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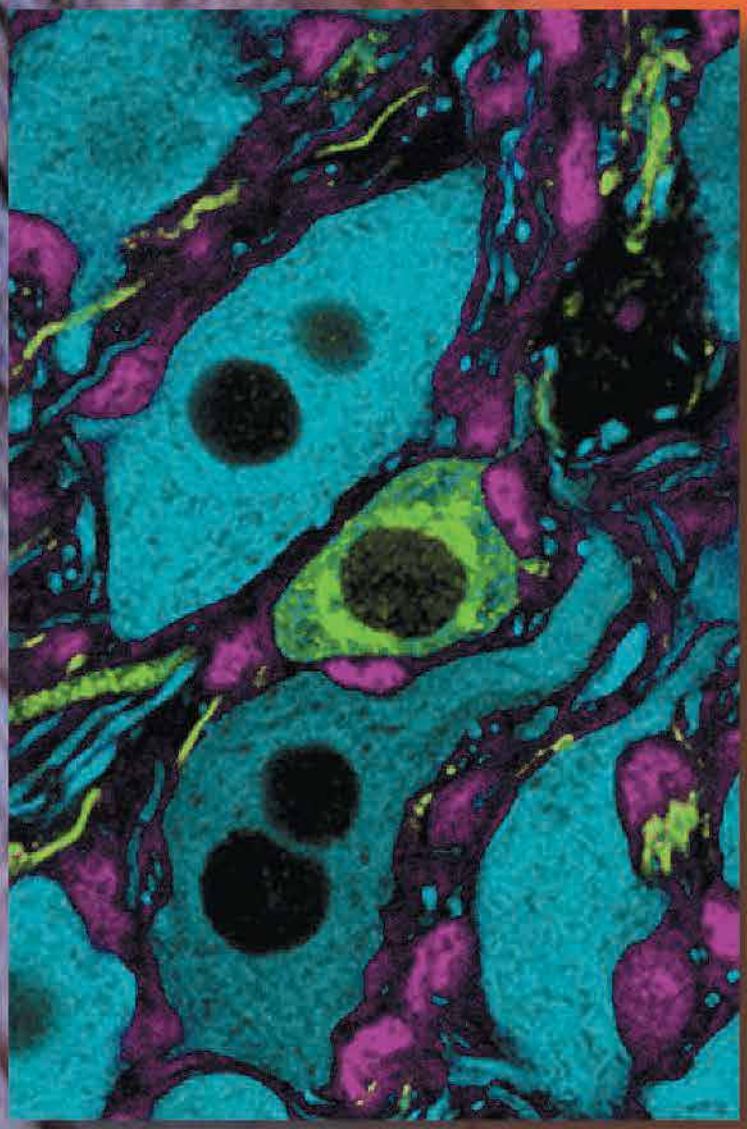

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ORIGINAL ARTICLES

OPINION ARTICLE

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CONTENTS

August 2019 Vol. 40 No. 3

Original Articles

Opinion Article

Regular Features

Advertising Index

AIMS Bursary and Grant Funding Opportunities

Instructions to authors *are available on the AIMS website www.aims.org.au*

Spurious mean cell volumes in hyperglycaemia on the UniCel DxH800 analyzer

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Abstract

The mean cell volume (MCV) is a key red cell parameter in the full blood count (FBC) that doctors use to determine the condition of a patient. Hyperglycaemia is known to cause spurious MCV results on impedence analysers. This is a product of the osmotic gradient caused once a hyperglycaemic sample is exposed to diluent which leads to swelling of red cells and a MCV result that is higher than the true value.

