AUSTRALIAN JOURNAL OF MEDICAL SCIENCE

2009

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Hereditary spherocytosis

TECHNICAL NOTE
Coagulase-negative Staphylococcus bacteraemia evaluation

HAEMATOLOGY UPDATE
A case of haemolytic anaemia occurring in a young child

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Introduction

Hereditary spherocytosis (HS) is considered to be the most common of the hereditary haemolytic anaemias among people of Northern European ancestry. It is also the most frequent cause of inherited chronic haemolysis in North America with a quoted incidence of 1 in 5000 births (Bolton-Maggs 2004). However, studies of osmotic fragility in blood donors suggest the existence of extremely mild or subclinical forms, raising the prevalence of HS to 1 in 2000 (Bolton-Maggs et al 2004). HS has also been found in other ethnic groups (in Africa, Algeria, Tunisia, Egypt, Japan, North India and Brazil); there are only rare cases reported in the black population (Bolton-Maggs et al 2004). While the disease is encountered worldwide, its incidence and prevalence in other populations such as in Australia, are not clearly established (Gonzalez 2006).

Clinical manifestation of hereditary spherocytosis

Anaemia, jaundice and splenomegaly, singly or together, are the most commonly encountered of the clinical features of HS. While two thirds of patients present in childhood with mild to moderate anaemia, signs and symptoms of the disease are highly variable, both with respect to age of onset and severity. As such, the degree of haemolysis varies widely, from severe transfusion-dependent patients to asymptomatic patients who are diagnosed incidentally.

At one end of this broad spectrum are those who present in infancy, often with severe neonatal jaundice and subsequent transfusion dependent anaemia. The anaemia and hyperbilirubinemia may be of such magnitude as to require exchange transfusion in the neonatal period. While the severity of the jaundice does not necessarily predict a severe subsequent course, children with severe hereditary spherocytosis (rare, about 5%) are constantly anaemic, and may be transfusion dependent, especially in the first few years of life (Bolton-Maggs 2004).

In asymptomatic patients, who comprise a subset of about 25%, the disorder may escape clinical recognition altogether, or, occasionally, HS can be detected for the first time in late life as a result of a family survey conducted
to document the hereditary nature of haemolytic disease in a younger relative. The compensated haemolytic state of asymptomatic patients may be diagnosed as late as the fourth to seventh decades of life (Smedley and Bellingham 1991).

**The erythrocyte membrane**

Before the pathogenesis of HS as related to abnormal erythrocyte cytoskeletal structure is described, it is necessary to review the structure of the normal red cell membrane. The red cell membrane is a small structure, less than 0.1% of the cell’s thickness and only approximately 1% of its weight. It is a multi-component structure that is responsible for many of the physiological functions and mechanical properties of the cell. The red cell membrane comprises a lipid bilayer, integral membrane proteins and a membrane skeleton (Tse and Lux 1999) (Fig. 1).

**Figure 1.** Schematic model of the red cell membrane, with the vertical and horizontal interaction of its components indicated. The relative position of the various proteins is correct, but the proteins and lipids are not drawn to scale (reproduced by kind permission of Blackwell Publishing from Tse and Lux 1999, Fig. 1, p. 2).

The red cell membrane skeleton is a multi-component complex formed by structural proteins including α- and β-spectrin, ankyrin, protein 4.1 and actin. The membrane skeleton proteins interact with the lipid bilayer and transmembrane proteins to confer strength and integrity upon the red cell membrane (Tse and Lux 1999). They also interact with each other to form scaffolding on the inner surface of the lipid bilayer. The abnormal morphology and shorter lifespan of the red cells in HS are attributable to a deficiency or dysfunction of one of the constituents of the red cell cytoskeleton (Delaunay et al 1996). Erythrocyte membranes from HS patients demonstrate qualitative and/or quantitative abnormalities of these proteins, most commonly combined spectrin and ankyrin deficiency, followed by band 3 deficiency, isolated spectrin deficiency, and protein 4.2 deficiency (Gallagher 2005).

Alpha and β-spectrin interact side-to-side to form flexible rod-like heterodimers which self-associate head-to-head to form tetramers. The tetramers are linked by ankyrin to the cytoplasmic domain of the integral membrane protein band 3. Protein 4.2 binds to band 3 at the same position and may enhance the ankyrin-band 3 interaction. Multiple spectrin tetramers interact at their tail ends and with actin protofilaments, tropomyosin, tropomodulin and adducin to form junctional complexes. Protein 4.1, which also binds to the integral membrane protein glycoporphin C, interacts with β-spectrin at the actin-binding domain and increases the affinity of the spectrin-actin binding. The primary molecular defects in HS reside in membrane skeleton proteins, particularly the proteins whose “vertical interactions” connect the membrane skeleton to the lipid bilayer: spectrin, ankyrin, protein 4.2, and band 3 (anion exchange protein) (Handin et al 2003).

**The genetics of hereditary spherocytosis**

As mentioned above, HS exhibits considerable phenotypic heterogeneity. The disease demonstrates both dominant and recessive phenotypes and affected cases are often compound heterozygotes. In early genetic studies, Morton and co-workers (1962) cited autosomal dominance as the inheritance pattern. More recently autosomal dominance has been attributed to occur in seventy five per cent of cases and it is thought that a major proportion of the remaining cases represent de novo mutations (Smedley and Bellingham 1991). A very small group of patients exhibiting autosomal recessive inheritance have also been identified recently. As a general rule, those patients homozygous for the autosomal recessive genotype have a severe clinical course.

The autosomal dominant disease produces a wide clinical spectrum (Smedley and Bellingham 1991) but the link between clinical severity and inheritance is complex, and there is a degree of overlap between these two broad groups. Homozygotes for dominant HS are very rare; from the frequency of the disease, one would estimate that they should occur approximately once in 100 million births (Handin et al 2003).

In approximately 20% to 25% of HS cases, both parents are clinically normal, although some of these patients have an autosomal recessive form of the disease.

The disease phenotype is represented by a variety of different clinical and genetic lesions. The variation in the clinical picture is a reflection of pronounced heterogeneity in the genetic basis of each of the cytoskeleton abnormalities. HS is caused by mutations in at least five genes presented below in order of frequency:

1. The ANK1 gene (encoding ankyrin 1)
2. The EPB3 gene (encodes for protein AE1, or Band 3)
3. The SPTB gene (encodes for the spectrin β-chain)
4. The ELB42 gene (encodes for protein 4.2)
5. The SPTA1 gene (encoding spectrin α-chain)

The main features of these genes are provided in Table 1. Very different molecular mechanisms can generate the same membrane protein deficiency in HS. Most of the reported protein gene mutations in HS are ‘private’ or
Sporadic occurrences, i.e. they are specific to one family or found in a few families from different countries.

**Spectrin defects**

In general, HS caused by α-spectrin defects is a recessive trait, and HS due to β-spectrin mutations is a dominant trait. This is due to the fact that α-spectrin exceeds β-spectrin synthesis by approximately three or four to one (Handin *et al.* 2003) and the excess alpha chains normally are degraded. Heterozygotes for α-spectrin defects produce sufficient normal α-spectrin to balance normal β-spectrin production. Therefore, α-spectrin deficiency would be evident only in the homozygous state, while defects of β-spectrin are more likely to be expressed in the heterozygous state, as synthesis of β-spectrin is the rate-limiting factor.

**Alpha-spectrin defects**

Defective α-spectrin production in one allele is not expected to cause a disease phenotype, and α-spectrin mutations have not been described in patients with dominant HS. Defects in both α-spectrin alleles have nonetheless been implicated in the recessive form of HS with severe spectrin deficiency (Wichterle *et al.* 1996).

