that most patients with only gastrointestinal symptoms and not bacteraemia do not have an underlying disorder (Tee et al. 1998). This patient can also be noted as having no known underlying illness and this seems to conflict with comments made by McNeil et al 1987 that states patients who developed bacteraemia had underlying illness, such as alcoholism, atherosclerosis, malignancy, diabetes mellitus, dental caries, recent surgery and other gastrointestinal disorders. However it was noted the medical practitioner was querying ischaemic colitis, although this was never diagnosed or proved.

Optimal antibiotic therapy for A. succiniciproducens remains a challenge, as there are no known recommendations (Fadzilah et al. 2009). The patient was given a course of ceftriaxone and gentamicin and it was not known if this cured her bacteraemia.

It was also not known why the first, second and fourth set of blood cultures were negative, as antimicrobial therapy was only commenced after the fourth set of blood cultures
were collected. Speculation as to the possible explanation for the negative blood cultures was put down to the patient having a transient bacteraemia, with the initial portal of entry being the faecal oral route from a dog or cat she happened to have come into contact with. As far as can be noted she did not present to the hospital again, and made a full recovery.

In conclusion, it is important to be aware of the laboratory diagnosis of this rarely encountered organism. The negative reaction for oxidase and no growth under microaerophillic conditions should be prompt enough to prevent confusion between the similar Gram-stain appearance of both *Anaerobiospirillum* spp. with the more commonly encountered organism *Campylobacter jejuni* that also causes gastrointestinal symptoms. Therefore, increased awareness from the medical scientist and laboratory perspective, as well as a consideration of *Anaerobiospirillum* spp. infection when a patient presents with gastrointestinal symptoms on the part of medical practitioners, will further help understanding of its epidemiology, treatment and diagnosis, and thus further lead to decreased patient morbidity, mortality and patient recovery times.

References


Australian Institute of Medical Scientists
Immunohaematology Quality Assurance Program

- Runs bi-monthly starting in June at the beginning of each financial year
- Level of difficulty to suit both small & large laboratories
- Includes blood grouping, antibody screening / identification & compatibility testing
- Ability to send **multiple** returns on the one subscription

- The reports provide participants’ own results along with graphical representation of the results of their peers allowing for easy comparison and analysis by supervising staff
- Absolute confidentiality of results is assured

For enrolment enquiries contact
STEVE MACKAY  E-mail: aimsqap@dspl.com.au
Helicobacter pylori: Molecular Genetics and Cellular Biology
Yoshi Yamaoka
Caister Academic Press
Hardcover 261 pages
ISBN: 978-1-904455-31-8
USD: $319

Helicobacter pylori is an important and common human pathogen, almost unique in its site of infection and highly specific to the human host. This book represents another review of molecular and genetics aspects of an important human pathogen by Caister Academic Press, commissioned in response to the publication of a third H. pylori genome in 2007.

The first chapter provides a brief overview of the history, physiology, epidemiology and disease outcomes of H. pylori. It also provides some discussion of the role of this organism as a class 1 carcinogen (akin to tobacco smoking and lung cancer) for gastric carcinomas. It represents a solid introduction to the area. The focus in this chapter, as throughout the text, is primarily on the organism itself, and therefore clinical details such as the presentation and investigation of H. pylori pyloric ulcerative disease and duodenal ulcerative disease, the management of these cases and their significance for the patient are not discussed.

Chapter two is focused on the lipopolysaccharide (LPS) membrane of H. pylori. The chapter represents a general review of the outer surface lipids and their role in disease, immune avoidance and chronicity. Discussion of lipid structure and roles and comparison between the two (major and minor) species of H. pylori and with those of Escherichia coli is undertaken. A fascinating area covered is the capacity of O-chain polysaccharides to mimic Lewis blood group antigens, and the role of this in immune evasion and potential involvement in the processes leading to gastric atrophy and gastric cancer. Asian isolates express homologues of Lewis-a and Lewis-b and western isolates express homologues of Lewis-x or Lewis-y.

Chapter three focuses on the outer membrane proteins (OMPs) of the organism. Four per cent of the H. pylori genome encodes OMPs and this chapter discusses the five major OMP families of the species. Included in this discussion is the relatedness of these protein families to function and clinical outcomes of infection in strains expressing different OMPs.

