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Original Article
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Christopher J. McIver and Sydney M. Bell

Microbiology Department (SEALS), St George Hospital, Kogarah, New South Wales and School of Medical Sciences, University of New South Wales, Sydney, New South Wales

Abstract

Cerebrospinal fluid (CSF) taken from suspect cases of meningitis are commonly screened for *Neisseria meningitidis* by real-time quantitative PCR (rtqPCR). The volume of this invasive sample is not always sufficient to undertake the wide range of pathology tests often requested. We investigate the feasibility of circumventing conventional extraction for an rtqPCR assay by testing a centrifuged deposit of CSF suspended in 50.0μL of buffer and thereby conserving supernatant for other pathology tests. We also explored the effect on assay performance when two different template volumes were used to detect this microorganism. The amplification efficiency and sensitivity were improved when the template was increased from 5.0μL to 10.0μL in a 20.0μL reaction mixture. The rtqPCR assay showed a limit of detection for *N. meningitidis* of 3.6–7.1x10^1 CFU/mL when 10.0μL templates from centrifuged deposits of 29 spiked-CSF were tested. This approach also enabled detection of target DNA in a suspension of the microorganism (1.6x10^7 CFU/mL) in pooled CSF after 16 d storage at 4°C. Thus, testing for *N. meningitidis* in centrifuged CSF using an improved rtqPCR assay proved to be a viable approach to testing small volumes (≥100μL) of CSF with the added advantage of conserving sample.

Keywords: Molecular diagnostics, rapid testing, *Neisseria meningitidis*, real-time PCR

Introduction

Real-time quantitative PCR (rtqPCR) assays are commonly used for rapid diagnosis of meningococcal meningitis which is required for timely clinical management and public health intervention. Early treatment of suspect cases with antibiotics is recommended and often thwarts cultivation of the putative agent and therefore molecular detection of *Neisseria meningitidis* DNA in the cerebrospinal fluid (CSF) may be the only opportunity for laboratory diagnosis. Further, the ability of the molecular assay to detect low levels of this microorganism (herein referred to as sensitivity) is essential for accurate diagnosis.

Samples of CSF taken during acute illness are often required for a range of diagnostic tests and distribution may compromise the volume available for molecular detection assays. The DNA in samples is extracted before molecular testing and usually requires a volume of ≥200 μL. In this study, we investigated the feasibility of testing for *N. meningitidis* DNA in centrifuged deposits of CSF to circumvent conventional extraction. We also explored the effect on the performance of an rtqPCR assay for this microorganism using either 5.0μL or 10.0μL templates of a buffered suspension of this centrifuged deposit. The resultant procedure allows for rapid and sensitive testing of small samples of CSF comparable in performance to testing extracted templates and with the added advantage of conserving volume for other pathology tests.

Materials and methods

**Molecular detection of Neisseria meningitidis**

**Extraction**

Samples of CSF (210.0μL) were extracted using a silica membrane-based method (QIAamp DNA Blood Mini Kit, Qiagen) facilitated by a robotic processor (Qiacube, Qiagen).

**Standard rtqPCR assay**

A 20.0μL reaction mixture comprising of 2.0μL FastStart DNA Master HybProbe (Roche Diagnostics); 0.6μM each ctrA forward and reverse primers (Corless *et al* 2001); 0.2μM probe (TaqMan) (Corless *et al* 2001); 4.0μL 25mM magnesium chloride (Roche Diagnostics); 1.0U AmpErase (Uracil-N-glycosylase) (Roche Diagnostics); and balanced with water to 10.0μL or 15.0μL for a 5.0μL
and 10.0μL template, respectively. A standard volume of 5.0μL template is recommended by the manufacturer (Roche Diagnostics). Assays are performed in real-time using the LightCycler 2.0 (Roche Diagnostics) programed at 40°C for 10 min; 95°C for 10 min; 50 amplification cycles (95°C for 10 sec and 60°C for 30 sec); and cooling to 40°C for 30 sec.

A second assay is performed in parallel using template spiked with a 1μL-saline suspension (approximately 10⁷ CFU/ml) of *N. meningitidis* NCTC 8554 (used throughout study) as a test for sample inhibition. The 95% CI of crossing points previously measured for 18 spiked-CSF extractions was 35.2–36.2 cycles. Failure to amplify target, or crossing points in excess of this range indicates sample inhibition.

### Assessing performance of rtqPCR assay using different template volumes

#### Standard curve analysis

A standard curve analysis was used to assess the differences in PCR performance when 5.0μL and 10.0μL templates were tested by the above rtqPCR assay. For this purpose, extractions of 10-fold saline serial dilutions of *N. meningitidis* were tested. The resultant crossing points were plotted against the logarithm of the viable count of each suspension as determined by a standard surface count technique (Collins and Lyne 1984). The correlation coefficient and slope of the curves of best-fit were resolved using a computer program (Prism 5, Version 5.0d, GraphPad® software). The slope value was used to determine amplification efficiency (-1+10(-1/slope)) using an online calculator (http://www.thermoscientificbio.com/webtools/qpcrefficiency/) (Thermo Scientific).

Optimal amplification efficiency (100%) equates to a doubling of the amplicon per PCR cycle (Hellemans and Vandesompele 2011).

### Reproducibility

To assess reproducibility, the above extracted 10-fold suspensions containing ≤10⁵ CFU/mL were tested five times using 5.0μL and 10.0μL templates to determine the number of positive reactions and the central tendency of the crossing point values (mean, coefficient of variation, standard error of mean and confidence limits) for each volume.

### Testing spiked-clinical samples

The crossing points were measured for the inhibition control (above) of assays for 15 CSF and 14 EDTA blood samples extracted as for the standard curve and tested using 5.0μL and 10.0μL extracted templates. The paired *t*-test was used to test statistical difference (p <0.05) between crossing points for each sample type.

### Direct testing of CSF

Twenty CSF samples (culture-negative) were tested directly (without prior extraction) with and without added 2.0μL of 1.9x10⁵ CFU/mL *N. meningitidis* suspension (spike) to the 20.0μL reaction mixture (final volume). Samples were tested using 5.0μL and 10.0μL template volumes.

### Testing centrifuged deposits of CSF by rtqPCR

A volume of CSF is centrifuged to precipitate bacteria, supernatant removed (available for other tests) and replaced with a small volume of buffer to preserve DNA. The rtqPCR assay is performed using a 10.0μL template from a single centrifuged deposit of a CSF sample. Microbial DNA is released from the bacteria during the PCR assay when polymerase is activated at 95°C for 10 min. In this study, CSF of no less than 100μL volume was centrifuged at 13,000 g (Heraeus Biofuge Pico, ThermoFisher Scientific) for 15 min, supernatant removed (and stored), and precipitant suspended in 50μL of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0) (Qiagen). AE buffer is commonly used in commercial extraction methods and we have not found this buffer to antagonize PCR reactions when used in 10.0μL template rtqPCR assays (data not shown).

### Limit of detection of rtqPCR assay using CSF centrifuged deposits

The limit of detection of *N. meningitidis* for 30 samples of spiked-CSF (culture-negative) was determined for the rtqPCR assay using template derived from a centrifuged deposit. A saline suspension of *N. meningitidis* (1.8x10⁶ CFU/mL) was serially diluted two-fold 13 times. A 20.0μL volume of each suspension was added to 980.0μL of each CSF to provide final concentration range from 8.9 x10⁰–3.6x10⁴ CFU/mL. A 100.0μL aliquot of each *N. meningitidis*-spiked CSF was centrifuged (as above) and the assay performed using 10.0μL templates. The assay was repeated for samples providing a negative result when initially tested.