The first reported α-spectrin mutation was the amino acid substitution alanine to asparagine at residue 309 of the α-spectrin chain. This variant α-spectrin chain, designated αIIA- or α-spectrin Burghill (Tse *et al.* 1997), represents a linked polymorphism. Wichterle and co-workers (1996) have described a low-expression α-spectrin variant, designated α-spectrinLEPRA (low-expression Prague) in a patient with severe non-dominant HS (Fig. 2). Alpha-spectrinLEPRA contains a splicing mutation that causes premature termination of translation and a marked decrease in α-spectrin production. The α-spectrin Burghill polymorphism is linked to the α-spectrinLEPRA mutation in a number of cases. The recessive HS phenotype is associated with either α-spectrinLEPRA homozygotes or compound heterozygotes with another α-spectrin mutation in trans (on the opposite chromosome), such as α-spectrin Prague - α-spectrin Prague encodes a truncated peptide ([Fig. 2]).

Patients with recessive HS and α-spectrin mutations are rare - probably less than 5% of HS (Handin *et al.* 2003). Such patients have moderately severe to severe HS that is sometimes transfusion dependent.

---

**Table 1.** Main features of the HS genes and the corresponding mRNAs involved. (reproduced by kind permission of the Ferrata Storti Foundation from Iolascon *et al.*, 1998, *Table 1*, p. 245).

<table>
<thead>
<tr>
<th>GeneBank accession</th>
<th>Gene symbol</th>
<th>Chromosome location</th>
<th>Gene size (kb)</th>
<th>Exon number</th>
<th>mRNA size (kb)</th>
<th>Coding sequences (amino acid number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrin α-chain</td>
<td>M61887</td>
<td>SPTA1 1q22-q23</td>
<td>80</td>
<td>52</td>
<td>8.0</td>
<td>2429</td>
</tr>
<tr>
<td>Spectrin β-chain</td>
<td>J05500</td>
<td>SPTB 14q23-q24.2</td>
<td>&gt;100</td>
<td>32*</td>
<td>7.5</td>
<td>2137</td>
</tr>
<tr>
<td>Ankyrin 1</td>
<td>X16609</td>
<td>ANK1 8p11.2</td>
<td>-160</td>
<td>42</td>
<td>9.0-7.2</td>
<td>1880</td>
</tr>
<tr>
<td>Protein AE1</td>
<td>M27819</td>
<td>EPB3 17q12-q21</td>
<td>17</td>
<td>20</td>
<td>4.7</td>
<td>911</td>
</tr>
<tr>
<td>Protein 4.2</td>
<td>M30646</td>
<td>ELB42 15q15-q21</td>
<td>20</td>
<td>13</td>
<td>2.4</td>
<td>691</td>
</tr>
</tbody>
</table>

*additional exons are spliced in within skeletal muscle (in the 3’-side of the coding sequence).*
Figure 2. Two α-spectrin mutations associated with recessive HS are shown next to a schematic representation of the α-spectrin peptide (reproduced by kind permission of Lippincott, Williams and Wilkins from Handin, Lux and Stossel, 2003, Fig. 51-30, p. 1758).

Beta-spectrin defects

Deficiency of the scarce β-spectrin chains should limit the formation of spectrin heterodimers; β-spectrin production is the rate-limiting step in biosynthesis of the membrane skeleton (Hanspal and Palek 1987). For this reason, most of the spectrin defects in HS are in the β-spectrin gene. Twenty mutations have been described, of which 19 are dominant (Handin et al 2003) (Fig. 3).

Beta-spectrin Kissimmee represents the first β-spectrin point mutation demonstrated in HS (Becker et al 1993). This de novo mutation results in a point mutation near the N-terminus of β-spectrin, close to the protein 4.1 binding site, leading to the protein being unstable and defective in its capacity to bind protein 4.1. Monoallelic expression of β-spectrin occurs frequently in HS patients with spectrin deficiency (Miraglia del Giudice et al 1998), suggesting that the defective allele is often not expressed (null mutation). Approximately 15 null mutations have been described in patients with dominant HS. The defects include initiation codon disruption, frameshift and nonsense mutations, gene

Figure 3. β-spectrin mutations associated with HS are shown next to a schematic representation of the β-spectrin peptide. Null mutations, arising from frameshifts, nonsense mutations, large deletions, or substitution of the initiation codon, are shown in shaded rectangles. Missense mutations are shown in open ellipses (reproduced by kind permission of Lippincott, Williams and Wilkins from Handin, Lux and Stossel 2003, Fig. 51-31, p. 1759).
deletions, and splicing defects. Some of these mutations result in impaired β-spectrin synthesis. Others produce unstable β-spectrins or abnormal β-spectrins that do not bind to ankyrin and undergo proteolytic degradation. Examples of such mutations include spectrin Promissão: a mutation in the translation initiation codon prevents translation of the peptide (Handin et al. 2003). In spectrins Bergen, Houston, Ostrava and Philadelphia, frameshift mutations close to the N-terminus of β-spectrin result in unstable transcripts and spectrin deficiency (Hassoun et al. 1997). In spectrins Baltimore and Tabor, nonsense mutations result in the same effect. In spectrin Durham, a 4.6-kb genomic deletion results in a truncated peptide that is inefficiently incorporated into the red cell (Hassoun et al. 1995). A splice site mutation in spectrin Winston-Salem leads to exon skipping and an unstable truncated β-spectrin peptide (Hassoun et al. 1996). Several missense mutations in the β-spectrin gene have been described in dominant HS. In most of these cases, it is not known if the mutation causes a functional defect or destabilises the mRNA or protein.

**Ankyrin defects**

HS is described in patients with a translocation involving chromosome 8 or deletion of the short arm of chromosome 8 (band 8p11.2) where the ankyrin gene is located. It follows that patients with HS and the deleted chromosome 8 are shown to have a decrease in red cell ankyrin content. Ankyrin is the principal binding site for spectrin on the red cell membrane. This globular protein, which is divided into three functional domains, provides the primary linkage between the spectrin-actin-based erythrocyte membrane skeleton and the plasma membrane by attaching tetramers of spectrin to the cytoplasmic domain of band 3 (Fig. 4).
In HS caused by ankyrin deficiency, a proportional decrease in spectrin content occurs, although spectrin synthesis is normal. In fact, 75% to 80% of patients with autosomal dominant HS have combined spectrin and ankyrin deficiency and the two proteins are diminished equally (Pekrun et al. 1993). Ankyrin defects are considered to be the most common cause of HS. McMullin (1998) states that some 70% of cases of HS are associated with ankyrin mutations. In 1995 the work of Jarolim and co-workers showed that one ankyrin allele had reduced expression in one-third of the HS patients they tested along with combined spectrin and ankyrin deficiency. This may be caused by either the reduced transcription of one of the ankyrin alleles or the instability of its mRNA. It has also been shown that de novo mutations leading to the decreased expression of one ankyrin allele are frequent in HS patients without a positive family history.

Null mutations predominate in dominant HS. These are nonsense or frameshift mutations that result in either unstable ankyrin mRNA transcripts or truncated peptides. Mutant ankyrins Bari, Bugey, Duisburg, Einbeck, Marburg, Napoli I, Osterholz and Stuttgart are truncated in the band 3 binding domain, ankyrins Anzio, Napoli II and Porta Westfalica are affected in the spectrin binding domain, and ankyrins Bovenden and Saint-Etienne 1 and 2 have premature termination within the regulatory domain (Tse and Lux 1999).

Several ankyrin defects have been found in patients with recessive HS. Unlike the null mutations found in dominant HS, these are usually missense or promoter mutations.

### Band 3 (anion exchange protein 1) defects

Band 3 is the major integral protein on the red cell membrane that interacts with the membrane skeleton. Deficiency of band 3 is found in about 20% of American and European patients with HS, but is more common in Japanese (Tse and Lux 1999). The disease is inherited as a dominant trait with relatively mild anaemia and spherocytosis. Many changes in the band 3 gene that result in null mutations have been identified (Fig. 5).
Such mutations include nonsense mutations, single nucleotide insertions and deletions, and splicing defects. These mutations presumably lead to mRNA instability and protein deficiency (Tse and Lux 1999). Missense mutations or short in-frame deletions affecting residues in the transmembrane domain of band 3 have been identified and presumably impair insertion of the mutant band 3 into the membrane. Missense mutations in the cytoplasmic domain of band 3 can interfere with its binding to other membrane skeleton proteins, resulting in a functional defect (Tse and Lux 1999).