The next chapter provides a review of how H. pylori moves about - flagella, motility and chemotaxis. The H. pylori flagellum, is similar in structure to those of coliforms, but has a vastly more complex hierarchical regulatory system. Clearly flagella have a significant role in infection, as flaA- and flaB- mutants are unable to maintain infection in gnotobiotic piglets. Comparison of the flagella associated genes in two sequenced H. pylori strains is included, but the third sequenced strain was not discussed, despite this being a rationale for the production of this volume. With regard to chemotaxis, bacteria of the species are attracted to urea, amino acids and bicarbonate and will move along acid gradients towards areas of lower pH.

Vacuolating cytotoxin (VacA) is the subject of chapter five. This cytotoxin plays an important role in the virulence of H. pylori, and is unlike any other described bacterial cytotoxin. It has a wide range of interactions with host gastric epithelia as well as immunomodulatory (both immunosuppressive and proinflammatory). A thorough discussion of this toxin is entered into in this most informative chapter. The different pathology of infection with strains possessing type s1 and type s2 VacA, with s1 increasing the possibility of gastric cancer development was particularly interesting, and made me wonder about the potential role of s2 as a target for vaccination.

The three type IV secretion systems of H. pylori are discussed and compared in chapter six. Both defined and putative T4SS factors are reviewed, including comparison of the ComB and Cag secretion apparatuses. Cag represents a particularly important factor in this due to its association with more pathogenic strains of H. pylori.

Chapter seven reviews the capacity of H. pylori to acclimatise and remain viable in the harsh environment of the human stomach. The review focuses on the molecular and proteomic mechanisms by which this impressive feat is achieved. This is followed by a chapter discussing the mechanisms of metal and other ion metabolism, acquisition and maintenance. Iron, nickel, potassium, sodium and copper metabolic pathways are discussed in turn. Following this, the cellular processes of replication and cell division specific to H. pylori are well reviewed in chapter nine.

The tenth chapter discusses the evolution of Helicobacter species. Using data acquired from MLST studies of numerous strains of H. pylori, and other species of Helicobacter, a fascinating picture of the evolution of the genus may be determined. As H. pylori is an ancient and specifically human pathogen, sequence data has been used to assist in mapping the migrations of humans throughout the world.
Unexpectedly the most similar species of *Helicobacter* to *H. pylori* is *H. acinonychis*, found in large African cats, whereas Helicobacters of domesticated animals such as *H. felis* are more distant in evolutionary terms. This leads to interesting speculation on how humans became infected with a common precursor from large cats, or large cats from humans.

A penultimate chapter on genomotyping by microarray delves into transcriptional structures and gene regulation and the many proteins essential to the survival and pathogenesis of *H. pylori*. Importantly, the chapter then reviews the converse proteomic aspects of immune response in the host organism, including immunexression in progression to MALT lymphoma.

The final chapter is a brief discussion of potential future directions in *H. pylori* research and the implications of new technologies for such research. Included is a discussion of potential vaccine targets for this organism.

This book represents a solid overview of molecular and genetic aspects of *H. pylori*. It is a represents a useful addition to the library of any individual studying specifically this area of microbiology. The book's scope is the organism biology and genetics, and hence it does not attempt to address clinical or diagnostic aspects of *H. pylori*. Future editions would benefit from inclusion of a chapter on the interactions of cholesterol and *H. pylori* pathogenesis and antimicrobial resistance. Whilst it is now a few years old, this still represents an excellent reference text for any individual undertaking post research into this organism.

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**Monographs in Clinical Cytology**  
*Volume 21:* FNA Cytology of Ophthalmic Tumors  
Charles V Biscotti, Arun D Singh  
Karger  
Hardcover  99 pages  
ISBN: 978-3-8055-9870-5  
USD $167

Ophthalmic cytology is typically part of routine practice in anatomical pathology laboratories associated with Eye Institutes but much less commonly in practices without this relationship. Most ophthalmologists don’t practice in formal institutes so why do we not see more of ocular/ueval or orbital specimens from abnormalities in the eye? This is due in part to the accuracy of the diagnosis of ophthalmic disorders made by the direct visualization methods of ophthalmologists. Nevertheless diagnostic cytology has an important role in the confirmation of the disorders that require corroborative evaluation between cytologist and eye specialist. A testament to this important collaboration is revealed in the new Monograph in Clinical Cytology edited by Doctor Charles Biscotti and Professor Arun Singh who have harnessed the expertise of 12 eminent contributors from within the United States, United Kingdom and Europe to crystalise contemporary ophthalmic cytopathology and provide some insight into this diagnostic subspecialty.