### Limit of detection of rtqPCR assay using different template preparations of CSF

The ability of the above rtqPCR assay to detect *N. meningitidis* in serial dilutions (in pooled CSF) using different template preparations of CSF was compared. Doubling serial dilutions of *N. meningitidis* (8.0x10⁴ CFU/mL) in pooled CSF (comprised of 30 culture-negative samples) were prepared and tested by rtqPCR assay using: 5.0 and 10.0μL extracted templates.
derived by the QiaAmp Blood DNA extraction method (Qiagen); a 10.0μL template of centrifuged deposits derived from 100.0μL and 200.0μL aliquots of the pooled CSF; and a 5.0μL template taken directly from the pooled CSF directly (without preliminary extraction). The limit of detection of target DNA of each method was compared.

Testing stored CSF

The feasibility of testing centrifuged deposits of stored CSF was investigated. We determined this method's ability to detect target DNA in different sample types stored at 4°C for 16 d. Sample types included pooled CSF (30 culture-negative samples), sterile saline (Baxter), and sterile water (Baxter) inoculated with *N. meningitidis*. A 10.0mL volume of each sample type was inoculated with an isolated colony from a fresh *N. meningitidis* chocolate agar culture. Before inoculation, the pooled CSF was filter-sterilised (0.2μm microfilter, Millipore). The pooled CSF was shown to have antibacterial activity which was detectable when an aliquot of 25.0μL was added to a sterile filter 6mm disc (Whatman) placed on the surface of a dried chocolate agar plate seeded with *N. meningitidis* (approximately 10^7 CFU/mL suspension and drained), and incubated in carbon dioxide for 48 h. Antimicrobial activity tested under the same conditions was not detected in the saline and water used in this experiment.

The viability of *N. meningitidis* in each sample type was tested initially (day 1) and until extinction during storage (as above). Aliquots of 210.0μL and 200.0μL were taken at the start and during storage at 1, 2, 5, 7, 9 and 16 d for extraction by QIAamp DNA Blood Mini Kit (Qiagen) and for centrifugation, respectively. The elution from the extracted CSF and the centrifuged deposit (as prepared above) were tested for *N. meningitidis* by rtqPCR assay using a 10.0μL template, in duplicate and with an inhibition control test (above).

Clinical cases of meningococcal meningitis

The ability to detect *N. meningitidis* in centrifuged deposits of CSF from clinical cases of meningococcal meningitis is considered. CSF from two cases were tested by rtqPCR assay using 10.0μL templates of extracted CSF and centrifuged deposits derived from 100.0μL of each sample. The CSF aliquots were cultured by inoculating onto blood and chocolate agar plates, incubated in 5% CO₂, and read after 48 h, whilst the broth was sub-cultured onto the same plate media (under the same incubation conditions) after 5 d incubation. The same assay was used to test extractions of available EDTA blood samples taken from each patient during the acute phase of the illness.

Results

The effect on performance of the rtqPCR assay using different template volumes

Standard curve analysis

A standard curve analysis showed that increasing the template volume from 5.0 to 10.0μL improved PCR efficiency from 92.0% (slope = -3.53; r² = 0.999) to 103.6% (slope = -3.24; r² = 0.995) and thereby increasing product amplification from 1.92 to 2.04 times per cycle (Figure 1).

Reproducibility

The above efficiency was also demonstrable in five repeated tests for PCR-extractions of 10-fold series (1.9x10^1–10^3 CFU/mL, prepared above). A 10.0μL template assay detected target DNA in all five measurements of crossing points for each suspension and with a comparatively lower CV%, SE (mean) and narrow confidence intervals than was achieved using a 5.0μL template (Table 1).

Testing spiked-extracted CSF

No significant difference in crossing points was detected in assays using either 5.0 or 10.0μL spiked-extracted templates in 15 CSF samples (p= 0.7937, paired t-test). However, a significant difference was detected in spiked-extractions of 14 EDTA blood samples (p= 0.0180, paired t-test) and hence precludes the use of a 10.0μL template for this sample type when using the above rtqPCR assay.

Direct testing of CSF

Target DNA was not detected in 20/20 CSF samples by the direct-PCR assay without added target DNA (controls) using either 5.0μL or 10.0μL of unextracted sample. However, when the CSF was spiked with *N. meningitidis*, the target DNA was detected in 20/20 and 3/20 samples using 5.0μL or 10.0μL templates, respectively. This shows the inhibitory property of CSF, which is evident when testing CSF directly (without extraction) using a 10.0μL template.

Testing centrifuged deposits of CSF

Limit of detection of rtqPCR using centrifuged CSF

Target DNA was detected in 29 of 30 spiked-CSF samples using a 10.0μL template derived from a centrifuged deposit. The negative-sample was heavily blood-stained and the presence of red cells in the template may account for this failure.
Table 2. Comparison of the limit of detection of Neisseria meningitidis in serial doubling dilutions of 8.0 x 10⁴ CFU/ml prepared in pooled CSF, as measured by rtqPCR assays using different templates.

<table>
<thead>
<tr>
<th>Suspension CFU/ml</th>
<th>Template volume</th>
<th>5 µL</th>
<th>10 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 x 10¹</td>
<td>Mean</td>
<td>31.5</td>
<td>30.2</td>
</tr>
<tr>
<td></td>
<td>CV%</td>
<td>0.60</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>SE (mean)</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>CI 95%</td>
<td>31.28 – 31.76</td>
<td>30.10 – 30.38</td>
</tr>
<tr>
<td>1.9 x 10²</td>
<td>Mean</td>
<td>35.0</td>
<td>33.42</td>
</tr>
<tr>
<td></td>
<td>CV%</td>
<td>2.51</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>SE (mean)</td>
<td>0.39</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>CI 95%</td>
<td>34.20 – 35.88</td>
<td>33.13 – 33.67</td>
</tr>
<tr>
<td>1.9 x 10¹</td>
<td>Mean</td>
<td>38.7</td>
<td>38.08</td>
</tr>
<tr>
<td></td>
<td>CV%</td>
<td>4.60</td>
<td>3.44</td>
</tr>
<tr>
<td></td>
<td>SE (mean)</td>
<td>1.03</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>CI 95%</td>
<td>34.28 – 43.12</td>
<td>36.83 – 39.33</td>
</tr>
</tbody>
</table>

Table 1. Reproducibility of crossing-points of rtqPCR assay for suspensions of Neisseria meningitidis crA conducted with 5 and 10 µL templates from extracted elutes, repeated five times.

<table>
<thead>
<tr>
<th>Suspension CFU/ml</th>
<th>Template volume</th>
<th>5 µL</th>
<th>10 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 x 10³</td>
<td>Mean</td>
<td>31.5</td>
<td>30.2</td>
</tr>
<tr>
<td></td>
<td>CV%</td>
<td>0.60</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>SE (mean)</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>CI 95%</td>
<td>31.28 – 31.76</td>
<td>30.10 – 30.38</td>
</tr>
<tr>
<td>1.9 x 10²</td>
<td>Mean</td>
<td>35.0</td>
<td>33.42</td>
</tr>
<tr>
<td></td>
<td>CV%</td>
<td>2.51</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>SE (mean)</td>
<td>0.39</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>CI 95%</td>
<td>34.20 – 35.88</td>
<td>33.13 – 33.67</td>
</tr>
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<td>1.9 x 10¹</td>
<td>Mean</td>
<td>38.7</td>
<td>38.08</td>
</tr>
<tr>
<td></td>
<td>CV%</td>
<td>4.60</td>
<td>3.44</td>
</tr>
<tr>
<td></td>
<td>SE (mean)</td>
<td>1.03</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>CI 95%</td>
<td>34.28 – 43.12</td>
<td>36.83 – 39.33</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the limit of detection of Neisseria meningitidis in serial doubling dilutions of 8.0 x 10⁴ CFU/ml prepared in pooled CSF, as measured by rtqPCR assays using different templates.