**Protein 4.2 defects**

Partial deficiency of protein 4.2 is often seen in patients with deficient ankyrin or band 3, secondary to the underlying defect. However, some HS patients have an isolated deficiency of protein 4.2 in their red cell membranes (Tse and Lux 1999). The most common mutation is protein 4.2 Nippon (Fig. 6). This mutation is common in Japanese HS patients. It arises from a missense mutation that causes mild haemolytic disease in homozygous form in Tunisian, Portuguese, and French patients, respectively (Hayette et al 1995 (A); Hayette et al 1995 (B); Beauchamp-Nicoud et al 2000). Clinically, only a small fraction of HS is due to mutations in protein 4.2 in Europeans (probably <5%), but the proportion is much higher in Japanese (45% to 50%) (Yawata et al 2000).

**Pathophysiology**

The mechanism by which these protein abnormalities result in premature red cell destruction is not well understood. It has been suggested that the protein defects leave microscopic patches of the lipid bilayer inner surface bare of proteins. Protein-deprived areas are the starting point of microvesiculisation (Iolascon et al 1998). Small expanses of cell membrane are thus lost, reducing the cell’s surface area and impairing its flexibility. This theory is supported by the finding that in vitro incubated erythrocytes of patients with HS shed portions of membrane as microvesicles until they assume a spherical shape (Smedley and Bellingham 1991). Clearly, a prime determinant of haemolysis in these patients is splenic destruction. Proposed mechanisms include physical entrapment in splenic vessels and ingestion by cells of the mononuclear phagocyte system. Both of these theories are confirmed by morphological studies of diseased spleens with light and electron microscopy; these show red cell trapping in splenic cords and, to a lesser extent, phagocytosis by cordal macrophages (Smedley and Bellingham 1991). Smedley and Bellingham (1991) also suggest that further theories rely on the concept that there is an unfavourable environment in the spleen, which damages erythrocytes passing through it. Low pH or glucose deficiency, or both, may affect cell metabolism, while high local concentration of toxic free radicals produced by adjacent phagocytes could directly affect the cytoskeleton.

Irrespective of these clearly complex cellular events, and certainly others yet to be described, the net result is extravascular haemolysis, which is largely corrected by splenectomy (Bolton-Maggs et al 2004).

**Diagnosis**

Diagnosis of red cell membrane protein disorders, particularly HS, is normally straightforward and is based upon a combination of clinical and family histories, physical examination (splenomegaly, jaundice) and laboratory data (full blood count, especially red cell indices, reticulocyte count) and peripheral blood smear examination.

Typical HS patients have obvious spherocytes lacking central pallor on the peripheral blood smear (Fig. 7). Less commonly, only a few spherocytes are present or, in severe cases, there are numerous small, dense spherocytes and bizarre erythrocyte morphology with anisocytosis and poikilocytosis. Molecular studies have shown that specific morphologic findings are associated with certain membrane protein defects such as pincered erythrocytes (band 3), spherocytic acanthocytes (β-spectrin), or spherostomatocytes (protein 4.2). The key features are spherocytes on the blood film and a raised reticulocyte count with or without anaemia. The bilirubin level (unconjugated) is often raised, the mean cell
volume (MCV) is usually normal (even though the cells appear smaller on the blood film due to their spherical shape), and there is an increased mean cell haemoglobin concentration (MCHC). The differential diagnosis is from autoimmune haemolytic anaemia (AIHA); a direct antiglobulin test (for the detection of antibodies on the red cells) will be negative in HS and usually positive in AIHA. The clinical context is important. People with HS are usually clinically well and often have a positive family history; AIHA is rare in children and mostly associated with an acute viral infection (Bolton-Maggs 2004).

Figure 7. Spherocytes on a peripheral blood smear (reproduced by kind permission of the Massachusetts Medical Society from Bain 2005 Fig. 2(c) p.501)

The automated red cell parameter MCHC can be used to predict or identify HS with a typical clinical presentation during the routine full blood count without requiring additional laboratory tests to confirm the diagnosis. Additional testing will primarily be helpful in situations where there is an atypical morphology or a lack of family history. When the diagnostic criteria are not met other causes of haemolysis need to be excluded (Bolton-Maggs 2004).

Historically, the first-line clinical laboratory tests for screening red cell membrane disorders, mainly for the diagnosis of HS, have included the osmotic fragility test, the auto-haemolysis test, and the acid glycerol lysis test. These additional screening tests exploit the decreased surface area-to-volume ratio of spherocytic red cells by determining the extent or the rate of red cell lysis over a period of time. The osmotic fragility test, which has been regarded by Streichman and Gescheidt (1998) as the classic way to detect HS, has some disadvantages. The main one is poor sensitivity, particularly in patients with relatively mild defects where as many as 25% of cases may be missed, according to Smedley and Bellingham (1991). The acidified glycerol lysis test has provided an alternative method for overcoming problems associated with the osmotic fragility test (Smedley and Bellingham 1991). The acidified glycerol lysis method is based on the measurement of the rate rather than the extent of haemolysis. Nonetheless drawbacks remain, not only in the form of incomplete specificity (positive in pregnancy and renal failure) but in a degree of inter-operator variability in sensitivity (Smedley and Bellingham 1991). Consequently, as both of these tests do not have a high degree of sensitivity and specificity, milder and atypical cases of HS are often missed (Korones and Pearson 1989).

In 1990 Streichman, Gescheidt and Tatarsky described a new method, 100% sensitive in the diagnosis of all patients, including asymptomatic obligate carriers with hereditary spherocytosis. Unlike other methods designed for the diagnosis of HS, this test does not depend on the cells’ surface-area-to-volume ratio (Streichman, Gescheidt and Tatarsky, 1990). This diagnostic test, as stated by Streichman and Gescheidt (1998), “probably reflects the dependency of the cryohemolysis on factors that are more related to the primary membrane molecular defects and less by the surface area to volume ratio”. Despite the superior sensitivity and specificity of this diagnostic test, compared with that of the osmotic fragility test and the autohaemolysis test (Streichman and Gescheidt 1998), this form of diagnostic testing can also detect red cells with rare membrane disorders, such as aberrant band 3 proteins (i.e. some congenital dyserythropoietic anaemia type II (CDAII) and Melanesian elliptocytosis) (Bolton-Maggs 2004).

More recently, a flow cytometric method has been described for quantitating the fluorescence intensity of intact red cells after incubation with the dye eosin-5'-maleimide (EMA), which binds covalently to the anion transport protein (Band-3) at lysine-430. A reduction in fluorescence intensity is seen in patients with HS (Kedar et al 2003). Research by King and co-workers (2000) suggests that the EMA dye method is a reliable, speedy diagnostic test for HS (two hours from sample collection to result), with a sensitivity of 92.7% and a specificity of 99.1%. Cooper and co-workers (2007) also found the EMA flow cytometric assay to be both a rapid and simple test, with high levels of sensitivity and specificity (values not provided), and to be “more appropriate than the hypertonic cryohemolysis and acidified glycerol lysis assays for a routine clinical laboratory”. The use of this method as a first-line screening test for the diagnosis of HS in routine haematology would allow a prompt diagnosis of even mild HS that would permit physicians to avoid extensive and unnecessary diagnostic studies for other anaemias, an advantage recognised by Korones and Pearson (1989). Furthermore, correct diagnosis allows for more effective management of anticipated problems such as cholelithiasis and its complications, the need for splenectomy, and the examination of family members for the disease (Korones and Pearson 1989). However, it seems that with the EMA dye methodology some rare red cell disorders such as CDAII, Melanesian elliptocytosis and cryohydrocytosis can result in similar fluorescence readings to the red cells of HS – fortunately these can be distinguished from HS on the basis of their distinct clinical features (Bolton-Maggs 2004).
The British Committee for Standards in Haematology recommend both the cryohaemolysis test and the EMA binding test as screening tests in the diagnosis of HS, because of the high predictive value of both techniques (Bolton-Maggs 2004). However, the availability of a flow cytometer may be the deciding factor for choosing the EMA binding method.