The monograph comprises 10 chapters that collectively provide an interesting overview of the history of ophthalmic cytology, current methods and future prospects in the diagnostic setting.

Chapter 1 provides a thorough review of the history, indications and contra indications, techniques of clinical examination, specimen collection and limitations of the ocular cytopathology specimen. While intraocular biopsy for histopathology has been reported since 1868, FNA has been adapted more recently and since 1979 has been proven to be a generally safe and reliable means of obtaining a diagnostic sample.

Chapter 2 addresses techniques of preparation including methods for direct smears, liquid based cytology and cell blocks. The extent of application of adjunct tests including opportunities for FISH and Flow Cytometry and cytogenetics are well covered.

Chapters 3 and 4 are dedicated to uveal metastases and uveal lymphoma respectively. The diagnostic evaluation and differential diagnosis of these lesions is presented using examples of case studies that are clearly portrayed with the support of ophthalmic images, radiological evidence and micrographs of cytological features and adjunct testing results.

Chapters 5 and 6 address aspects of uveal melanoma. In chapter 5 the cytology of melanoma in representative uveal samples is described as being similar to melanoma elsewhere and the characteristic diagnostic features are clearly elucidated in text and images. Uveal melanomas frequently metastasize and the content of Chapter 6 describes current research attempting to identify prognostic indicators based on karyotyping, FISH and oncogene expression profiling.

Chapter 7 describes the collection and interpretation of benign, inflammatory and neoplastic conditions encountered in vitreous fluid specimens using cytology smears, liquid based cytology and cell block samples. The authors emphasize the importance of a team approach by the various specialists involved in the collection and management of these typically small samples to achieve the optimum outcome.

Chapters 8 and 9 cover the FNA of retinal tumors and orbital tumors in detail with some extraordinary images of ophthalmic lesions as seen in the clinic and the microscopic morphological correlation in a series of case presentations. The skill necessary to perform the test on lesions in these sites cannot be understated and the commitment of special-
ist and patient to the procedures for collection of samples for cytology is evident in the graphic images that are presented.

The final chapter deals with future directions of ophthalmic cytology and in essence predicts the future of many areas of cytodiagnosis with projected improvements in specimen collection the evolving impact of digital imaging and cell profiling that may enhance traditional methods and improve diagnostic certainty.

The monograph is well indexed and each of the chapters is supported with comprehensive reference lists that direct the reader to other resources related to this specialty area. While this publication applies to a niche market it provides a valuable contemporary presentation of current concepts of ophthalmic cytology and would be a valuable addition to the reference collections of pathologists, cytologists and medical specialists with an interest in ophthalmic disease.

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Insulin A Voice for Choice
Arthur Teuscher
Karger
Soft cover  XIV + 82 pages
ISBN: 978-3-8055-8353-4
AUD $28.00

Professor Arthur Teuscher is a diabetologist based in Europe. He founded the European Diabetes Epidemiology Group and the European Diabetes and Nutrition Study Group. The book “Insulin – A Voice for Choice” he describes as a review on the debate on the introduction of analogue insulin for the last 20 years.

Insulin A Voice for Choice was published in 2007 and a few things have changed since in the world of diabetes to 2011.

The book is broken into 7 chapters. Initially he gives a brief background on the condition of diabetes in the first chapter. There is then an overview of insulin therapy and interesting history on the changes in insulin production from animal insulin to the more common analogue insulin which is used today. The remaining chapters discuss hypoglycaemia then hazards, research and marketing of the analogue insulin used today.

The author discusses how the “new” analogue insulin when introduced in 1982 didn’t suit all patients with diabetes. Hypoglycaemia unawareness appeared to be a consequence to some patients he reviewed, who switched to the new analogue style of insulin. Hypoglycaemia unawareness does occur in patients with diabetes after having the condition for a number of years. The author highlights some cases where the hypoglycaemic occurrence resulted in the death of the patient, but he chooses not to highlight hypoglycaemic deaths by those who are taking animal insulin or have hypoglycaemic unawareness before changing to analogue insulin. The condition is an individual response and there would be cases in the literature.

The book is written using language that is today considered inappropriate. The term “diabetic” which is used throughout defines the individual as their health condition, rather than a persons ability to live with diabetes. The terms of non insulin dependent diabetes mellitus (NIDDM) and insulin dependent diabetes mellitus (IDDM) are also no longer used, with Type 2 and Type 1 Diabetes replacing them. The book was published in 2007, and these changes have been in the last few years.