<table>
<thead>
<tr>
<th>Template type</th>
<th>Extracted elute¹</th>
<th>Centrifuged deposit²</th>
<th>Direct testing³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 µl</td>
<td>200 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>Template volume (µL)</td>
<td>5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Limit of detection (CFU/ml)</td>
<td>40</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>

¹ Extracted using QIAamp® DNA Mini and Blood Mini (Qiagen).
² Centrifuged deposits suspended in 50 µl of AE buffer (derived from 100 and 200 µl volumes of CSF).
³ Without extracting (above) or centrifuging.
Table 3. Testing pooled CSF, saline, and water inoculated with Neisseria meningitidis NCTC 8554 for viability and target DNA detection using 10 μl-templates derived from extraction and centrifuged deposits.

<table>
<thead>
<tr>
<th>Time / Day</th>
<th>Sample</th>
<th>Viable count</th>
<th>Extraction(^1)</th>
<th>Centrifuged deposit(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>CSF</td>
<td>1.6 x 10⁷</td>
<td>17.2</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>1.1 x 10⁷</td>
<td>17.5</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>1.6 x 10⁶</td>
<td>17.3</td>
<td>18.7</td>
</tr>
<tr>
<td>10h</td>
<td>CSF</td>
<td>2.8 x 10⁶</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>1.4 x 10⁶</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>1.6 x 10⁶</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1d</td>
<td>CSF</td>
<td>2.3 x 10⁵</td>
<td>17.0</td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>2.4 x 10⁵</td>
<td>17.5</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>3.4 x 10⁴</td>
<td>17.6</td>
<td>19.2</td>
</tr>
<tr>
<td>2d</td>
<td>CSF</td>
<td>6.2 x 10⁴</td>
<td>17.0</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>3.0 x 10⁴</td>
<td>17.5</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>3.0 x 10⁴</td>
<td>16.8</td>
<td>18.1</td>
</tr>
<tr>
<td>5d</td>
<td>CSF</td>
<td>3.0 x 10³</td>
<td>19.9</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>3.0 x 10⁴</td>
<td>17.9</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>3.0 x 10⁴</td>
<td>17.1</td>
<td>18.2</td>
</tr>
<tr>
<td>7d</td>
<td>CSF</td>
<td>0</td>
<td>19.7</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>0</td>
<td>17.8</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0</td>
<td>17.1</td>
<td>19.6</td>
</tr>
<tr>
<td>9d</td>
<td>CSF</td>
<td>0</td>
<td>19.3</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>0</td>
<td>17.7</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0</td>
<td>17.2</td>
<td>18.7</td>
</tr>
<tr>
<td>16d</td>
<td>CSF</td>
<td>0</td>
<td>20.9</td>
<td>21.1</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>0</td>
<td>18.8</td>
<td>20.7</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0</td>
<td>17.2</td>
<td>18.6</td>
</tr>
</tbody>
</table>

\(^1\) Extracted using QIAamp\(^\text{®}\) DNA Mini and Blood Mini (Qiagen).

\(^2\) Centrifuged deposits suspended in 50 μl of AE buffer (derived from 200 μl volumes of CSF).
In the remaining samples, target DNA was detected in 29/29 containing 7.1x10^1 and 23/29 containing 3.6x10^1 CFU/mL suspensions of *N. meningitidis*, respectively. For 5/29 positive-samples containing 7.1x10^1 CFU/mL, five were repeated to return a positive result. Similarly 5/23 positive-samples containing 3.6x10^1 CFU/mL also were repeated. This indicates the need to perform the rtqPCR assay in duplicate to improve sensitivity.

**Limit of detection of rtqPCR using different templates of CSF**

The limit of detection for *N. meningitidis* in serial dilutions prepared in pooled CSF by testing directly and using templates from extracted elutes and centrifuged deposits is shown in Table 2. All methods detected suspensions of at least 80 CFU/mL. Sensitivity of the assay using templates derived from extracted elutes and centrifuged deposits was comparable considering experimental error. Also shown is the improvement in sensitivity when increased volumes for templates (10.0μL) and for centrifuging CSF (>100μL) are used. Testing the pooled CSF directly and using a 5.0μL template volume was the least sensitive method.

**Testing stored CSF**

The viability of *N. meningitidis* and detection of specific DNA in pooled CSF, saline and water is shown in Table 3. Extinction of viability is first evident in pooled CSF as predicted by a positive antimicrobial activity test. The consecutive crossing points recorded for each sample type showed a different trend over the 16 d storage. Results from molecular assays using extracted elutes and centrifuged deposits were not significantly different for pooled CSF (p= 0.1053, paired t-test) over this timeframe. However significant differences were detected between these template preparations for saline and water (p=0.003 and 0.0001, respectively). After 16 d storage, the crossing points for pooled CSF increased by 3.7 and 2.3 cycles for templates of extracted elutes and centrifuged deposits, respectively (indicating a decrease in target DNA). Similarly an increase of 1.3 and 2.1 cycles respectively was measured for saline. However no significant change was measured for water.

**Application in two clinical cases of meningococcal meningitis**

Target DNA was detected in the CSF of both cases of meningococcal meningitis using extracted elutes and centrifuged deposits for templates. Crossing points for the extracted templates (32.3,18.8) were lower than recorded for templates derived from the centrifuged deposit (38.1, 23.9) for both respective cases. Only 100.0μL of CSF was available from both cases for centrifugation and may account for this difference in sensitivity. In both cases *N. meningitidis* was not isolated by culture. However target DNA was detected by the rtqPCR assay in extracted EDTA blood samples collected within 24 h of the lumbar puncture.

**Discussion**

Direct testing of CSF has been described previously (Baethgen et al 2003) but to our knowledge, the centrifugation approach to concentrate and extract *N. meningitidis* (as evaluated here) has not been previously reported. Small reaction volumes of ≤50μL are commonly used in diagnostic rtqPCR assays and are vulnerable to experimental error, which may compromise detection of low levels of target DNA (Buggraf et al 2005). Further, small reaction volumes also limit the opportunity to vary template volume and reaction mix ratio to dilute inhibitory substances present in clinical samples (Bastein et al 2008). Nonetheless, we improved the amplification efficiency of a rtqPCR assay by increasing template volume from 5.0μL to 10.0μL. This increased volume amounts to half the total volume of reaction mixture used for this assay and is contrary to the manufacturer’s recommendation (Roche Diagnostics 2006), as higher template volume increase potential for PCR inhibition. However, this was not evident when we compared crossing points of 5.0μL and 10.0μL-extracted templates for assays of CSF samples identically spiked with *N. meningitidis*. Conversely, the same experiment with EDTA blood samples showed significant differences in crossing points (between the two template volumes) indicating inhibition of the PCR assay and therefore precludes the use of a 10.0μL template for this sample type.

The concept of testing centrifuged deposits of this invasive sample was explored whilst utilizing a 10.0μL template. A minimum of 100.0μL of CSF was centrifuged to concentrate bacteria and the supernatant removed (now available for other clinical tests) and replaced by AE buffer, which is commonly used for elution in commercial extraction methods. This buffer does not inhibit PCR reactions and allows for prolonged storage of suspended DNA at -20°C or 2–8°C (Oxford Gene Technology 2011; Qiagen 2012). In this study, we showed that testing centrifuged deposits enables detection of *N. meningitidis* in CSF at concentrations of about 10–100 CFU/mL using a 10.0μL template. However, it was found not to be suitable for testing CSF samples containing blood as it is inhibitory and this phenomenon has previously been described (Byrnes et al 1975).