**Conclusion**

HS is a familial haemolytic disorder with marked heterogeneity of clinical features. The morphologic hallmark of HS is the spherocyte, which is caused by loss of membrane surface area, and an abnormal osmotic fragility *in vitro*. Investigation of HS has afforded important insights into the structure and function of cell membranes and into the role of the spleen in maintaining red blood cell integrity. Defective erythrocyte membrane proteins can be consequent to intrinsic genetic defects. Four abnormalities in red cell membrane proteins have been identified in HS; each is associated with a variety of mutations that result in different protein abnormalities and varied clinical expression. The abundance of new information, dealing principally with molecular and genetic aspects of pathophysiology, is beginning to have implications for the investigation and management of HS.

**References**


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TECHNICAL NOTE

Coagulase-negative *Staphylococcus* bacteraemia evaluation: lack of clinical information and prevalence of methicillin resistance

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South West Pathology Service, Albury, NSW

Abstract

Coagulase-negative *Staphylococcus* (CoNS) contamination of blood cultures is well recognised. This issue is frequently faced by microbiologists and a well documented investigation of whether this contamination is device-related needs to be carried out, in case of complaints by patients. Results from three years of blood culture performed by our laboratory were reviewed to determine the prevalence and significance of methicillin-resistant CoNS. A total of 5216 sets of blood culture specimens were tested. Of these 159 grew CoNS, of which 62% were methicillin-resistant. No clinical information was provided in 73% of cases. This represents the proportion of cases where the medical microbiologist had inadequate information to decide if the isolate was a contaminant or the cause of infection. Acquiring this information would be extremely time consuming. It is important for laboratory services to continue to request that clinical information be provided on the request form. The practice of suppressing antibiotic susceptibilities and adding the comment such as “possible contaminant, sensitivities available on request” also remains a valid precaution to avoid blame in the case of a complaint.

Keywords: blood culture contaminants, clinical information, methicillin-resistant coagulase-negative *Staphylococcus*, precaution against litigation.

Introduction

Bacteraemia is a common ailment in a hospital setting. Accurate laboratory reports depend on the care taken in interpreting positive results (Magadia and Weinstein 2001). Knowledge of which organisms are frequently found as contaminants in blood cultures aids interpretation. Potential contaminants, including environmentally ubiquitous *Bacillus* spp, and skin colonisers coagulase-negative *Staphylococcus* (CoNS), *Micrococcus* spp and *Propionibacterium acnes* are usually not treated (Wilson et al. 2007). When treatment is considered, penicillinase-resistant β-lactams are administered empirical (Araya et al. 2007; Averbuch et al. 2008). However, methicillin-resistant CoNS would generally require vancomycin (Kan et al. 2003).

Patients’ families sometimes demand explanations from the health service (Greater Southern Area Health Service 2009) when nosocomial infection is proven. Therefore, laboratory staff must ask: if a complaint arise from a case of CoNS isolations from blood culture, is the laboratory fully prepared to defend itself?

CoNS do sometimes present as pathogens but are more frequently considered contaminants. The decision as to whether CoNS is present as a pathogen is fraught with uncertainty. Patients with immunosuppression and malignancy, as well as those in intensive care are at risk; especially those that have complex medical problems (Hall and Lyman 2006). Clinical signs of infective endocarditis may involve heart failure (Revilla et al. 2005). Fever, leukocytosis, leukopenia, and thrombocytopenia are common presentations in true CoNS infection. Every microbiology laboratory should be prepared to explain the interpretation of a CoNS isolation from blood cultures.

Research objective

Given that CoNS in blood cultures sometimes present as a pathogen, but most often as a contaminant (Araya et al. 2007; Falcone et al. 2007; Kan et al. 2003, Wilson et al. 2007), a three-year review of blood culture reports was performed. This was technically a health impact assessment of our standard operational procedures and included a review of communication with the clinician, and the follow up of cases of potential bacteraemia owing to CoNS. A secondary objective was...
to evaluate the prevalence of contaminants in our blood cultures, especially methicillin-resistant CoNS.

**Materials and methods**

**Ethical and protocol considerations**

This evaluation of de-identified patient data was approved by the HREC Chair through the Manager Partnerships & Research for Greater Southern Area Health Service. The study protocol followed the methods of Saul et al (2008).

**Blood culture processing**

The South West Pathology Service Albury receives blood samples for culture predominantly from the Albury Base Hospital and clinics from Albury-Wodonga communities. Samples were collected by nursing staff or medical officers at the hospital. When out-patients presented at the laboratory a dedicated phlebotomist collected the blood samples.

Samples were cultured using BD BACTEC™ blood culture bottles – ‘PLUS + Aerobic/F’ and ‘Lytic/10 Anaerobic/F’ for adults, or ‘PEDS PLUS/F’ for paediatrics – and incubated on the BACTEC 9120 (Becton Dickinson Pty Ltd, NJ, USA).

Documented standard operational procedures for venting blood culture bottles (vials) that were positive were followed. A Gram stain was performed on positive vials and the samples were sub-cultured when organisms were seen on the Gram stain. CoNS isolates were identified using Vitek® 2 (bioMerieux, Marcy l’Etoile, France), DNase and Staphylase tests (Oxoid, Adelaide, Australia). A CoNS was confirmed by combined negative results of DNase and Staphylase tests. Methicillin resistance or susceptibility was determined as previously published (Nwose et al 2008).

**Data extraction**

Data were acquired by downloading three years of archived Albury South West Pathology blood culture results from the Auslab Laboratory Information System. All results from blood cultures for the period of time between 1 July 2005 and 30 June 2008 were included. The results of organisms isolated were sorted.

Further information on CoNS-positive cases was sought, including information about antimicrobial susceptibility of isolates, number and types (adult or paediatric) of sets of blood cultures collected, and if there were positive catheter tips culture associated with infection. Results obtained from one patient were further critically reviewed as a case study.

**Results**

A total of 5215 blood samples were collected for culture. One hundred and seventy-nine results were reported as possible contaminants (Fig. 1).

**Table 1. Breakdown of CoNS bacteraemia results.**

<table>
<thead>
<tr>
<th>Total (N*)</th>
<th>Oxacillin susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multiples</td>
</tr>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Adults</td>
<td>1187</td>
</tr>
<tr>
<td>Paediatrics</td>
<td>412</td>
</tr>
</tbody>
</table>

Key: *Number of cases associated with positive catheter tip cultures from same patient; R = oxacillin resistant, S = oxacillin susceptible.

Definitions: multiples are positive cases from same subject; singles are positives cases with at least one negative blood cultures from the same patient; loners are cases with no other blood culture performed on the patient.
A retrospective review of laboratory records, patients’ notes (including the available clinical notes of the medical microbiologist) was undertaken to determine how cases of bacteraemia due to CoNS were followed up. It was observed that clinical information was provided in 43 cases only. Therefore, no clinical notes to support diagnostic judgment were available in 73% cases of the CoNS isolation.

**Case presentation**

Three of the 30 mixed cultures involving CoNS were from one patient, constituting six methicillin-resistant CoNS positive blood cultures over a period of twenty days, of which three cultures grew mixed organisms. No clinical information was provided. A retrospective review of microbiology test records show that a catheter tip specimen from the patient was positive for CoNS two weeks prior to the first positive blood culture.

**Discussion**

Our result shows that between 1 July 2005 and 30 June 2008, 179/5216 representing 3.4% of total blood collections for culture have been reported as having possible contaminants. This included 21 samples that were associated with either other positive blood cultures, or positive catheter tip culture (Table 1). Technically, a decision that these 21 cases are not contaminants but true infections still leaves 158/5216, representing 3.0% of total blood collections, as being possibly contaminated. This contamination rate is similar to that reported by Saul et al (2008) who observed 3.6% of sets of blood cultures to be positive for *Bacillus* spp, CoNS, *Micrococcus* spp, and *Propionibacterium acnes*.