Pharmaceutical companies do at times have a reputation for being able to use their considerable financial backing to push their respective items with the medical community. The author does discuss the hazards of insulin in one chapter and the marketing of the newer analogue insulin by pharmaceutical companies.

Some points Dr Teuscher makes in the book are:

- States / criticises the study results of some of the pharmaceutical companies.
- Uses isolated case studies to highlight his point of view on animal insulin versus analogue insulin.
- The view that what the pharmaceutical companies promised with the new analogue insulin has not delivered and instead the complications that he has seen in patients.
- When written in 2007, production of animal insulin was to be ceased by 2011 (4 years left).
- When discussing patients with diabetes driving a motor car on the road, he makes it appear that all patients with diabetes are a risk to other drivers on the road due to their condition and the risk of a “hypo” on the road.

The author presents no studies to confirm his statements on animal insulin, just isolated cases to back up his claim that analogue insulin produces hypo unawareness whilst animal insulin does not.

Overall, health professionals may find the information Dr Teuscher presents on the various insulins thought provoking and educational for patients they may consult with, that have had diabetes for a long period of time. The book does give background history on insulin and the changes that have
Humans started arming themselves approximately 2.5 million years ago, during the Palaeolithic Age, edged tools such as flint and obsidian emerged as the weapons of choice for hunting purposes. These weapons were used for fighting with other animals but also for defensive and offensive activities with other humans. The quest for better hunting grounds, water holes and the best caves transformed these hunting tools into effective combat weapons. The combination of continuous technological improvements and adaptation to ever-changing battlespace conditions in the past 3,000 years led to the recent development of super-accurate weapons and high mobility. Nevertheless, why are we still unable to defeat hostile bacteria in the microbiological battlespace? Two main questions for us: first, are we investing sufficient and suitable resources in order to provide effective responses to multidrug resistant (MDR) bacteria and secondly, are we inventing new ‘super-accurate weapons’ to mount counter-attacks to recover infected areas? The first edition of Emerging Trends in Antibacterial Discovery: Answering the Call to Arms was compiled and developed with the intent of providing answers to these two fundamental questions. This publication is a summary of several major concepts and pioneering strategies currently being used to develop new ‘super-accurate weapons’ to fight these hostile bacteria. The aim of this book is to offer new ways of thinking about antibiotics and technical solutions for resistance problems. The text has been edited by two subject matter experts from Antibacterials Research Unit, Pharmatherapeutics Division, Pfizer Worldwide Research and Development, Groton, Connecticut: Alita A Miller, Senior Principal Scientist; and, Paul F Miller, Vice President and Chief Scientific Officer. These two editors have been published extensively for the past 30 years in internationally reputable journals. The main text is contributed by 43 expert authors. Four of these authors are based in Australia: John DF Hale, Monash University; Elizabeth J Harry, University of Technology, Sydney (UTS); Leigh G Monahan, UTS; and Ronald J Quinn, Griffith University. The focus of this book occurred but as one reads the book, a feeling of bias against analogue insulin is very strong. If the aim of the book was to make health professionals aware of the past, the book has achieved that aim. If it was to give scientific facts on the pros and cons of analogue insulin vs animal insulin the book disappointingly fails.

Emerging Trends in Antibacterial Discovery: Answering the Call to Arms
Edited by AA Miller and PF Miller
Caster Academic Press
Hardcover VIII + 460 pages
USD $350.00

Part I ‘Answering the Call to Arms’ consists of two short chapters that set the introductory scene for the entire book (Chapters 1 and 2; pp. 1-32). Basically, we have been using one of the defensive techniques of ‘counterattack’ to deal with infections for the past decades. An effective counterattack requires the use of suitable weapons to neutralise or destroy a threat force from the required distance. So do we now have appropriate weapons and ammunition to destroy, delay, isolate, neutralise or suppress the bacterial threats? This basic question led to several organisations, including the Infectious Diseases Society of America, to raise a ‘call to arms’ to encourage everybody to collectively address the emergence and dissemination of antibiotic resistance throughout the world. It is becoming apparent that we will no longer be able to provide effective treatments to infections on a global scale. Major concerns include Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species collectively known as ESKAPE, the multiresistance of methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus and increasing rates of extended spectrum β-lactamase production among enteric organisms. Obviously, a significant investigation is required to address this issue urgently. Instead of injecting money directly into research, the United States decided to have regulatory reforms and financial incentives: first, the approval of the Orphan Drug Act which allowing incentives to manufacturers to invest in research and development of drugs with a small market; second, the formation of the Biomedical Advanced Research and Development Authority for coordination of the development and purchase of treatments and diagnostic tools for public health emergencies; third, the extension of patents, allowing pharmaceutical companies to prolong the marketing exclusivity of their products. The overall aim is to reduce the human and economic burden of antibiotic-resistant bacteria to the world.