Given that CSF may be refrigerated for long periods before testing, we investigated reliability of using centrifuged CSF as templates when samples were stored up to 16 d at 4°C. Predictably in this study, the viability of *N. meningitidis* in pooled CSF during storage declined rapidly because of the presence of antimicrobial activity. Loss of viability in pooled CSF had preceded the gradual decline evident in suspensions in water and saline. It has also been shown previously that the viability of *N. meningitidis* in CSF naturally declines over time when stored in air at 37°C, 4°C and at room temperature (Gunniffe et al 1996). However even in the absence of viable *N. meningitidis*,...
target DNA was detected by rtqPCR in pooled CSF, saline and water after 16 d storage at 4° C using templates from extracted elutes or centrifuged deposits, although a gradual decline in target DNA had occurred in pooled CSF and saline but not water. Detection of target DNA at 16 d may be attributed to the high starting concentration of \( N. \) meningitidis \((10^6–10^7 \text{ CFU/mL})\) and possibly not have been achievable with a lower initial inoculum. In this study, the decline in detectable target DNA in pooled CSF over 16 d was more evident in the templates from the extraction compared to templates from the centrifuged deposit. Importantly we have shown that \( N. \) meningitidis DNA deteriorates during storage at 4° C albeit slowly, and that early testing of CSF is important for diagnosis when testing templates of extracted elutes or centrifuged deposit.

In this study, we tested CSF from two cases of meningococcal meningitis where the putative organism was not cultivatable, although \( N. \) meningitidis DNA was detected in blood samples. The target DNA was detected in CSF in both cases using 10.0μL templates from the extraction and centrifuged deposit. Lower crossing points were achieved again with the extracted templates and reflected the more sensitive method in samples tested soon after collection. However, in other experiments, we have shown that the results using templates of centrifuged deposits become increasingly comparable to results using templates of extracted elutes when a greater volume of CSF sample is available for centrifugation.

We have therefore shown that centrifuged deposits can be used as templates for rtqPCR testing of CSF circumventing the need to extract (requiring at least 200μL of sample) and thereby conserving sample volume. The method explored here enables templates to be prepared from small volumes of CSF (≥100μL) and allows for residual supernatant to be available for other tests (if suitable). Further, \( N. \) meningitidis DNA can be detected when the organism is exposed to antimicrobial activity and when non-viable during storage at 4° C. It is uncertain as to how \( N. \) meningitidis DNA is detectable in centrifuged pooled CSF, saline and water when the organism is non-viable given that this method relies on the precipitation of intact cells. However, since \( N. \) meningitidis DNA concentration declines during storage, it is recommended to perform an rtqPCR assay soon after lumber puncture when testing templates derived from either extracted elutes or centrifuged deposits.

**Acknowledgements**

The authors are appreciative for the support of colleagues in the Microbiology Department (SEALS), St George Hospital, Kogarah, New South Wales.

**References**


Qiagen. 2012. QIAamp® DNA Mini and Blood Mini Hand-...
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P57 staining in molar pregnancy
Piero Nelva

Anatomical Pathology, Monash Health, Monash Medical Centre, Clayton, Victoria

Introduction
Molar pregnancy (MP), or hydatidiform mole results from aberrant fertilization and leads to an over-production of the tissue that is supposed to develop into the placenta. MP’s can occur in one of two forms:

• Partial molar pregnancy: There is an abnormal placenta and some foetal development.
• Complete molar pregnancy: There is an abnormal placenta but no foetal development.

Signs of MP can include
• Abnormal growth of the uterus
  ◦ Excessive growth in about half of cases
  ◦ Smaller than expected growth in about a third of cases
• Extreme nausea and vomiting that may result in dehydration and hospitalisation
• Abnormal vaginal bleeding during the first 3 months of pregnancy
• Symptoms similar to pre-eclampsia occurring in the 1st or early 2nd trimester (this is almost always a sign of a hydatidiform mole, as pre-eclampsia is extremely rare so early in a normal pregnancy).

Initial investigation often shows an abnormally high serum β-HCG level. Subsequent pelvic examination may show signs similar to a normal pregnancy, but the size of the uterus may be abnormal and foetal heart sounds are absent. An ultrasound may show an abnormal placenta with or without foetal development.

Treatment is removal of abnormal tissue via dilation and curettage or, in rare cases, hysterectomy. Over 80% of hydatidiform moles are benign. If untreated, hydatidiform moles can develop into invasive moles, which grow into the uterine wall. They may rupture the wall of the uterus, resulting in haemorrhage. Invasive moles are aggressive and have malignant potential, and molar tissue can spread to distant organs. Malignant complications include the development of choriocarcinoma. This aggressive cancer occurs in 15% to 20% of complete moles and in 2% to 3% of partial moles.

Case Report
In November 2013 a 31-year-old woman underwent a uterine dilation and curettage as treatment for an ultrasound-detected molar pregnancy.

The histology laboratory received suction curettings measuring 100x90x10 mm in aggregate. No foetal parts were identified. The curettings associated with a molar pregnancy are often described as being “bunch of grape-like” in appearance (Figure 1). Original macroscopic photographs from this case were not available. Representative samples of the material were processed for routine histology.

Under microscopic evaluation, the sample showed a population of abnormal placental villi. Most of these displayed circumferential, filigree-like trophoblastic proliferation (Figure 2), compared to the normal placental villi which consist of two single layers (Figure 3).

The abnormal villi also showed karyorrhectic cellular debris, also known as apoptotic cellular debris (Figure 4). The final diagnosis was of a complete hydatidiform molar pregnancy.

P57 is a cyclin-dependent kinase inhibitor associated with loss or point mutation of p57. P57 shows strong paternal genomic imprinting, resulting in expression predominantly from the maternal allele.

The p57 antibody is a nuclear stain. In normal placenta, immunoreactivity is seen in the nuclei of cytotrophoblasts (Figure 5), decidua and villous stromal cells. Syncytiotrophoblasts are negative.
Figure 1. Macroscopic appearance of molar pregnancy curettings showing the edematous, fluid filled chorionic villi. This is classically referred to as having a “grape-like” appearance. Image Copyright Jian-Hua Qiao (http://www.pathxchange.org/files/pd/users/user-2470/case-21137/images/M1.jpg).

Figure 2. Villi from hydatidiform pregnancy, showing florid circumferential hyperplasia of cytotrophoblastic layer.

Figure 3. Normal chorionic villi showing two single layers of epithelial covering.

Figure 4. Karyorrhectic debris from apoptotic cells.

Figure 5. P57 staining of normal placental chorionic villi. Staining is seen in the inner, cytotrophoblastic layer.

Figure 6. Cytotrophoblastic hyperplasia with loss of p57 staining. This is a characteristic of hydatidiform mole.
Complete hydatidiform moles are diploid and formed exclusively from paternal DNA and do not express p57 (Figure 6). By contrast, partial moles are triploid and consist of one maternal and two paternal haploid genomes. Partial moles show a normal p57 staining pattern.

Acknowledgements

Thanks to Drs S. Dayan and D. Lenghaus for their assistance in preparing this report.

Bibliography

http://e-immunohistochemistry.info/web/p57.htm
http://www.pathxchange.org/files/px/users/user-2470/case-21137/images/M1.jpg

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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: amsgqap@dspl.com.au
A case of severe anaemia in a sixteen-year-old male

Gillian Rozenberg

South Eastern Sydney & Illawarra Area Health Service, Prince of Wales Hospital, New South Wales

A 16-year-old male presented to the Emergency Department with nausea and vomiting, diarrhoea, haematuria and jaundice. He had been feeling unwell for a week prior to his admission to hospital. He was of Southeast Asian descent and gave a history of having a haemoglobinopathy.