Figure 1 also shows that 159 CoNS reports were released. We observed that of the 159 CoNS, 62% were methicillin-resistant while 73% cases had no clinical information provided. Without clinical information, it is difficult for the microbiologist to offer advice regarding the significance of the possible contaminant, especially when the interns and/or on-call registrar contact the laboratory for such advice.

In the case study it is difficult to conclude that the CoNS cultures were contaminants. As the positive catheter tip culture was tested two weeks prior to the positive blood cultures, it is difficult to assess the relationship. Taking into account the positive catheter tip culture result, plus ascertaining that the positive blood sample is from a peripheral vein may indicate no causative relationship.

The physician may not have requested six blood cultures in 20 days, presumably waiting until the correct antibiotic treatment was effective. However, even if antibiotic treatment had commenced immediately, the laboratory department could have advised of methicillin resistance, which could have facilitated a change to a more appropriate choice of antibiotics.

It was observed that for the entire three-year period, there was no case of methicillin-susceptible CoNS presenting twice in any patient (Table 1). Instead, 100% cases of multiple CoNS bacteremia were methicillin-resistant, while 62% of all CoNS were methicillin-resistant. In our opinion, this indicates a system-wide problem, which could be due to carriage of methicillin-resistant CoNS (Ibrahim et al 2009).

**Limit of the medical microbiologist’s responsibility**

CoNS are potential pathogens, especially in cancer, dialysis and endocarditis patients (Araya et al 2007; Falcone et al 2007; Kan, Thomas and Heath 2003), as well as an opportunistic infection in catheterised patients (Casey et al 2007). The interpretation of the significance of CoNS bacteraemia is a troubling one for microbiologists (Hall and Lyman 2006). Most laboratories report these organisms, but suppress antibiotic susceptibilities results and add comments such as “possible contaminant, susceptibilities available on request.” This is a valid strategy, because there is the concern that clinicians receiving a report naming a bacterium and reporting sensitivities may regard that as a recommendation to treat. In intensive care, immunosuppressed or malignancy patients, it may be valid to report the susceptibility results with note that “it is the responsibility of the clinician to decide to treat.”

There has been controversy regarding collection of blood culture samples from intravascular catheters. It has been reported that intravascular catheter samples have better negative predictive value, lower specificity and positive predictive values and have a more sensitive diagnostic value than peripheral samples (Falagas et al 2008). This study did not investigate differences in the site of blood collection. However, it is noted that, if a patient has a CoNS sepsis and blood for culture is drawn through an IV line the CoNS will be isolated. The difficulty is that there is uncertainty as to whether it is a true pathogen or a coloniser of the catheter.

In our opinion, selection of the blood culture collection site should be determined by the objective for the test i.e., whether investigating nosocomial infection due to some medical device or other forms of bacteraemia. For instance, investigation for infective endocarditis cannot be appropriately diagnosed by isolating CoNS from blood cultures drawn from IVC-line. On the contrary, a contaminated IVC causing nosocomial infection is better investigated by culturing the IVC-line line itself. In a situation where a patient on IVC suddenly presents with cardiovascular collapse and fever, it may be appropriate
to collect both peripheral and IVC-line blood samples for cultures. If blood drawn through the catheter only grows CoNS, then it is likely to be a coloniser of the catheter. If the peripheral blood sample grows the same organism, it is likely the CoNS culture is significant and the catheter is the source. There is suggestion that analytical profile index biotyping, antibiograms and pulsed-field gel electrophoresis are required to show whether CoNS isolates drawn through catheters and peripheral blood were the same (Casey et al 2007).

What this technical note emphasises is that the prevalence of methicillin-resistant CoNS is as high as 62% of all CoNS blood culture isolates. It is imperative that the medical microbiologist seek to determine from clinical notes or consultation with the treating physician whether treatment may be required and full antibiotic susceptibility of the CoNS reported (unsuppressed) for blood and tip culture specimens. However, where clinical information is unavailable, the responsibilities start with handling specimens according to standard operational procedures and finish with issuing of accurate results. This caution will protect the laboratory in cases of complaint from patients.

Conclusion

This technical note presents observation of a substantial lack of clinical information that could hamper appropriate interpretation and subsequent comment regarding CoNS isolations from blood cultures. It is emphasised that the necessity for provision of clinical information and indication of the site of specimen collection for every blood culture should be reinforced by the medical microbiologist in order to adequately advise on any case. This is important to protect against any unforeseen complaints that might require explanation.

Acknowledgements

All authors are employees of the Albury’s South West Pathology of the Greater Southern Area Health Service. We appreciate the privilege granted us, by both the Senior Microbiologist and the Operations’ manager to do this study. The intellectual advice of the Medical Director, Dr Lance Meng is also appreciated.

References


A three-year-old female child presented at the casualty department. She had been unwell with tonsillitis for the past week. A full blood count revealed the following results:

Hb 67 g/L, WBC 24.2 x 10^9/L and platelets 452 x 10^9/L.

The blood film was leucoerythroblastic with a reticulocytosis and a moderate number of spherocytes. There was an absolute neutrophilia of 14.0 x 10^9/L. A preliminary diagnosis of autoimmune haemolysis was made. A direct antiglobulin test (DAT) was performed with a positive result. The LDH was raised at 666 IU/L demonstrating acute haemolysis. Urinalysis revealed dark brown urine with a small number of red cells.

As this was an acute presentation (the child was well two weeks previously) the possibility of paroxysmal cold haemoglobinuria (PCH) was considered. A Donath-Landsteiner test was performed and the result was positive.

PCH is a disease characterised by the sudden presence of haemoglobin in the urine after exposure to cold temperatures. It occurs mostly in young children under the age of five years. There is a male to female ratio of 3.5 to 1.

In 1904 Julius Donath and Karl Landsteiner described an autoimmune haemolytic anaemia characterised by the presence of a biphasic antibody that attaches to red cells in the cold inducing haemolysis when the red cells are subsequently warmed. The Donath-Landsteiner (D-L) antibody is a polyclonal IgG binding to various red cell antigens such as I, i, p and P on the red cell surface. The P antigen is its primary target. This polyclonal IgG anti-P autoantibody binds to red blood cell surface antigens in the cold. When the blood returns to the warmer central circulation, the red cells are lysed with complement, causing intravascular haemolysis. The attachment of C3d on the red cells results in a positive DAT. The resultant blood picture is that of a normochromic normocytic anaemia with spherocytes, reticulocytes, especially in the recovery phase, auto agglutination and nucleated red cells. Monocytes and granulocytes sometimes show erythrophagocytosis.

**Peripheral blood (LP) showing auto agglutination**

**Peripheral blood (HP) showing spherocytes**
The D-L antibody test is performed as follows: The method involves preparing three tubes: 1) the patient’s serum, 2) a mixture of patient’s serum and normal serum and 3) normal serum. To each tube is added P-positive red cells at 4°C. After 30 min the tubes are then heated to 37°C and observed visually for haemolysis, indicative of a positive result.

PCH is associated with upper respiratory and gastrointestinal symptoms. It may occur following measles, mumps, influenza, varicella zoster virus, cytomegalovirus, Epstein-Barr virus, adenovirus, parvovirus B19, Coxsackie A9, Haemophilus influenzae, Mycoplasma pneumoniae and Klebsiella pneumoniae. It also may occur post vaccination for measles.

PCH is a self-limiting disease. If promptly diagnosed and appropriately treated with supportive care, most patients recover within days to a few weeks. Supportive treatment may include pre-warmed packed red blood cells as well as steroids although steroids have not been shown to shorten the course of the disease.

The patient in this case study was transfused warm packed red blood cells. She remains well.