In 2008 a study was conducted at the Dandenong Hospital on the Beckman Coulter LH750 and MAXM analysers and a threshold of 40 mmol/L glucose was determined to be the threshold level over which the MCV was affected by more than 2 femtolitres (fL). These analysers have now been replaced with Beckman Coulter UniCel DxH800 (DxH800) analysers and the MCV appears to be affected at a lower glucose level. This study determined the new threshold level of glucose to be 26 mmol/L. Methods for correction including saline replacement and dilution were evaluated and the method that proved most consistent with less sources of error was either a 1:1 or 1:5 dilution with 0.9% saline. Using the DxH800 diluent to perform the dilutions was shown to over-correct the MCV and cause spuriously high MCHC results. A review of a previous 12-month period showed that significant numbers of MCV errors were detected once the glucose level was >26 mmol/L, some of which made the result appear spuriously macrocytic when it was in fact normal or spuriously normocytic when the cells were actually microcytic. Some errors were also found with a glucose level of between 20 and 26 mmol/L. A threshold of a glucose level of 26 mmol/L is subsequently advocated as trigger to guide the investigation of a possible spurious MCV result. It would be worthwhile for haematology laboratories to determine their own threshold specific for their particular analyser and determine the rate of spurious MCV results in their laboratory.

Keywords: hyperglycaemia, mean cell volume, osmolar

Introduction

The mean cell volume (MCV) is an important red cell parameter used in conjunction with other red cell parameters in the FBC to diagnose a clinical condition. A raised MCV in anaemia may indicate megaloblastosis as a cause, whereas a low MCV may lead to investigation of iron deficiency or haemoglobinopathy. A normocytic normochromic anaemia requires investigation of possible blood loss or haemolysis. In hyperglycaemia the MCV may be erroneous and misleading and lead to unnecessary investigation of macrocytosis or may preclude investigation of iron deficiency (Bock *et al* 1985; Cornbleet and Myrick 1983; Savage and Hoffman 1983; Strauchen *et al* 1981).

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High serum glucose levels have been known to cause spuriously high MCV measurements on impedance cell counters (Beautyman and Bills 1982; Bock *et al* 1985; Cornbleet 1983; Evan-Wong and Davidson 1983; Holt *et al* 1981; Morse *et al* 1981; Planas *et al* 1985; Savage and Hoffman 1983; Strauchen *et al* 1981; Van Diujnhoven and Treskes 1996;Ward 2000; Zandecki *et al* 2011). Holt *et al* (1982) and Planas *et al* (1985) determined that glucose levels above 35 mmol/L cause a spuriously high MCV. Glucose is a polar osmotically active substance which readily crosses the red cell membrane (transit time (T½) 25secs), however not as readily as water (T½ 7msec) (Savage 1983). When the red cell, which is in equilibrium with its hyperglycaemic environment, is exposed to the isotonic environment of the red cell diluent a net influx of water into the red cell will occur. If left in the isotonic environment the glucose and water will move out of the red cell until equilibrium with its new environment is restored. Unfortunately, this equilibrium cannot always be achieved in the time period within which the MCV is measured on impedance analyzers. This can lead to spuriously high MCV values (Holt *et al* 1981; Morse *et al* 1981; Planas *et*

al 1985; Savage and Hoffman 1983; Van Diujnhoven and Treskes 2000).

These spurious results may be highlighted by a spurious mean cell haemoglobin concentration (MCHC) result that is below the normal range as this value is calculated using the MCV (Berda-Haddad *et al* 2017; Cornbleet1983; Morse *et al* 1981; Strauchen *et al* 1981; Van Diujnhoven and Treskes 1996; Zandecki *et al* 2011). However, this is not always the case, as the spurious MCV may cause a lowering of the MCHC, but at the same time, the MCHC remains within the normal limits. Thus, the presence of a low MCHC cannot be relied upon to detect all spurious MCV results.

In 2008, a small study was conducted on the Beckman Coulter LH750 and MAXM analyzers and determined that the threshold glucose level at which the MCV was significantly affected by more than 2 fL was 40 mmol/L (Whiting *et al* 2008). This has been used as a trigger value at Monash Pathology (Victoria) to alert the laboratory of a possible spurious MCV and was shown to affect a small number of patients. However, since the implementation of the DxH800 analyzers it has been observed that the threshold appears to be lower than 40 mmol/L. If this is indeed so, a larger number of patients may be affected.

The aim of this study is to determine the glucose level or threshold at which the MCV is significantly increased by more than 2 fL from the true value on the DxH800 and introduce a new approach for the detection and correction of spurious MCV.