A full blood count, electrolytes, liver function tests and LDH were performed. The results were as follows:

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<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Hb</td>
<td>76</td>
<td>RR 130–180 g/L</td>
</tr>
<tr>
<td>Hct</td>
<td>0.265</td>
<td>RR 0.40–0.54</td>
</tr>
<tr>
<td>MCV</td>
<td>75.3</td>
<td>RR 80–100 fl.</td>
</tr>
<tr>
<td>MCH</td>
<td>21.6</td>
<td>RR 26.5–33.0 pg</td>
</tr>
<tr>
<td>WBC</td>
<td>9.7</td>
<td>RR 3.5–11.0 x 10⁹/L</td>
</tr>
<tr>
<td>Plt</td>
<td>111</td>
<td>RR 150–400 x 10⁹/L</td>
</tr>
<tr>
<td>Retic %</td>
<td>1.6</td>
<td>RR 0.2–2.0%</td>
</tr>
<tr>
<td>Retic Abs</td>
<td>55.1</td>
<td>RR 20–80 x 10⁹/L</td>
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<tr>
<td>Urea</td>
<td>8.2</td>
<td>RR 2.9–7.1 mmol/L</td>
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<tr>
<td>Creatinine</td>
<td>132</td>
<td>RR 60–110 umol/L</td>
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<tr>
<td>Bilirubin Total</td>
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<td>RR 0–25 umol/L</td>
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<tr>
<td>LDH</td>
<td>4430</td>
<td>RR &lt;250 IU/L</td>
</tr>
</tbody>
</table>

The above results demonstrated severe haemolysis. A direct antiglobulin test (DAT) was performed with a negative result.

The blood film showed a red cell picture that was consistent with oxidative haemolysis with a marked number of bite cells present.

A urinary haemosiderin, a test of intravascular haemolysis, was also performed. The result was positive confirming the patient in this case study had intravascular haemolysis.

A glucose-6-phosphate dehydrogenase (G6PD) screen was also performed. The result showed ‘deficient’ activity. This was a case of G6PD deficiency post exposure to an oxidant.

The patient was now passing ‘black’ urine. The serum in the chemistry specimen was also ‘black’. Note that the reticulocyte count was normal. Haematopoiesis was unable to compensate for the rapid red cell destruction.

G6PD is the red cell enzyme which catalyses the first step of the pentose phosphate pathway which in turn leads to the formation of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). G6PD/NADPH protects red cells against damage from oxidising free radicals that cause oxidative damage.

G6PD deficiency is inherited as an X-linked recessive disorder. Symptomatic patients are almost exclusively males however should a female have one affected X chromosome she may also be symptomatic to the same degree as the male.

G6PD deficiency occurs most commonly in African, Middle Eastern and Southeast Asian populations.
Individuals with G6PD deficiency have the potential to demonstrate a severe non-immune haemolytic anaemia when subjected to certain causes, namely, infection, drugs, chemicals and certain foods such as fava or broad beans. The drugs include antimalarial drugs such as primaquine and chloroquine; sulphonamides such as sulphanilamide and sulfamethoxazole and some non-sulpha drugs such as dapsone and the antibiotic Bactrim. Neonates, especially premature neonates, are susceptible to naphthalene present in moth balls. All of the above are oxidants capable of inducing a haemolytic anaemia referred to as oxidative haemolysis. Haemoglobin is denatured, transformed from a ferrous (Fe^{2+}) to a ferric (Fe^{3+}) state resulting in the formation of a Heinz body. Heinz bodies, together with some of the red cell membrane and red cell content are removed as they pass through the spleen resulting in the formation of a bite cell. Should the red cell membrane re-connect after the Heinz body has been removed, a blister cell will result. Thus bite and blister cells characterise oxidant haemolysis.

As the patient in this case study gave a history of having a haemoglobinopathy and his full blood count showed a low MCV and MCH, haemoglobin electrophoresis was performed. The results were as follows:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
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<tr>
<td>HPLC</td>
<td>Peak suggestive of HbH</td>
</tr>
<tr>
<td>HbA2</td>
<td>&lt;1.0 RR 2.0–3.5%</td>
</tr>
<tr>
<td>HbF</td>
<td>1.0 RR &lt;1%</td>
</tr>
<tr>
<td>HbH inclusions</td>
<td>Positive</td>
</tr>
<tr>
<td>Isopropanol instability</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Haemoglobin electrophoresis was diagnostic of haemoglobin H (HbH) disease.

Under normal conditions, patients with HbH disease have haemoglobin levels ranging from 90–100 g/L. During a haemolytic crisis the haemoglobin level may drop significantly; the patient can develop shock and renal shutdown. HbH disease is prevalent in African, Middle Eastern and Southeast Asian populations.

The patient in this case study spent a considerable amount of time in the renal unit at the Prince of Wales Hospital. The oxidant to which he was exposed is unknown however it was thought to be fava beans in a casserole his mother prepared for him.
ELECTION OF PRESIDENT AND DIRECTORS 2014
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 2014 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 18th JULY 2014
AIMS National Scientific Meeting Program

As this is AIMS centenary year, other professional societies were asked to join our celebrations by nominating an eminent presenter to speak on relevant topics. We are delighted to include these speakers in our program and thank the Australian Society of Cytology, the Fertility Society of Australia, the Australian Society for Microbiology, the Australasian Association of Biochemists, the Australasian Society of Thrombosis and Haemostasis and the Human Genetics Society of Australasia.

Thursday 4th September, 2014

"PAST"

Focus - Long standing diseases

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<td>9:30 AM</td>
<td>Official opening</td>
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<td>10:30 AM</td>
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<td></td>
<td>Professor Matthew Brown</td>
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<td>Director, The University of Queensland Diamantina Institute</td>
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<td>The history of haemophilia</td>
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<td>Dr Kevin Bisgard</td>
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<td>Heart disease - old or new?</td>
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<td>Assoc Prof Tony Badcock</td>
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AIMS National Scientific Meeting Program

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Friday 5th September, 2014
"PRESENT"

Focus - Obstetric and neonates, World pathology

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<td>AIMS AGM</td>
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<td>The diagnosis of acute leukaemia</td>
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<td>Gillian Rosenberg, Charnaine Marsh &amp; Pauline Daltell</td>
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<td>Paediatric tumours</td>
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<td>Treatment of childhood malignancies</td>
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<td>Aditi Vedi</td>
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<td>5:30 PM</td>
<td>SJOG Pathology Outreach program in East Timor</td>
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<td></td>
<td>Kyle Jackson Brown</td>
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<td>7:00 PM</td>
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<td>Conference Dinner</td>
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AIMS National Scientific Meeting Program

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Saturday 6th September, 2014

"FUTURE"

Focus - Cancer markers, genomics, lab management and systems

<table>
<thead>
<tr>
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<th>Time To</th>
<th>Activity</th>
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<tr>
<td>9:00 AM</td>
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<td>New diagnostics for cancer</td>
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<td></td>
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<td>Prof. Sandra O’Toole</td>
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<tr>
<td>9:30 AM</td>
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<td>Genomic sequencing</td>
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<td>TBC</td>
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</tr>
<tr>
<td>11:00 AM</td>
<td>11:40 AM</td>
<td>Gene assay testing: what is being tested for, the applicability and the implications</td>
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<td>Masterclass 5 (continued)</td>
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Questions relating to ‘A novel approach to testing Neisseria meningitidis in small volumes of CSF by a real-time quantitative PCR assay’, page 42 of this issue.