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Department of Haematology
Prince of Wales Hospital
Barker Street
Randwick NSW 2031
Email: gillian_rozenberg@yahoo.com

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The Fred Hollows Foundation
Infectious Diseases – Atlas, Cases
by Robin A. Cooke
Hard cover, 503 pages
ISBN: 9780070159068
AU$159.00

This unique text gives the reader an insight seldom seen into infectious disease. Written with a wealth of clinical experience and beautifully illustrated the reader is taken on a journey through history from the 1800’s to the present in just over 500 pages.

Professor Cooke approaches the task from the perspective of the clinical and physical presentation of the disease, causative organism(s), pathology and diagnosis. The photographs include macroscopic anatomical, macroscopic cultures, microscopic wet and stained microbiological preparations and histological sections.

There are 12 chapters covering a broad range of topics.

Chapter 1, Historical Overview (7 pages, 17 photographs) describes the emergence of infectious diseases as a discipline in the time of Jenner, Pasteur and Koch through to modern times touching on the discoveries and observations of Semmelweis, Snow, Lister, Fleming, Florey and Chain. This brief overview supported by photographs sets the scene for the following chapters.

Chapter 2, Bacterial Infections (30 pages, 114 photographs) covers a rather eclectic group including meningitis, β haemolytic streptococcal infections, Streptococcus pneumoniae, Staphylococcus aureus, Enterobacteriaceae, Typhoid fever, Helicobacter, Diphtheria, Rhinoscleromater, Clostridial infections, Anthrax and Melioidosis. Each is given a brief synopsis with generous use of case studies and photographs illustrating the disease processes.

There are unfortunately some notable omissions including Cholera, however the text is not meant to be a comprehensive bacterial infectious diseases text.

Chapter 3, Tuberculosis (25 pages, 89 photographs) is given more comprehensive treatment, again using the case study approach and well supported by excellent photographs.

Chapter 4, Leprosy (39 pages, 154 photographs) is also given a fairly detailed treatment and may well reflect the fact that the author spent considerable time in areas where the disease was, and still is endemic.

The chapter on Sexually transmitted infections (STIs), Chapter 5 (40 pages, 149 photographs) begins with Gonorrhea and covers NSU, Syphilis, Donovanosis, Pubic Lice & Scabies and Mobiluncus.

Of these syphilis is given the most comprehensive coverage with the effective use of sometimes graphic photographs showing the physical effects of this disease not often seen in all its stages in today’s modern Australia.

Chapter 6, Atypical Mycobacterial Infections, Rickettsial infections, Yaws and Colonic Spirochetosis (27 pages, 82 photographs) gives the reader an overview of these conditions through pictures and a brief but interesting history lesson of these diseases and how they have been discovered, diagnosed, treated and managed over the ages.

The mixture of organisms is further expanded to include Vibrio vulnificus infections as while not caused by one of the Atypical mycobacteria, the clinical features and appearances are similar.

The section of the chapter covering the rickettsial infections includes the history of the disease Q Fever which of course, has a Queensland flavour.

Yaws is given significant coverage with many historical photographs showing the clinical effects of this disease while colonic spirochetosis is only mentioned briefly, though the photomicrographs are impressive.

Fungal infections, Chapter 7 (60 pages, 223 photographs) starts with the superficial mycoses dealt with in quite some detail and moves to Actinomycosis, Histoplasmosis, Aspergillosis, Cryptococcosis and Mucormycosis. It then goes on to cover ‘geographical’ infections such as Coccidiomycosis, Rhinosporidiosis, Blastomycosis (both North and South American), Basidobolus and Pneumocystis. The chapter is well illustrated with many photographs as seen throughout the text.

Viral infections, Chapter 8 (51 pages, 162 photographs) begins with a brief description of viral taxonomy explaining DNA and RNA viral groups and also mentions prions. It then discusses a number of viral diseases in detail including Chicken Pox, Shingles, Measles, Herpes simplex, HPV, Molluscum contagiosum, CMV, Rubella, Chlamydia infections (C. trachomatis, C. psittaci & C. pneumoniae), Hepatitis viruses (A, B & C), Yellow fever, Lyssaviruses (Rabies & Australian bat lyssavirus) and prion diseases – CJD, vCJD, BSE & Kuru, EBV and Polio.
Each of these diseases is discussed in terms of history, clinical presentation, diagnosis, treatment or management and sequelae. Again the use of excellent colour photographs helps the reader to gain a better understanding of the topic discussed.

Parasitic infections covers two chapters, Chapter 9 (68 pages, 212 photographs) discusses the protozoa while Chapter 10 (94 pages, 298 photographs) discusses Trematodes, Nematodes and Cestodes. My personal favourites, these two chapters cover the blood borne and intestinal parasites with excellent use of photographs and covers the history of parasitic disease investigation and management which in itself is a fascinating story. The author's obvious love of the history of disease is well demonstrated with photographs of sites of significance from all around the globe, many taken by the author himself during his extensive travels.

Chapter 11 (13 pages, 27 photographs) touches on Pandemic diseases from the ancient (Plague and Smallpox) to the modern (Bird flu). This could easily have been combined with Chapter 12 Emerging viral infections (24 pages, 59 photographs) as this covers AIDS and SARS however it also includes Monkeypox and the Hendra viruses, not pandemic per se.

These final chapters give a glimpse into the future while the majority of the text gives the reader an excellent view of the past.

The difficult task with this text is to classify it, perhaps the sensible thing is not to try. It is part historical text, part atlas, part clinical text, part case study collection but most of all it is an entertaining and informative journey through the fascinating world of infectious diseases.

Written in a relaxed narrative style and brilliantly illustrated it is a very worthwhile addition to any library and will find readers in many stages of their careers from novice student to vastly experienced practitioner.

The illustrations, many of which are the work of Brian Stewart with whom the author has worked for many years and on a number of other publications including the Colour Atlas of Anatomical Pathology and Clinical Parasitology – A Slide Seminar are excellent and help the reader understand the complexity of the diseases discussed.

Many of the photographs used in the current text can also be found in these other publications however there are many, many additional photographs to compliment the new text.

I would wholeheartedly recommend this text to all those interested in Infectious diseases and believe it to be a valuable addition to any laboratory library collection. At the RRP of $159.00 I believe the book offers excellent value particularly as it contains over 1500 colour photographs of outstanding quality.

Wayne Pederick
Laboratory Manager
QML Pathology
Rockhampton, Queensland

Real-time PCR
Current Technology and Applications
Edited by Julie Logan, Kristin Edwards, and Nick Saunders
Caister Academic Press, 2009
Hard cover, 284 pages.
US$310

This book is a compilation of basic and advanced concepts of real-time PCR applications for the novice and experienced user. It comprises 17 chapters written by international authors expert in specific technical principles and applications. The chapters are concise and divided into sections with diagrams and tables. It begins with an introduction to the technology and an overview of PCR platforms. Subsequent chapters describe essential features such as the use of fluorescent chemistries, internal and external controls for reagent validation, and software for data analysis and reference gene validation. The applications include mutation detection, clinical microbiology, invasive fungal infections, biodefence, food microbiology as well as specialised areas of molecular halotyping, nucleic acid sequence-based amplification and analysis of mRNA expression.

Medical scientists would find the chapter on applications in clinical microbiology particularly useful. This chapter reviews real-time PCR in clinical bacteriology, virology and parasitology. It includes tables of assays developed for detection of a wide range of micro-organisms including the genes targeted and sensitivity. However, it does not provide protocols but will refer the reader to references. Assays frequently used for detection of respiratory and genitourinary infections, malaria, enteric parasites and common viral infections are discussed. A critique of these assays highlight the issues often encountered such as PCR inhibition, cross-reaction of primers, loss of sensitivity when multiplexing, and comparison between commercial and in-house developed assays.

A separate chapter on invasive fungal infections describes the advantage of a real-time platform over the standard PCR-based amplification methods with the added improvement of an enclosed system to prevent contamination. Topics such as multiplex PCR assays, rapid
identification of drug resistance, false-positive reactions due to contamination, and nucleic acid extraction methods are also considered.