Chapter 1 is a summary of 16 chapters and identifies knowledge gaps for further consideration. The first two chapters provide the necessary introductory information for readers. Part I is particularly useful for medical scientists who wish to revise their current knowledge in antimicrobial literature.

Part II ‘Novel Targets and Sources’ consists of six chapters (Chapters 3 to 8; pp. 33-192). Chapters 3 to 6 (pp. 33-147) cover the latest concepts on several new vulnerable points as antibacterial targets. As Sun Tzu in 500 BC has stated ‘avoid what is strong, attack what is weak’. These chapters provide a detailed analysis of the search for new targets for antibiotics to act effectively. Several new promising targets include cell division proteins, multicomponent efflux pumps from Gram negative bacteria, metabolite-sensing riboswitches and secretion systems from Gram negative bacteria. Chapters 7
to 8 cover the active exploration on natural products that may lead to discovering new bacterial inhibitors in the near future. These chapters provide detailed discussions enabling readers to explore new antibiotic reaction sites and new natural products as inhibitors.

Part III ‘Microbial Communities and Interactions with the Host’ consists of four chapters (Chapters 9 to 12; pp. 193-279) which cover our current understanding on the defensive mechanisms involved in antimicrobial resistance, which include drug indifference, tolerance, persistance and the biofilms resistance to antibacterial agents. Different types of antibiotic survival and biofilm infection mechanisms are extensively reviewed and discussed. Part III is particularly useful for medical scientists who wish to gain further understanding regarding microbial resistance.

Part IV ‘Biological Agents for Antibacterial Therapy’ consists of five chapters (Chapters 13 to 17) which cover several promising agents for suppressing and controlling infectious diseases. Evidently, fluoroquinolones and cephalosporins are becoming ineffective against certain types of infections caused by MDR Gram negative bacteria, therefore new agents need to be identified to overcome this issue. One solution is the use of vaccines. Recent insights into vaccines manufactured with purified components, including capsular polysaccharides and their conjugates to protein carriers, toxoids and proteins are providing promising futures. It is pleasing to know that new vaccines for *Neisseria meningitidis* (serogroup B), *Staphylococcus aureus* and *Clostridium difficile* are being developed. Further new considerations such as the importance of choosing the correct adjuvants to enhance vaccine efficacy are being addressed. Other innovative concepts such as strengthening the defence by enhancement of cationic host defence peptides, new monoclonal antibody therapy and new bacteriophage therapy are extensively reviewed. Part IV is very useful for the revision of current knowledge in the search for non-antibiotic treatments.

Part V ‘Enabling Antibacterial Discovery’ consists of three chapters (Chapters 18 to 20) which review the pharmacokinetic-pharmacodynamic models that have been in use to quantitatively characterise the distribution of the administered drugs in vivo. These models are very important because they enable the assessment of the impact of the drugs on health and disease. The emerging diagnostic techniques are listed and reviewed accordingly. Chapter 18 is highly relevant to medical scientists who wish to keep up-to-date on the latest methods available for rapid identification. Advantages and disadvantages of various techniques such as deep sequencing, microarray science, quantum dots, atomic force microscopy, carbon nanotubes and polymerase chain reaction electrospray ionization mass spectrometry are reviewed in detail.

This book has achieved a grade of ‘high distinction’ in our opinion. It displays consistent standards of excellence throughout. There is a wide range of relevant information gathered; a total of 2,659 references are available for further readings. It identifies the problems in a systematic and insightful manner and has an impressive depth of analysis and discussion of the relevant issues. There is a clear and logical flow throughout the chapters which makes it well organised, easy to read and interesting. The book is professionally presented using appropriate academic style, referencing and grammar. The technical presentation of this book is almost perfect. However, there are two weaknesses identified. The book does not contain a subject index and several chapters are not collaboratively written. Hence, there are three areas which could be improved in the next edition. First, a list of abbreviations used in this text should be provided to aid readers in clarifying commonly used terms. Second, a subject index should be included enabling readers to locate the relevant sections effectively. Third, each part should be collaboratively written resulting in more uniform presentation with minimised repetition.