1. Real-time quantitative PCR (rtqPCR) assays are commonly used for rapid diagnosis of meningococcal meningitis. True/False

2. The viability of *N. meningitidis* in CSF naturally does not decline over time when stored in air at 37 ºC, 4 ºC and at room temperature. True/False

3. Early treatment of suspect cases with antibiotics often prevents the cultivation of *Neisseria meningitidis* in the CSF. True/False

4. Small reaction volumes of ≤ 50 μl are commonly used in diagnostic rtqPCR assays and are vulnerable to experimental error, which may compromise detection of low levels of target DNA. True/False

5. No significant difference in crossing points was detected in assays using either 5.0 or 10.0 μl spiked-extracted templates in 15 CSF samples (paired t-test = 0.7937). True/False

6. The authors showed that increasing the template volume from 5.0 to 10.0 μl for CSF had no effect on the PCR efficiency. True/False

7. The ability of the molecular assay to detect low levels of *Neisseria meningitidis* is essential for accurate diagnosis. True/False

8. The 10.0 μl template is also suitable for EDTA blood sample testing. True/False

9. The target DNA was detected in CSF in two cases using 10.0 μl templates from the extraction and centrifuged deposit, even though the organism was not able to be cultivated. True/False

10. Target DNA was not detected by rtqPCR in pooled CSF, saline and water after 16 day storage at 4 ºC. True/False

Name: ________________________________________________________________

Email: ________________________________________________________________

Please photocopy this page or print it from the electronic AJMS which is stored in the AIMS 'Member centre' under the heading 'Journal' at www.aims.org.au. Circle your answers then post, fax or scan and email to us:

AJMS APACE Questions, AIMS National Office, PO Box 1911, Milton Qld 4064. Facsimile: 61 7 3876 2999
Questions relating to ‘P57 staining in molar pregnancy’, page 50 of this issue.

1. A complete molar pregnancy will require some foetal development. True/False

2. There is smaller than expected growth in about half cases. True/False

3. Pre-eclampsia is extremely rare in the early 2nd trimester. True/False

4. In molar pregnancy there will often be abnormally high serum β-HCG levels. True/False

5. Treatment of molar pregnancy often results in hysterectomy. True/False

6. Aggressive cancer development is more common in partial moles. True/False

7. Abnormal villi can show apoptotic cellular debris. True/False

8. In normal placenta, immunoreactivity is not seen in the nuclei of villous stromal cells. True/False

9. Complete hydatidiform moles are formed from paternal DNA and do not express p57. True/False

10. Partial moles consist of one maternal and one paternal haploid genomes. True/False

Name: _________________________________________________________

Email: _________________________________________________________

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AJMS APACE Questions, AIMS National Office, PO Box 1911, Milton Qld 4064. Facsimile: 61 7 3876 2999
YEAR 2014

JULY 19–20
AIMS Tasmanian Branch Annual Meeting
The Riverfront Motel and Villas,
11 Strathaven Drive
Rosetta   TAS   AUSTRALIA

JULY 20–25
AIDS 2014 - 20th International AIDS Conference
Melbourne   VIC   AUSTRALIA
www.aids2014.org/

AUGUST 7–9
AIMS/RCPA QAP Haematology Morphology Workshop (II)
7th: Malaria & Introduction to microscopy component
8th & 9th: Morphology component
Sydney   NSW   AUSTRALIA

AUGUST 8
Allied Health Victorian CCC Cancer Care Symposium
Ella Latham Lecture Theatre
Parkville   VIC   AUSTRALIA
Queries can be addressed to: carol.jewell@mh.org.au

SEPTEMBER 4–6
AIMS National Scientific Meeting 2014
Celebrating the Centenary of AIMS
Rydges World Square
Sydney   NSW   AUSTRALIA
http://www.aomevents.com/AIMS2014

SEPTEMBER 14–18
15th International Conference on Systems Biology (CSB)
Melbourne   VIC   AUSTRALIA
www.icsb14.com

OCTOBER 3–6
Australian Society of Cytology
44th Annual Scientific & Business Meeting
"Cytology on the Frontier"
Darwin Convention Centre
Darwin, NT, Australia
The Society will be holding the 2014 Tutorial after the Scientific Meeting on 7-10 October.
http://www.cytology-asc.com/meetings/index.htm

OCTOBER 11–12
Australian Bioinformatics Conference
Melbourne   VIC   AUSTRALIA

OCTOBER 19–22
Haematology Society of Australia & New Zealand,
The Australian & New Zealand Society of Blood Transfusion & The Australasian Society of Thrombosis & Haemostasis
Perth Convention centre
Perth   WA   AUSTRALIA
http://www.haa2014.com/

OCTOBER 27–29
Australasian Association of Clinical Biochemists
52nd Annual Scientific Conference
Adelaide SA   AUSTRALIA

OCTOBER 28–31
AusBiotech 2014
Gold Coast   QLD   AUSTRALIA

NOVEMBER 3–7
Association of Biosafety for Australia & New Zealand
Softiel
Sydney   NSW   AUSTRALIA

NOVEMBER 10–12
Lab Management Conference 2014
Venue TBA
Sydney   NSW   AUSTRALIA

NOVEMBER 16–19
Australian Health and Medical Research Congress
Melbourne   VIC   AUSTRALIA
www.ahmrcongress.org.au/

DECEMBER 1–5
Australasian Society for Immunology 44th Annual Scientific Meeting
Wollongong   NSW   AUSTRALIA
www.asi2014.org/

DECEMBER 6–9
American Society of Hematology
San Francisco   USA
http://www.hematology.org/Meetings/Annual-Meeting/11522.aspx

YEAR 2015

JUNE 20–25
International Society of Thrombosis and Hemostasis
Toronto   CANADA
http://www.isth.org/?page=ISTHCongresses
BOOKS FOR REVIEW

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.

As per our agreement with the book publishing companies, complimentary books are submitted to the Institute provided that all reviews are published in the Australian Journal of Medical Science. These reviews must be of a high quality as buying decisions and the reputation of the book and author are important considerations.

Books not requested will be allocated at discretion of the Editors for the Australian Journal of Medical Science. Reviews should be 300 to 700 words depending on the volume of the book. Time limit for return of review is six weeks.

Unfortunately AIMS is unable to send books overseas.

Please send your request to: Australian Institute of Medical Scientists  PO Box 1911  Milton  Qld  4064
Tel: (07) 3876 2988  Fax: (07) 3876 2999  Email: aimsnat@aims.org.au

Latest additions:


25. Knowing One's Medical Fate Challenges for Diagnosis and Treatment, Philosophy, Thics and Religion edited by G. Pfleiderer, M. Battegay & K. Lindpaintner. Karger. vi + 122 pages.


Yearbook of Pediatric Endocrinology 2013
Edited by K. Ong & Z. Hochberg
Karger 2013
Hard Cover xii + 258 pages
ISBN: 978-3-318-02506-4
RRP: USD $82.00

This book is a collection of excerpts from journal articles from 2013. It is edited by two primary editors (Ken Ong and Ze’ev Hochberg), and a number of associate editors.

The chapters are topic based, and include 12 sections, plus chapters titled Editor’s Choice and Science & Medicine, followed by the author and subject index.

The 12 Sections cover; Neuroendocrine, Pituitary, Thyroid, Growth and Growth factors, Growth Plate, Bone and Mineral Metabolism, Reproductive Endocrinology, Adrenals, Oncology and Chronic Disease, Type 1 Diabetes, Obesity, Type 2 Diabetes, Metabolic Syndrome and Lipids.

This is the 10th edition and the Editor’s preface state they “look forward to playing a role in the dissemination of new discoveries and the discussion of controversies and topical issues”. So it was with this in mind that I tackled the book.