The use of real-time PCR for the detection of infectious agents in a biodefence setting is similarly presented with concise discussions on assay validation, PCR workflow, clinical assays, analysis of liquids and foods, analysis of environmental samples, and multiplex PCR assays.

A chapter on the application to food authenticity and legislation is relevant to food scientists with concise discussions on detection of food pathogens, food spoilage organisms, allergens, and genetically modified organisms. The concept of measurement of uncertainty is also briefly discussed but avoids elaboration of the statistical parameters commonly used to determine quality.

The book serves as a useful compendium of basic and advanced applications for laboratory scientists. It is an ideal introductory text book and a will serve as a practical handbook in laboratories where the technology is employed.

Christopher J. McIver
Principal Hospital Scientist,
Microbiology Department (SEALS),
Prince of Wales Hospital,
Randwick. NSW
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www.alloccasionsgroup.com/AIMSNSM09

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SEPTEMBER 28-30: IBMS Biomedical Science Congress. ICC, Birmingham. www.ibmscongress.com


OCTOBER 30-NOVEMBER 1:


NOVEMBER 1-4: The Australasian Flow Cytometry Group 32nd Annual Meeting. Brisbane Convention and Exhibition Centre, Southbank, Brisbane. Contacts: grace.chojnowski@qimr.edu.au; paula.hall@qimr.edu.au. www.afcg.org.au


DECEMBER 5-8: American Society of Hematology Annual Meeting. Ernest N Morial Convention Center, New Orleans, USA. Contact: ash@hematology.org www.hematology.org.

YEAR 2010


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Workshop Sessions
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Wednesday 14 to Friday 16 October 2009

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MEDICAL SCIENCE - TODAY AND TOMORROW
<table>
<thead>
<tr>
<th>Time</th>
<th>Presenter(s)</th>
<th>Workshop Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.00-12.30</td>
<td>Robyn Wells</td>
<td><strong>Malaria Morphology</strong>&lt;br&gt;This workshop will focus on the morphological characteristics of the five Plasmodium species. The features of each of the stages and species will be discussed and examples of thick and thin films will be shown. A number of cases will be presented and the species determined with input and interaction from the participants.</td>
</tr>
<tr>
<td>09.00-12.30</td>
<td>Steve Davis</td>
<td><strong>FULL DAY WORKSHOP</strong>&lt;br&gt;<strong>Classical approach to fungal identification: macroscopic and microscopic</strong></td>
</tr>
<tr>
<td>13.30-17.30</td>
<td>Brian Matthews</td>
<td><strong>Fundamental blood cell morphology</strong></td>
</tr>
<tr>
<td>13.30-17.30</td>
<td>Vicki McGill Neil McGill</td>
<td><strong>RCPA Synovial Fluid QAP</strong>&lt;br&gt;This workshop includes:&lt;br&gt; - An overview of clinical presentations in which synovial fluid examination is useful&lt;br&gt; - The basics of polarised light microscopy&lt;br&gt; - Synovial fluid collection and handling – avoiding the pitfalls&lt;br&gt; - Description of crystals and other particles detectable in synovial fluid</td>
</tr>
<tr>
<td>13.30-17.30</td>
<td>Craig Cox</td>
<td><strong>Immunohaematology</strong></td>
</tr>
<tr>
<td>18.30 -20.00</td>
<td>Liz Clark</td>
<td><strong>FULL DAY WORKSHOP</strong>&lt;br&gt;<strong>Dangerous Goods Packaging (CASA Accredited)</strong>&lt;br&gt;Training Course for Shippers of Infectious Substances, Genetically Modified Micro-organisms, Genetically Modified Organisms and Dry Ice</td>
</tr>
<tr>
<td>18.30 -20.00</td>
<td>Michael Harrison</td>
<td><strong>Causes of Elevated LFTs</strong></td>
</tr>
</tbody>
</table>

18.30 -20.00 **A Tasting Tour of Scottish Whiskies: Venue TBA**
<table>
<thead>
<tr>
<th>Time</th>
<th>Workshop</th>
</tr>
</thead>
</table>
| 09.00-12.30  | **Robyn Wells** Morphological challenges  
This workshop presents cases that many blood film reviewers find challenging. Those cases will be reviewed, the features discussed and differential diagnoses considered. Helpful characteristics that lead to the correct diagnosis will be demonstrated. Cases presented will include childhood leukaemias, Hereditary Spherocytosis, lymphoma cases, acute promyelocytic leukaemia and viral infections. |
|              | **Dennis Mok** Leadership                                                |
|              | **Brian Smith** Becton Dickinson  
**Phlebotomy**  
The workshop will include two sessions:  
- The preanalytical phase-a logical focus for quality improvement  
- It only takes one. Treating NSI seriously  
The two sessions will cover the preanalytical variables and best practice to avoid NSI in Healthcare workers. The information will be suitable to both scientists as well as phlebotomists. There will be enough time for open forum for discussion as well. |
| 13.30-17.30  | **Tim Carroll** Snake venom detection                                     |
|              | **Dennis Mok** Managing change                                            |
|              | **John Stirling** Scientific writing and publication  
The aim of this workshop is to improve critical writing skills and increase understanding of the publication process. The workshop will focus on what is suitable for publication and the basic skills required to write journal articles (with a focus on case studies). The mechanics of publication will also be covered. The workshop is suitable for all inexperienced authors including students, 'bench' scientists and technologists and senior staff, in fact anyone who would like to publish or would like an opportunity to improve their writing skills. |
<p>| 18.00-19.30  | <strong>Opening Cocktail Reception: Exhibition Hall Adelaide Convention Centre</strong> |</p>
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
</table>
| 09.00-09.30 | **Official opening**  
His Excellency Rear Admiral Kevin Scarce AC CSC RANR  
AIMS Award presentations |
| 09.30-09.15 | **Keynote address**  
*Peter Rathjen*  
Stem Cell Research; Today and Tomorrow |
| 10.15-11.00 | **Saal-Foley lecture**  
*John Glasson*  
Innovation, Automation and Art |
| 11.00-11.30 | **Morning break** |
| 11.30-13.00 | **Plenary session 1**  
*Susan Bramford*  
Chronic myeloid leukemia  
*Stuart Blacksell*  
TBA |
| 14.00-15.30 | **Session 1 Biochemistry**  
*Helen Martin*  
Case studies  
*RCPA QAP*  
Lisa Jolly  
Using chemical pathology reports to troubleshoot your method  
*Sue McLennan*  
Blood salvage  
*Hamish Scott*  
Towards your personal genome sequence  
*Graeme Suthers*  
Diversity of genetic testing in Australia  
*Hamish Scott*  
Epigenetic hypermutation  
*Jan Bell*  
MBLs and ESBLs  
*John Merlino*  
MRSA  
*Jan Kay*  
VRE |
|            | **Session 2 Microbiology**  
*Jan Bell*  
MBLs and ESBLs  
*John Merlino*  
MRSA  
*Jan Kay*  
VRE |
|            | **Session 3 Haematology**  
*Ross Brown*  
Multiple myeloma  
*RCPA QAP*  
Gail Earl, Ros Bonar and Fifin Intan  
Advances in haematology QAP including virtual microscopy  
*Colin Story*  
Uncertainty of measurement |
|            | **Session 4 Histopathology**  
*Janette Thurley*  
Neuronal and axon guidance in brain development  
*Ruth Davies*  
IHC Case studies  
*Sharin Prakash*  
Lynch Syndrome (HNPCC) – the role of Immunohistchemistry for mismatch gene protein in screening of patients |
|            | **Session 5 Transfusion and Transplantation**  
*Sue McLennan*  
Blood salvage  
*Jason Carlesso*  
Evaluating methods for detecting low Hb levels in blood products  
*Suzanne Candy*  
Evaluating platelet additives |
|            | **Session 6 Molecular Pathology**  
*Hamish Scott*  
Towards your personal genome sequence  
*Graeme Suthers*  
Diversity of genetic testing in Australia  
*Hamish Scott*  
Epigenetic hypermutation |
| 15.30-16.00 | **Afternoon break** |
### Wednesday 14 October 2009