The book has certainly achieved its aim to provide innovative ways of thinking about the use of antibiotics and technical solutions for the resistance problems. In sum, it is highly suitable for any medical scientist wanting to gain insight into the rapidly changing world of bacteriology. The cost of this text is justifiable for such well compiled information. Overall, it is a perfect companion for keeping up-to-date with the latest developments on antibacterial topics.

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Medical Scientist
Sullivan Nicolaides Pathology
Cairns Queensland
Following is a list of books available for review by resource consultants and members of the Institute with particular expertise in the field. The reviewer is invited to retain the complimentary copy of the book once the review is received.

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- Stress Response in Microbiology
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</table>


43. The Regulatory Genome: Gene Regulatory Networks in Development & Evolution author EH Davidson. Elsevier Australia. 289 pages.


YEAR 2012

JULY 22 - 25
Human Genetics Society of Australasia
36th Annual Scientific Meeting
National Convention Centre
Canberra ACT AUSTRALIA
www.hgsa.org.au Email: hgsa@wsn.com.au

JULY 30 - AUGUST 2
HISA
Sydney Convention & Exhibition Centre
Sydney NSW AUSTRALIA

AUGUST 2 - 4
RCPA AIMS Morphology Workshop
Australian Technology Park
Redfern NSW AUSTRALIA

AUGUST 4 - 5
AIMS/AACB
Tasmanian Branches Combined Annual Meeting
Shearwater Resort
Shearwater TAS AUSTRALIA
secretary.aims.tas@gmail.com

AUGUST 18
30th World Congress of Biomedical Laboratory Science
Berlin GERMANY

AUGUST 18 - 19
AIMS Victorian Branch, Haematology Discussion Group & Histology Group of Victoria Joint Meeting
Cape Schanck Resort VIC AUSTRALIA

AUGUST 27 - 31
NZIMLS ANNUAL SCIENTIFIC MEETING
Wellington Town Hall
Wellington NEW ZEALAND
http://www.eenz.com/nzimls12/

SEPTEMBER 5
European Congress of Immunology
Glasgow UNITED KINGDOM

SEPTEMBER 24 - 27
AIMS National Scientific Meeting
Darwin Convention Centre
Darwin NT AUSTRALIA

OCTOBER 14 - 17
Australasian Flow Cytometry Group Meeting
Melbourne VIC AUSTRALIA

OCTOBER 20 - 21
AIMS NSW/ACT Branch Scientific Meeting
Crowne Plaza Hotel
Canberra ACT AUSTRALIA

OCTOBER 27
ASTH Scientific Workshop 2012
Melbourne Convention Centre
Melbourne VIC AUSTRALIA

OCTOBER 28 - 31
HAA Meeting
Melbourne VIC AUSTRALIA

NOVEMBER 2 - 4
AIMS NSW North Coast Annual Conference
Darlington Beach Resort
Arrawarra NSW AUSTRALIA
neil.horton@hnehealth.nsw.gov.au

NOVEMBER 15 - 18
AACB Annual Scientific Meeting
Melbourne VIC AUSTRALIA

NOVEMBER 15 - 18
IFCC General Conference
Kuala Lumpur MALAYSIA

NOVEMBER 25 - 28
Australian Health and Medical Research Congress
Adelaide Convention Centre
Adelaide SA AUSTRALIA
www.ahmcongress.org.au

DECEMBER 8 - 11
American Society of Hamatology Annual Meeting
Atlanta GEORGIA USA
http://www.hematology.org/

YEAR 2013

MAY 19 - 23
Euromedlab
Milano Convention Centre
Milano ITALY
http://www.milan2013.org/

AUGUST 22
15th International Congress of Immunology
Rome ITALY

OCTOBER 6 - 9
Asia-Pacific Federation for Clinical Biochemistry and Laboratory Medicine Congress
Bali Nusa Dua Convention Centre
Bali INDONESIA

OCTOBER 20 - 23
HAA Meeting
Gold Coast QLD AUSTRALIA
Australian Institute of Medical Scientists

ELECTION OF PRESIDENT AND DIRECTORS 2012

CALL FOR NOMINATIONS

Background

Under the terms of the Constitution, the Board shall include at least three and not more than four Directors who are elected directly by and from amongst the voting members, comprising the President and up to three other Directors. There are therefore three positions for Elected Directors and one for President to be filled by election directly by voting members of the Institute. The elected President and Directors will take office from the 2012 AGM.