Each article is a summary of the full version, encompassing headings of; Background, Methods, Results and Conclusions, followed by a Summary of Findings.

The majority of articles are heavily genetics based and are beyond the scope of the general endocrinology laboratory. However, there are a variety of more general review articles that are much more relevant, including:

- Bioinactive ACTH causing glucocorticoid deficiency
- Cushing syndrome in Paediatrics
- Assessment of iodine nutrition in populations: past, present, and future
- Generic and brand-name L-thyroxine are not bioequivalent for children with severe congenital hypothyroidism
- Three-year follow-up results of bone mineral content and density after a school-based physical activity randomized intervention trial
- Androgen Insensitivity Syndrome
- A novel tool in the diagnosis and follow-up of (cyclic) Cushing’s syndrome: measurement of long-term cortisol in scalp hair
- The assessment of cortisol in human hair: associations with sociodemographic variables and potential cofounders
- Reduced cortisol metabolism during critical illness
- Childhood obesity in developing countries

The chapter on Oncology and Chronic Disease includes a number of articles investigating endocrine repercussions following treatment of childhood cancers.

Given the majority of articles focus heavily on the genetics of endocrine disease, this book would most benefit research scientists, or medical students specialising in Paediatric Endocrinology. The benefit of purchasing this book, would be that it brings together all the cutting edge research into one book, together with its original publishing journal, thereby allowing the reader to easily access the full article of interest.

Christine Gorringe MAIMS

The Road to Good Nutrition - A Global Perspective
Karger 2013
Soft cover 209 pages
ISBN: 978-3-318-02549-1
RRP: USD 68.00

The Road to Good Nutrition is like a Lonely Planet guidebook for those that wish to quickly gain knowledge on this multi-faceted subject.

It is written by a panel of experts who have compiled an easy to digest summary of the world’s nutritional status and the challenges that are faced in trying to improve it. The book aims to provide information on nutrition to the lay person and those who require or already have some knowledge; primarily those who work in public health, government or development.

It is arranged into 13 chapters that are clear, concise and packed with case studies, illustrations, photographs, tables, diagrams and quotes from researchers and world leaders. Each chapter begins with a box outlining the key messages and concludes with the personal views of each chapter author as well as references for further in-depth reading. Key definitions and statistics are contained throughout and facilitate first-pass clarity.

A wide and varied array of topics are addressed; stunting, food security, hunger, obesity, economics of nutrition, ways to improve nutrition, governance, advocacy, innovation, society and the evolving world of nutrition. An extensive index allows readers to easily
locate the wide and varied topics that fall under these main subject areas.

It delivers content in sufficient detail and avoids becoming too technical or too in depth.

This text serves as an excellent reference book for those already working in public health or development, particularly in developing country settings. It would also be a great text for students of policy, development studies, public health, nutrition and dietetics or sociology.

Anthony Draper MAIMS

Post-Genomic Cardiology
José Marín-García
Academic Press

Hard cover VIII + 684 pages
ISBN: 978-0-12-373698-7
RRP: AUD$200.00

The recent advancement of molecular biology (MB) in cardiovascular medicine can provide powerful insights into the aetiology and pathophysiology of a range of cardiovascular diseases (CVDs); and more importantly, leading to more effective diagnostic and therapeutic practices thereby reducing mortality and morbidity rates. The biggest impact thus far is the identification of genes responsible for both common and rare forms of cardiovascular anomalies. The 2007 edition of Post-Genomic Cardiology fills this vital gap in our knowledge of the MB of the heart, particularly in the basic underlying mechanisms of most CVDs. The aim of this publication is to present the current knowledge of cardiology at the cellular and MB levels. The text enables both a broad audience, including readers who are not routinely practising molecular techniques, to appreciate the current issues from a molecular perspective. The text is authored by a subject matter expert from The Molecular Cardiology and Neuromuscular Institute (TMCNI), Highland Park, New Jersey: José Marín-García, Director of TMCNI with the collaboration of MJ Goldenthal, TMCNI and GW Moe, University of Toronto. The publication is divided into 11 parts (Sections I–XI) and comprises 20 chapters in total. It has a table of contents (pp v–vi), a subject index (pp 657–680), a glossary section (pp 637–655) and is supported by a sufficient number of tables (95 tables) and figures (81 monochrome and 6 colour) throughout the text.

The information is broadly organised into four main topic areas. First, Sections I and II (Chapters 1–7), the fundamental and recent knowledge and techniques of MB are clearly explained and reviewed accordingly. Second, Sections III to VII (Chapters 8–17), followed by comprehensive analysis and discussions on the use of these techniques to identify the aetiology and pathophysiology of the major types of CVDs. Third, Sections VIII and IX (Chapters 18–19), the latest influential information on the contribution of gender factors and ageing processes to CVDs is presented and discussed. Fourth, Section X (Chapter 20), the present and near future diagnostic and therapeutic options available for the treatment of CVDs are presented for references.

Sections I and II (pp 3–208) cover the necessary background information required for understanding both cellular and MB, and the principles of recent techniques that are being used to identify abnormalities associated with the presence of CVDs. The fundamentals such as the gene structure, transcription and translation, are covered for readers. This is followed by an overview of standard methods that have been used to study cardiovascular genes and their expression. Genetic engineering techniques using restriction endonuclease digestion, followed by a range of cellular techniques are reviewed and discussed. Other routine techniques such as the use of gene profiling, Southern and northern blot hybridisation, metabolic imaging of cardiomyocytes and two-dimensional electrophoresis are evaluated. Finally, several developing techniques such as temperature gradient capillary electrophoresis and molecular beacons are presented. These seven chapters provide useful background for medical scientists (MSs) who wish to revise their cellular and MB knowledge, especially to those who do not routinely use these procedures. The explanation pitched at about the level of a third year undergraduate, so prior knowledge in this area is required for reading these chapters.

Sections III to VII (pp 211–552) cover the molecular, genetic and cellular events that are contributing factors to CVDs. The recent identification of susceptibility and effector genes that are related to CVDs is just the beginning in cardiology. It is important to note that it is highly likely multiple genetic factors and complex gene-environment interactions are actively involved in the cardiovascular disease processes. Specific CVDs included in these chapters include atherogenesis, thrombosis, coronary artery disease, myocardial infarction, acute ischaemia, vasculogenesis, angiogenesis, arteriogenesis, hypertension, cardiomyopathies, myocarditis, heart failure and cardiac dysrhythmias. These chapters provide valuable information for MSs who are interested in the expression of specific genes and their subsequent proteins in modulating specific disorders that lead to CVDs. The roles of these identified genes remain to be analysed extensively in further studies.

Sections VIII and IX (pp 555–616) cover gender differences and cardiac ageing in the aetiology of CVDs. It is established that the sex steroid hormones, especially oestrogen, progesterone and testosterone, play influential roles; however the specific molecular mechanisms underlying the gender and ageing factors in the incidence remains poorly understood. These two short chapters provide a bigger picture for MSs who appreciate the area of endocrinology, especially at the cellular and physiology levels.
Section X (pp 619–636) covers the future frontiers in post-genomic cardiology. The areas include proteomics, biomarkers, systems biology and mitochondrial medicine. The area of cardiac disease biomarkers provides the most relevant subject for MSs. Recent discoveries of biomarkers have indicated that they can be very useful in providing information in pathological, diagnostic and prognostic indications and risk stratification for patients. Several key examples are: ischaemia-modified albumin and myeloperoxidase in the diagnostic indication of acute coronary syndrome; troponin I, troponin T, heart-fatty acid binding protein for myocardial infarction; and soluble fibre as early marker of ischaemia in unstable angina. The continual combined use of biomarkers increases the reliability of cardiovascular disease diagnosis. This chapter is particularly well-written highlighting the usefulness of cardiac biomarkers. MSs should find this chapter contains a much better explanation than most clinical biochemistry publications.