Materials and methods

Methods for the correction of the spurious MCV currently in use at Monash Health (Victoria) are 1) saline or DxH800 diluent replacement of the plasma and 2) 1:1 dilution of the sample with saline or DxH800 diluent. The effectiveness of these methods was evaluated and verified. An additional quicker method for correction involving a 1:4 dilution in either saline or DxH800 diluent was tested and verified.

A retrospective study over a 12-month period was also undertaken to determine the significance of this new threshold and the frequency of affected MCVs. The effectiveness of the old threshold level of 40 mmol/L glucose in detecting the spurious result was also examined and the corrective measures used evaluated.

Low risk and Victorian Site Specific Ethics approval was obtained for this project .

Thirty normal participants were recruited. 20 x K2EDTA samples were collected from each participant. An attempt was made to spread the cohort evenly between male and female and over a range of age groups. The glucose concentration of the neat sample was required to fall within the random glucose Monash Pathology reference interval for adults of 3.3-7.7 mmol/L for further use in the study.

Part 1. Determination of the threshold

Pure glucose (Glucogen) was obtained and a 500 g/L solution was made up in isotonic saline (Fresenius Kabi Sodium Chloride for 0.9% for irrigation). For each set of 20 samples, the first sample would remain neat, then glucose solution was added in increasing volume such that the concentration of glucose would increase by approximately 5 mmol/L for each successive sample. The volume of sample collected in each tube showed some variance from the 3ml projected and thus the final concentrations were difficult to predict. The glucose concentration of each tube therefore was measured as outlined in Part 2.

The samples were incubated for 1 hour at room temperature to allow the red cells to equalize with the new hyperglycaemic environment. Each sample was then run on the DxH800 to determine the MCV. The change in MCV of each aliquot in fL from the neat sample or base level was then recorded against the glucose concentration in mmol/L (measured as described below) and the threshold glucose level determined as the point at which MCV change was greater than 2 fL.

Part 2. Validation of corrective measures

From each sample a 1:1 dilution was made with either isotonic saline or DxH800 diluent (15 participants each) and allowed to incubate at room temperature for 20 mins. These aliquots were then run on the DxH800 to determine the MCV. The MCV change from the true value (neat sample) of each aliquot versus that of each diluted sample were then compared to determine if the method of correction was effective. A further 1:4 dilution was made from each sample in either isotonic saline or DxH800 diluent (15 participants each) and incubated for 5 min. These samples were then analyzed through predilute 5 mode on the DxH800 and the MCV compared to the true value to determine if this method of correction was effective.

The remainder of the samples were centrifuged at 2000 rpm. The plasma was then removed and the glucose level for each sample determined on the Beckman Coulter AU680 analyzer by the Monash Pathology Hexokinase endpoint timed method. An equal volume of isotonic saline or DxH800 diluent (15 participants each) was added to the plasma that had been removed and this was then added to the remaining packed cells and mixed at room temperature for 20 minutes. These aliquots were then run on the DxH800 and the MCV determined.

It was decided to perform corrections with both saline and diluent as it was discovered that corrections were being performed within the network with both these solutions.

Despite the original validation being performed with 0.9% saline, the assumption had been made that the DxH800 diluent would perform in the same manner as the 0.9% saline and this required validation.

The neat MCV for each participant or true value from Part 1 was then compared with the MCV of each diluted aliquot and saline/diluent replaced aliquot to determine if the method of correction was effective. The aim was to return the MCV to within 2 fL of the true value, preferably the same value as measured in the neat sample from Part 1.

Part 3. Analysis of patient data

Patient data from the network was reviewed for glucose levels around and above the new threshold limit to determine the frequency of spurious MCV results. The MCV results were examined for discrepancies and possible clinical significance of those discrepancies.

Data above the previous threshold of 40 mmol/L was analyzed separately to determine if a) the spurious result was detected and b) if the corrective measures in use at present (saline replacement and 1:1 dilution) were adequately correcting the spurious result.

Results

The cohort consisted of 14 male and 16 female participants with ages ranging from 22 to 71 years. There was also a range of ethnic origins with 18 Caucasian, 8 Asian and 4 participants from other origins -2 Greek, 1 Tamil, 1 South African.