Call for Nominations

Nominations are hereby called for the positions of President and three Directors.

Under the terms of the constitution, nominees for the position of President must have been a Director or a member of the AIMS Advisory Council for at least 2 out of the past 4 years prior to standing for election, must be Professional Members of the Australian Institute of Medical Scientists (AIMS) and must be financial at the time of nomination.

Under the terms of the Constitution, nominees for the position of Director must be Professional Members of the Australian Institute of Medical Scientists (AIMS) and must be financial at the time of nomination.

Candidates must be nominated by at least TWO FINANCIAL VOTING members of AIMS.

Nominations should be on the nomination form. The first section of the form should be completed by the proposer and seconder of the nomination. The second section of the nomination form should be completed by the nominee.

If more than one nomination for the position of President is received by the closing date, a postal ballot shall be conducted on a first past the post basis. If more than three nominations for the three Director positions are received by the closing date, a postal ballot shall be conducted on a first past the post basis.

Information and nominations forms are on our website www.aims.org.au

Nominations must reach AIMS National Office
PO Box 1911 Milton 4064

by
4 pm AEST FRIDAY 27th JULY 2012
Fellowship of AIMS

The AIMS Fellowship is an attractive and highly competitive option to academic post graduate degrees.

The Fellowship is recognised by the Department of Health and Ageing as meeting the requirements for supervision of category GX and GY laboratories.

Qualification for the Fellowship is by EXAMINATION in one of the following disciplines:

- Transfusion Science
- Clinical Biochemistry
- Cytology
- Haematology
- Histopathology
- Immunology
- Microbiology
- General (including Core Laboratory)

To enrol in the fellowship program or for further information please contact the AIMS National Office: aimsnat@aims.org.au
Medical Training Solutions (MTS) Online available for all AIMS Members

AIMS members receive unlimited access to Clinical Laboratory Training and Competency Assessment from MTS provided by the University of Washington.

More than 30 training courses and 20 Competency assessments in:
- Safety
- Phlebotomy
- Haematology
- Microbiology
- Body fluids
- Coagulation
- Transfusion Services
- And more

Training

- Award-winning course content for initial training and cross training
- Engaging video, animation and narration of procedures and concepts
- Database of high-resolution specimen images

Competency Assessment

- Image-based multiple-choice questions by expert authors for objective testing of staff
- New sets of questions every six months on January 1st and July 1st
- Test results from previous periods stored in archives
- Thorough explanations for education and remediation provided after test completion.
- More information is available on the AIMS website. Members will need to register at MTS to use the service

APACE credits

- 1 APACE Credit for each hour of on-line instruction.
- 5 APACE Credit for each examination unit passed.

Save certificate for APACE audit and training records.
5 APACE credits per set of questions will be awarded if at least 8 out of 10 questions are answered correctly. 40 credits maximum per year can be claimed.

Journal-based CPD No.32
Page 1 of 2

Questions relating to 'A new approach to minimise the problem of patient to patient contamination in histology', page 42 of this issue.

1. Many histology work practices developed well over 50 years ago could be dramatically improved to reduce sample contamination rates.  
   True/False

2. The processing of histological specimens routinely involves the steps of: grossing, processing, embedding, microtomy and section staining.  
   True/False

3. Contaminants that show a greater staining intensity than the tissue sample may suggest that the source of the contamination may be from the staining bath.  
   True/False

4. There is no difference between the use of a cold or warm water bath to float out cut sections prior to mounting the sections onto a glass slide.  
   True/False

5. The removal of the use of “tissue stompers” from the embedding areas did not adversely impact the ability to embed tissue flat.  
   True/False

6. Completely emptying and refilling water baths used in the microtomy stage had a marked effect in reducing tissue contaminants.  
   True/False

7. Staining baths are rarely involved as a source of tissue contamination.  
   True/False

8. Changes to laboratory processes showed a reduction of contaminants from 10.6% to 3.8%.  
   True/False

9. The major contributor to reducing patient to patient tissue biopsy contamination was the introduction of single use gloves for those involved in tissue processing.  
   True/False

10. Autoclaving instruments used in tissue processing which are intended for reuse, renders any remaining contaminating tissue easily identifiable at the microscopic level.  
    True/False

Name: ___________________________________ Membership No: ___________________________

Email: ____________________________________________________________________________

Please photocopy this page or print it from the AJMS on the AIMS Member Library in the AIMS Member Lounge at www.aims.org.au, circle your answers and post, fax or scan and email to us by 31 August 2012 to:

AJMS APACE Questions, AIMS National Office, PO Box 1911, Milton Qld 4064. Facsimile: 61 7 3876 2999
Questions relating to ‘The Immature Platelet Fraction: an assessment of its application to a routine clinical laboratory’, page 48 of this issue.