Section XI 'Glossary' (pp 637–655) offers a comprehensive compilation of commonly accepted definitions and abbreviations that are used throughout the text. Most basic MB terms, such as exon, pseudogenes, restriction endonucleases and trans-acting elements, are all clearly defined and explained; and acronyms, such as BER, DISC, OH and YAC, are stated in full along with appropriate levels of explanation.

This book merits a grade of ‘high distinction’ in accordance with my criteria for assessment. The author conveys an authentic and engaging passion for his subject throughout the text, and consistent standards of excellence are displayed throughout. There is a wide range of relevant information gathered thorough the book; a total of 4,716 references are available for further reading. The book identifies the most relevant concepts and information, presenting them systematically and insightfully. The text is an excellent example of formal academic style and language used effectively. Precision and objectivity are retained while the clear and logical flow of the text makes it very readable. However, there are two weaknesses identified. First, the references could have been presented in a smaller font size to reduce the number of pages and the physical weight of the book by at least 10%. This is a common issue in many publications. Second, the accessibility of the text would be improved if the numerous acronyms and abbreviations used were listed separately rather than simply incorporated into the general glossary.

The first edition of Post-Genomic Cardiology addresses the topic well and shows evidence of sound expertise and critical analysis. It is recommended for MSs wanting to revise their knowledge in cellular and MB, and gain further insight into the world of molecular cardiology. However, it should be noted that the information presented by this publication is current up to 2007 only. There have been major advances in MB in the past five years, therefore readers may find that the text is not as up-to-date as they would like. Overall, this book is a valuable reference for MSs who routinely practise cardiology at bench level and for MSs who wish to keep current on the continual development of molecular techniques in pathology of the heart.

Dennis Mok MAIMS

Australia’s Poisonous Plants, Fungi and Cyanobacteria - A Guide to species of Medical and Veterinary Importance

Ross McKenzie

CSIRO Publishing 2012

Hard cover 976 pages


RRP: AUD$195.00

This book fills a gap in the toxicology realm in Australia. Previously there was Poisonous Plants of Australia by S.L. Everist in 1981 and more recently Pretty But Poisonous by R.C.H Shepard (2004). Everist’s book is out of print and Ross Shepard’s book, although good, (aimed at the person in the street) is not as comprehensive as Ross McKenzie’s book. Ross Shepard produced a Handbook in 2010 but it is not quite as comprehensive as Ross McKenzie’s book, but still a worthwhile text. There are numerous small fact sheets produced on this subject but they are just pamphlets with a narrower focus. The national herbarium started a project in 1984-85, but did not complete this. This work can be seen at http://www.cpbr.gov.au/poison-plants/index.html. The Therapeutic Guidelines book Toxicology and Wilderness concentrates on animals, drugs and chemicals and although it covers toxidromes does not address poisonous plants and fungi, and Cyanobacteria, in much detail. The online database http://www.toxinology.com/# is useful but you need to know the name of the plant or group for it to be fully useful in its clinical application and does not have the Australian geographic distributions of plants.

The book provides management and therapy, but as the book acknowledges itself, it should be used in conjunction with a continuously updated database such as TOXNZTM for clinical management. It also rightly directs people to the poisons information helpline for help.

The structure of the book is as follows:

The warning part of the book which provides the bounds of the book both professionally and legally.

Using this book which I would stress is mandatory to get the most out of the book; it gives the meaning of the pictograms, the structure of the description for each plant. Identification danger and distribution
Chapter 1 is an introductory chapter on plants and plant poisoning, which includes some details on profiling of susceptible individuals and groups, both human and animal.

Chapter 2 gives information on confirming tentative identifications, including the contact details of the state herbariums and how to collect and handle specimens for identification.

Chapter 3 describes the common poisoning profiles associated with plants fungi and blue green algae. The profiles are listed and a pictogram box for summary is included. The plant sources are listed followed by a description of the profile, followed by post mortem changes and treatment.

After the first three chapters comes, as I would describe it "the meat of the book". Part 1 Poisonous Cyanobacteria, Part 2 Poisonous Fungi and Part 3 Poisonous Vascular Plants.

All three chapters follow the sequence described in the section 'using this book'. All descriptions are accompanied by numerous good, clear photos and a distribution map except for the Poisonous Cyanobacteria section.

This part of the book has a high visual appeal and the pictures and geographical locations described would aid to the identification of the plant in question.

The book concludes with what I would consider the "dessert of the book".

Chapter 15 is the 'Digest of poisonous Cyanobacteria, algae, slime mould, macrofungi and plants in Australia'. Basically this chapter is a big table listing all of the entries, their scientific name, common name, toxin/s present, animals at risk, with the weight of evidence of their toxicity and their degree of danger. This provides a good summary of parts 1, 2 and 3.

This is followed by Appendix 1: Aids to identifying flowering plants, which provides a useful chapter on identification.

Appendix 2 is the top killers, or in another phrase the usual suspects in relation to human and animal deaths.

Appendix 3 is another chapter which helps in profiling and relates to poisoning hot spots in the home, in the garden and on the land.

Appendix 4 is what I would consider the most useful profiling chapter and is animals and the major species that poison them. This will lead the vet clinician to the most likely culprits in relation to poisoning.

Appendix 5 is the body systems affected by the major poisonous species. This is useful as in many cases the plant does not accompany the patient, and by asking the parent/carer/pet owner do you have any of these in your garden/property can be a clue to the poisoning.

Appendix 6 is a breakdown by state with regards to the major poisonous species; an example here would be that it is unlikely to find black soil blindness coral fungus outside the Northern territory of Western Australia.

A glossary follows explaining unfamiliar terms found in the book.

References and further reading follows after this and provides an excellent reference list with comments.

The index finishes off the book and should be used as a first point of call if the common or scientific name of the plant/fungi or blue green algae is known.

A useful part of the book is the pictographic representations of the toxicity and therapeutic actions available. Each species is accompanied by a distribution map which is accompanied by text on its geographical location. This book is designed to help in the identification of poisonous plants, fungi and blue green algae. It does this well and thoroughly for both people and animals.

In conclusion I would thoroughly recommend this book to any rural accident and emergency department, veterinarian, toxicologist (clinical and non-clinical). Farmers would also find this book useful. The book, is in my assessment, the most current up to date and comprehensive book on poisonous plants in Australia. If asked for ways to improve this book I would say future editions are required, as the geographical distribution of the plants will change over time, i.e., travel of plants and weed along highways, and stock routes. The only thing I would like to see in future editions would be a clear plastic template/overlay to go over the distribution maps found throughout the book including major cities and rural centres so we know where the dots are in relation to these centres.

Gary Smith MAIMS
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/ljieweb.pdf) using standard abbreviations from the ISSN List of Title Word Abbreviations (see: http://www.isssn.org/en/node/344) All authors should be given in the reference list.

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

Examples of the correct form for references are given below:
i) Journal Reference:

ii) Personal Author(s) of a book:

iii) Editor, Compiler, Chairman as Author:

iv) Chapter in Book:

v) Online documents:

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

* † ‡ § ¶ ** ††

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

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

Illustrations

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

When plotting points, the following symbols are preferred:

\[
\begin{array}{c}
\bigcirc \quad \triangle \quad \Delta \quad \square \quad \blacksquare
\end{array}
\]

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.

<table>
<thead>
<tr>
<th>Commonly used abbreviations</th>
<th>Standard Units of Measurement</th>
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<td>or Symbol</td>
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Additional information

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


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