Part 1. Determination of the threshold

For each participant, the MCV for each sample was plotted on the x axis vs the concentration of glucose (3 examples shown in Figures 1-3). The data was found to have a hyperbolic distribution and the formula then determined for each participant. The threshold glucose level was then calculated as the glucose concentration (y) over which the MCV would have increased by greater than 2 fL, that is when x=2.

The threshold level varied from individual to individual and ranged between 26 and 39 mmol/L. The average threshold was 33 mmol/L. When the threshold values were examined and compared with the age of the participants (Figure 4) no relationship or correlation was found to explain the differences in threshold.

There is a slight difference between male and female , with the threshold for men ranging between 29 and 39 with a mean value of 35 mmol/L. The threshold for females ranged between 26 and 35 mmol/L with a mean of 32 mmol/L, on average 3 mmol/L lower than the male cohort (Figure 5). However, due to the small sample size this would need further verification.

There does not appear to be any difference between ethnic groups, with the average threshold for Asian, Caucasian and other ethnic groups to be 34, 33 and 33 fL respectively (Figure 6). Further verification is again required due to the small sample size.

The base or neat MCV for each individual ranged between 82 and 99 fL and did not appear to show any correlation with the threshold glucose level (Figure 7). This confirms the findings of Holt *et al* (1981) who showed no correlation between the baseline MCV and the degree of change in MCV at different concentrations.

The threshold is significantly lower than the 40 mmol/L determined for the LH750 and MAXM analyzers (Whiting 2008). This threshold was a conservative level, the lowest threshold level from our study in 2008 (Whiting 2008). The lowest threshold level obtained in the current study was 26 mmol/L. By chance this was found to be the same individual with the lowest threshold in 2008 but the level was significantly lower in this study. Holt *et al* (1981) determined that the spurious MCVs were seen when concentration of glucose was greater than 35 mmol/L using Coulter S and Coulter S plus instruments which is again significantly higher than the lowest threshold found using the DxH800.

The cause of this difference cannot be found in the diluents used. Table 1 shows a comparison of osmotically active analytes found in the diluents for the LH750 and the DxH800 and there is little difference between the two. Holt *et al* (1981) and Planas *et al* (1985) also ruled out the diluent as a cause. The answer is most probably due to timing. It has been previously shown that the degree of change in the MCV due to hyperglycaemia varies with the instrument used and that this can be attributed to the length of time the red cells are exposed to the diluent before counting and measurement of the MCV commences. The shorter the time, the greater the effect. Water moves almost instantaneously into the red cell upon dilution, swelling the cells. This will equalize over time as glucose moves out of the red cell, water moves with it and osmotic equilibrium is obtained (Bock *et al* 1985; Holt *et al* 1981; Planas *et al* 1985; Strauchen *et al* 1981; Van Duijnhoven and Treskes 1996). On the LH750 and MAXM analyzers blood and diluent are mixed in the red cell bath by bubbling air through the mixture. The red cell count and MCV measurement does not begin until the bubbles dissipate at 12-22 secs (Whiting 2008). The DxH800 is faster as the blood is mixed with the diluent and via tangential flow. The rear aperture electrode and housings are primed for approximately 2 seconds or until the flow has stabilized prior to initiation of the count. Counting can begin as early as 2 sec after dilution and this

accounts for the much lower glucose threshold at which the MCV is affected. The count can be extended up to 20 sec if a low platelet count is present as these are counted in the same chamber. The variation in count time may also account for the variability of the threshold level. Figure 8 demonstrates the difference in timing and the effect on the MCV measurement for the DxH800 and the LH750.

Part 2. Validation of corrective actions

Three methods of correction were evaluated, each method was evaluated using both DxH800 diluent and 0.9% saline (15 participants each). Each of the 20 glucose adjusted samples for each participant was corrected and the MCV of the 'corrected' sample compared to the true or neat MCV to determine the effectiveness of the method over increasing glucose concentrations.