1. Less than 2% of patients with a normal platelet count had a raised immature platelet fraction. True/False

2. There is good agreement between published studies with respect to the reference interval and sample stability time of the immature platelet fraction measurement. True/False

3. The spread of immature platelet fraction results from normal individuals showed a normal Gaussian distribution. True/False

4. The highest values for the immature platelet fraction were found in two patients with megakaryocytic leukaemia. True/False

5. The immature platelet fraction is relatively stable if assayed within 8 hours of collection. True/False

6. With extended storage to 24-48 hours the immature platelet fraction falls sharply due to the degradation of RNA in the platelet. True/False

7. Patients with renal disease show high immature platelet fraction measurements. True/False

8. The immature platelet fraction is analogous to a reticulocyte count. True/False

9. One difficulty with monitoring immature platelet fraction assay validity is the lack of suitable control material across the reportable range. True/False

10. Reticulated platelets are rich in RNA and can be identified by staining the RNA with fluorescent dyes. True/False

Name: ___________________________ Membership No: ___________________________

Email: ____________________________

Please photocopy this page or print it from the AJMS on the AIMS Member Library in the AIMS Member Lounge at www.aims.org.au, circle your answers and post, fax or scan and email to us by 31 August 2012 to:

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Instructions to authors

The following instructions are based on the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals”, also known as the Declaration of Vancouver, and on the Australian Government Style manual: for authors, editors and printers, 6th edition, 2002. URLs were correct on September 29th, 2008.

Manuscripts that do not fully comply with the following ‘Instructions to Authors’ may be returned for revision before they are considered for publication.

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.

Papers published in the AJMS are in the form of:

- Review Articles
- Original Articles
- Brief Communications
- Technical Notes
- Case Studies
- Letters to the Editor
- Book Reviews

Articles submitted for publication are understood to be offered only to the AJMS and those accepted become the property of the AJMS.

All individuals listed as authors must have made a substantial contribution to the conception and design of the study, the acquisition of data or the analysis and interpretation of data; the drafting of the article or revising it critically for important intellectual content; and final approval of the version to be published. The corresponding author must take responsibility for obtaining permission from all the authors for the submission of any version of the manuscript and for any changes in authorship.

When the manuscript is submitted the authors must disclose any potential conflict of interest and/or commercial support.

Requirements & preparation of manuscripts

General

Articles should be submitted in electronic format to ajms@aims.org.au. If an article is too large to be submitted by email, it should be submitted on a CD.

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.

Authors should ensure that their manuscript communicates their ideas and concepts simply and clearly so that the article is easily read and understood. Authors are strongly recommended to refer to the recommendations on reporting standards as outlined in the statements and checklists of the CONSORT group (see: http://www.consort-statement.org/) and similar groups such as STARD (see: http://www.stard-statement.org/). The principles outlined in these standards may be used as general guidelines and not just as applied to clinical trials and diagnostic studies.

Title page

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 & 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/). 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 leading 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 & 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.

Results

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.

Discussion

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/ljweb.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:


v) Online documents: National Center for Biotechnology Information.

**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, below).

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.

**Commonly used abbreviations**

<table>
<thead>
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<th>Abbreviation</th>
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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.

- **Style manual for authors, editors and printers. 6th ed.** John Wiley & Sons Australia Ltd, 2002.
- **Day RA. How to write and publish a scientific paper. Philadelphia, Institute for Scientific Information Press, 1979.**
The Ultimate Physiological Function Assay of the Coagulation System

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HAEMATOLOGY UPDATE

A case of blastic plasmacytoid dendritic cell neoplasm

ORIGINAL ARTICLE

A new approach to minimise the problem of patient to patient contamination in histology

ORIGINAL ARTICLE

The Immature Platelet Fraction: an assessment of its application to a routine clinical laboratory