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<th>Session 9</th>
<th>Session 10</th>
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<tr>
<td>16.00-17.30</td>
<td>Biochemistry</td>
<td>Microbiology</td>
<td>Haematology</td>
<td>Histopathology</td>
<td>Changing patterns of disease</td>
<td>Immunology</td>
</tr>
<tr>
<td></td>
<td>Michael Metz</td>
<td>Andrew Lawrenc</td>
<td>Ram Suppiah</td>
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<td>Meningococcal disease</td>
<td>Paediatric haematology</td>
<td>SA Forensic Histopathology: from the beginning to now</td>
<td>Changing patterns of antimicrobial resistances in bacterial infections</td>
<td>A recent breakthrough in cancer research</td>
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<td>Neil Horton</td>
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<td>Immunology QAP and your laboratory</td>
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<td>Bone marrow donation &quot;the agony and the ecstasy&quot;</td>
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<td>Ros Escott</td>
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### Thursday 14 October 2009

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<tr>
<td>07.15-08.45</td>
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|            | Ulysses Balis:
|            | TBA |
|            | Brendan McMorran:
<p>|            | Platelets and Malaria |
| 10.30-11.00 | Morning break |</p>
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<tr>
<th>Time</th>
<th>Session 13</th>
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<td>Kay McCallum</td>
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<td></td>
<td>Penny Petinos</td>
<td>Epidemiology and diagnosis of Rickettsial disease in Australia</td>
<td>Case studies</td>
<td>Margaret Dimech</td>
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<td>KIMMS: a new QAP to measure pre- and post-analytical quality</td>
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<td>How far have we come in measuring laboratory performance?</td>
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<td>Colin Gordon</td>
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<td>AIMS AGM - Meet the AIMS Board</td>
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<td>Histopathology: Cut up</td>
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<td>Speaker 1 Heddy Zola</td>
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<td>Penny Coates</td>
<td>Advances in response to massive blood loss</td>
<td>Monoclonal Antibodies and flow cytometry - techniques made for each other and pathology.</td>
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<td>Kellie Madigan</td>
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<td>Howard Morris</td>
<td>Traceability and standardisation of immunoassays: a major challenge</td>
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<td>Barney Rudzki</td>
<td>Present and future directions in diagnostic molecular pathology</td>
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<td>14.30-15.30</td>
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<td>John McBride</td>
<td>Dengue fever</td>
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<td></td>
<td>Mr Hieu Van Le</td>
<td>Lieutenant Governor of South Australia and</td>
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<td>Chairman of the South Australian Multicultural and Ethnic Affairs Commission</td>
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Australasian Professional Acknowledgement of Continuing Education

Be acknowledged within your profession by accreditation and recognition through the APACE program.

The APACE (Australasian Professional Acknowledgement Continuing Education) scheme is a voluntary program that recognises continuing education, formal courses and a wide range of professional activities which contribute to your professional growth.

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www.aims.org.au
1. While two thirds of patients with HS present in childhood with mild to moderate anaemia, signs and symptoms of the disease are highly variable, both with respect to age of onset and severity. TRUE/FALSE

2. The eosin-5'-maleimide test which binds specifically to the anion transport protein (band 3) has been proved to be an ineffective screening test for red cell membrane disorders, particularly HS. TRUE/FALSE

3. While the disease is encountered worldwide, its incidence and prevalence in some countries, including Australia, are not clearly established. TRUE/FALSE

4. In asymptomatic patients, who comprise a subset of about 75%, the disorder may escape clinical recognition altogether. TRUE/FALSE

5. HS is caused by mutations in at least five genes; the ANK1 gene (encoding ankyrin 1); the EPB3 gene (encodes for protein AE1, or Band 3); the SPTB gene (encodes for the spectrin β-chain); the ELB42 gene (encodes for protein 4.2); and the SPTA1 gene (encoding spectrin α-chain). TRUE/FALSE

6. Defective α-spectrin production in one allele is expected to cause a disease phenotype. TRUE/FALSE

7. Beta-spectrin Kissimmee which represents the first β-spectrin point mutation demonstrated results in a point mutation near the N-terminus of β-spectrin, close to the protein 4.1 binding site, leading to the protein being unstable and defective in its capacity to bind protein 4.1. TRUE/FALSE

8. Patients with recessive HS and α-spectrin mutations are rare, probably less than 5% of HS, and demonstrate a moderately severe to severe form of the disease that is in some severe cases transfusion dependent. TRUE/FALSE

9. HS has been described in patients with a translocation involving chromosome 8 or deletion of the short arm of chromosome 8 (band 8p11.2) where the ankyrin gene is located. TRUE/FALSE

10. The investigation and management of HS is currently being enhanced by the abundance of new information, dealing principally with molecular and genetic aspects of pathophysiology. 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 or fax by 30 November 2009 to:

AJMS APACE Questions, AIMS National Office, PO Box 1911, Milton Qld 4064. Facsimile: 61 7 3876 2999
1. This evaluation reviews the results of 5216 sets of blood culture specimens tested over a three year period to determine the prevalence and significance of methicillin-resistant CoNS.  
TRUE/FALSE

2. A dedicated phlebotomist collected all the blood samples for culture.  
TRUE/FALSE

3. A total of 5215 blood samples were collected for culture, of these hundred and seventy-nine or 3.4 % of results were reported as possible contaminants  
TRUE/FALSE

4. A total of 98/159 (62%) CoNS were methicillin-resistant as indicated by oxacillin susceptibility testing.  
TRUE/FALSE

5. In this study, 100% cases of multiple CoNS bacteraemia were methicillin-resistant, while 62% of all CoNS were methicillin-resistant indicating a system-wide problem, which could be due to carriage of methicillin-resistant CoNS.  
TRUE/FALSE

6. In intensive care, immunosuppressed or malignancy patients, it may be valid to report the susceptibility results with note that “it is the responsibility of the clinician to decide to treat.”  
TRUE/FALSE

7. CoNS is in all cases considered to be a contaminant in blood cultures  
TRUE/FALSE

8. The most common symptoms in cases of true CoNS are pyrexia, leukocytosis, leukopenia, and thrombocytopenia.  
TRUE/FALSE

9. The contamination rate in this study contrasts greatly from that reported by Saul, Richards and Car (2008) who observed 3.6% of sets of blood cultures to be positive for Bacillus spp, CoNS, Micrococcus spp, and Propionibacterium acnes.  
TRUE/FALSE

10. A Gram stain was performed on all vials and the samples were sub-cultured when organisms were seen on the Gram stain.  
TRUE/FALSE

Name: ___________________________ Membership No: ___________________________

Email: ___________________________

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AJMS APACE Questions, AIMS National Office, PO Box 1911, Milton Qld 4064. Facsimile: 61 7 3876 2999
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 January 25th, 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 and 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/website%20stard/). 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 and Keywords

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

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

Text

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

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

• Introduction
• Materials and methods
• Results
• Discussion
• References

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

Introduction

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

Materials and methods

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

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

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

Examples of the correct form for references are given below:

i) Journal Reference:

ii) Personal Author(s) of a book:

iii) Editor, Compiler, Chairman as Author:

iv) Chapter in Book:

v) Online documents:

Tables

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

For footnotes, use the following symbols in this sequence:

* † ‡ § ¶ ** ††

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

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

Illustrations

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

When plotting points, the following symbols are preferred:

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

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

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

Legends for Illustrations

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

Abbreviations

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

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

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

Commonly used abbreviations

<table>
<thead>
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<td>liter, litre</td>
</tr>
<tr>
<td>m</td>
<td>meter, metre</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>mol</td>
<td>Mole</td>
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<tr>
<td>N</td>
<td>Newton</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>wk</td>
<td>week</td>
</tr>
<tr>
<td>yr</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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