Replacement of plasma with saline or diluent

This method of correction is currently in use at Monash Health. Correction was achieved with both DxH800 diluent and saline up to a certain point. Fifteen participant samples were treated with saline and 15 with DxH800 diluent.

Figure 9 shows the relationship between the original glucose concentration and the degree of correction or change in MCV from the true value (where X=0). The aim is to achieve correction as close as possible to this mark and within 2 fL. Adequate correction was achieved up to around 60 mmol/L where the difference in MCV begins to climb over the target 2 fL. The average correction up to this point is about 1fL above the true value.

Figure 10 shows the similar relationship between the original glucose concentration and DxH800 diluent replacement. Adequate correction is achieved up to approximately 80 mmol/L where the change in MCV begins to climb. The average correction up to this point is about 0.5 fL below the true value, a slight but insignificant over correction. Use of the DxH800 diluent in this method appears to be more effective at higher concentrations.

Use of the saline or diluent replacement methods over a number of years has highlighted some variables and sources of error in this method. Firstly, the corrective ability of this method is dependent upon the haematocrit of the sample. A normal haematocrit varies between 0.35 and 0.54 (Monash Pathology) and therefore the amount of plasma replaced in a sample varies from individual to individual. Glucose concentration is in equilibrium between plasma and red cells and this is equivalent to approximately 1:3 dilution when the haematocrit is 0.35 or a 1:2 dilution when the haematocrit is 0.54. An anaemic or polycythaemic patient will introduce greater variability. The effectiveness of the correction will thus be affected and is less reliable as the haematocrit increases .

The second and most significant source of error is in the method itself. If the intent is to use the corrected sample data to result parameters other than the MCV, the removal of plasma must be done carefully so that none of the buffy coat or red cells are removed from the sample and the volume of plasma accurately replaced so that the concentrations of the other parameters will not be affected. The white cell count, haemoglobin and platelet counts achieved can be compared to the original sample to assess the integrity of the results.

The integrity of the results released is dependent on the skill of the scientist performing the correction and their attention to detail when validating results. In practice it was found that platelet counts are almost always reduced in the corrected sample as it is almost impossible to remove the plasma without removing some platelets with it. White cell count and platelet counts are accepted from the original sampling, whilst the red cell parameters are accepted from the corrected sample, provided the haemoglobin results correlate.

A solution to this problem is to take the MCV from the corrected sample and use it to recalculate the haematocrit and MCHC of the original results. The MCV is a measured value on the DxH800 and is not affected by the abovementioned errors in the replacement method. Errors can of course be introduced in the calculation and manual entry of these parameters but should be checked by a second scientist on validation.

A significant disadvantage of this method is the time taken to perform the correction. The time taken to spin the sample, execute replacement of the plasma, allow 20 min for the sample to equalize and then run on the DxH800 can in practice mean a delay in the release of the FBC result by an hour or more. Most samples that require correction originate in emergency and this affects turnaround times and throughput for this department.

1:1 dilution with saline or diluent

This method is also currently in use at Monash Health. Sample sets from 15 participants each were diluted 1:1 with either saline or DxH800 diluent. Correction was achieved up to a point with both saline and the DxH800 diluent.

Figure 11 demonstrates the relationship between the original glucose concentration and the degree of correction or difference in MCV from the true value using a 1:1 dilution with saline. Correction was achieved in a similar pattern to the saline replacement up to about a glucose of 60 mmol/L after which the difference begins to rise. The average correction up to this point is 0 fL or equal to the true value. The change in MCV does not rise above 2 fL until glucose of 80 mmol/L is reached which shows improved correction over the saline replacement method.

Figure 12 demonstrates the relationship between original glucose concentration and the degree of correction towards the true value using a 1:1 dilution with DxH800 diluent. The data shows similar results to the DxH800 diluent replacement method. The MCV is over corrected by an average of 1 fL and up to 2 fL until the 80 mmol/L glucose concentration is reached and this means that the average correction is 1 fL and up to 2 fL lower than the true value. As the uncorrected glucose levels rise above 60 mmol/L the corrected value rises but does not rise above 2 fL higher than the true value. On first appearances this may seem superior to using saline for dilution however the overcorrection of the MCV creates a new problem as those over corrected results were accompanied by a rise in the MCHC above the reference range.

These results indicate that the use of saline to perform the dilution is preferable to using DxH800 diluent. The accuracy of the dilution is paramount as any error is amplified twofold when the results are calculated out. All the FBC results must be multiplied by 2 (the dilution) and entered manually both of which are subject to human error. Any results calculated and validated in this way should be checked by a second scientist.

Alternately the MCV from the dilution may be used with the red cell count and haemoglobin of the original sample to calculate the correct haematocrit and MCHC. This would require editing of only three points of data on the original results and less opportunity for error.

The advantages of this method over the saline/diluent replacement method are 1) that it is technically less difficult and therefore less subject to error; 2) the time taken to perform the correction is 10 to 15 min shorter due to the lack of a centrifugation step, thus improving turnaround times and 3) not subject to variability caused by the haematocrit of individuals.

1:4 dilution with saline or diluent

This method is currently not in use. It was designed by the author in attempt to reduce the amount of time required to perform a correction and to reduce the margin of error incurred by the methods currently in use. T½ for glucose is 25 sec (Savage 1983) and from this it is concluded that an incubation time of 5 min would be ample to allow for equalization. The 1:4 dilution was chosen because it would provide better correction at higher glucose concentration and also because it could be run on predilute mode on the DxH800 analyzer.

Figure 13 shows the relationship between the uncorrected glucose level and the difference in MCV corrected by 1:4 dilution with saline from the true value. The correction is more consistent over all dilutions with the average correction being -0.5 to -1.0 fL from the true value. That is a slight over correction, still within acceptable limits.

Figure 14 similarly shows this relationship when the method is performed using DxH800 diluent. The correction is again more consistent over all glucose levels however the average correction is -2.0 fL and up to -3.0 fL. This over correction leads to MCHC results being greater than the reference range and therefore a spurious result. Consequently the use of saline would be preferable to DxH800 diluent for correction by this method.

Advantages of this method over the previous methods are:

1) technically less difficult than saline/diluent replacement method reducing sources of error;

2) much quicker resolution of the spurious result and vast improvement in turnaround time;

3) not subject to variability caused by the haematocrit of the sample;

4) use of Predilute 5 mode on the DxH800 means the results are automatically calculated out and transmitted directly to the LIS to be validated.

Disadvantages of this method are:

1) predilute 5 mode on the DxH800 does not perform a differential white cell count and so the differential from the uncorrected sample should be used and the white cell count, haemoglobin and platelet count of the original sample should be used to assess the integrity of the dilution performed;

2) any error in dilution is multiplied fivefold therefore the accuracy of the dilution is paramount and is dependent on the skill of the scientist performing the dilution and the accuracy of the pipette used.

The over-correction was observed when DxH800 diluent was used in all three methods tested and may be attributed to other osmotic factors at play due to the composition of the diluent. The levels of solutes known to affect the MCV was measured in both the saline and DxH800 diluent in an effort to determine the cause. Sodium levels were measured by the ISE module on the Beckman Coulter AU680 analyzer and osmolality and pH were also measured . Table 1 shows the osmotic elements at play in both saline and DxH800 diluent. Note that the sodium level in the DxH800 diluent is significantly higher than in the saline. The potassium level is also higher and this results in a higher osmolality. The red cells actively maintain an ionic gradient and membrane potential across the red cell membrane by active transport known as the sodium-potassium pump. This maintains a higher potassium level within the red cell and conversely a higher sodium level outside the red cell or in the plasma. The sodium-potassium pump requires ATP

as an energy source and takes time to operate. If the cells are exposed to a higher sodium environment than the cell is used to, such as in the DxH800 diluent, this affects the electrochemical gradient. Osmotic forces come into play and water will move out of the cell to compensate (Khan Academy 2018) and this could explain the overcorrection of the MCV using DxH800 diluent.

Previous articles have mentioned that the spurious MCV can be corrected by either extending the time in the diluent by pre-diluting the sample for 5 min (Holt *et al* 1981; Morse *et al* 1981; Planas *et al* 1985; Savage and Hoffman 1983; Zandecki *et al* 2011) or by performing a spun haematocrit (Morse *et al* 1981; Savage and Hoffman 1983) and calculating the MCV. This project has verified that the pre-dilution of the sample with saline and 5 min incubation prior to sampling give a correction of the spurious MCV result close to the true value. The differences in the 1:1 and 1:4 dilutions indicate that for full correction the dilution must bring the glucose down below the threshold value. The pre-dilution with DxH800 diluent can lead to over correction of the MCV.

Part 3. Analysis of patient data

Patient data the period from May 2017 to May 2018 was retrieved for patients with glucose levels above 20 mmol/L. The FBC data for these patient episodes was reviewed and the change in MCV tracked as the glucose levels normalized.

The 40 mmol/L threshold has been used at Monash Pathology as a trigger to perform corrections by either saline/diluent replacement or 1:2 dilution since 2008. Analysis of the data for glucose levels above this threshold revealed 48 episodes, with 43 of these episodes having accompanying FBC results. The data show that the issue of spurious MCV results above this threshold is significant with a total of 35 (85%) of these episodes exhibiting a significant decrease in MCV decreasing from 3 fL to 16 fL upon normalization. Only two FBC results showed no change (Figure 15). The remaining six episodes had insufficient data with no follow up FBC to determine if a change had occurred.

Figure 16 shows the performance of a laboratory with regards to the correction of the spurious MCV results. Corrections were attempted on 24 of the 35 spurious results with only 10 of these achieving full correction. The remaining 14 exhibited partial correction. Possible causes for this are 1) the corrective method may not have reduced the glucose concentration below the threshold level for that person (as previously described the threshold varies from individual to individual and the effectiveness of the corrective measures currently in use decreases as the glucose concentration rises above 60 mmol/L) and 2) other osmotic influences at play as many patients with glucose levels in this range exhibit ketoacidosis with acidic pH and low bicarbonate levels. Low sodium levels and high potassium levels are also factors which affect the ionic gradient and exert osmotic influence when the red cells are exposed to diluent or saline that have much higher sodium levels (Evan-Wong and Davidson 1983; Khan Academy 2018; Philipsen and Madsen 2015; Zandecki et al 2011).

If the new threshold for action was to be set at 26 mmol/L, which is the lowest threshold for our participants, then a total of 160 episodes were found with glucose levels above this threshold which could potentially have spurious MCV results. This is 112 more episodes than if the 40 mmol/L threshold is used. Fifteen extra episodes with spurious results MCVs were confirmed between this new threshold and the old threshold of 40 mmol/L with changes in MCV ranging from 3 to 8 fL. This represented 16% of the episodes in this range. Forty-three of these episodes had no follow up so it could not be determined if there was a spurious result. This amounts to 38% of the total number of episodes with glucose levels between 26 and 40 mmol/L making determination of the impact of glucose levels in this range difficult. Forty showed no significant difference in MCV (Figure 17).

Ten episodes exhibited spurious MCVs between glucose levels of 20 and 26 mmol/L with variance of 3 to 6 fL, representing 8% of total number of episodes in this range. Only two of these episodes exhibited a low MCHC and could be detected in this manner. Given the high numbers with no follow up FBC (37%) it is difficult to ascertain the true numbers of affected results.

A total of 64 spurious MCV results were confirmed for all episodes with glucose levels above 20 mmol/L when compared to the MCV result for a subsequent FBE with normalized glucose level. A proportion of these were corrected as described above. The clinical significance of these spurious results is dependent upon what degree the MCV changed and whether the true result moved into or outside the reference interval. In a good proportion of these results (64%) the MCVs were reduced but remained within the normal range. A significant number however (28%), changed from a macrocytic MCV to a normal MCV. One of these in particular the MCV dropped from 109 fL to 84 fL. Three (5%) changed from normocytic MCV to a microcytic MCV and one from a macrocytic MCV to a microcytic one with the MCV falling from 101 to 75 fL range 80-98 fL. The significance of the change can be minimal for one individual and quite significant for another. It was also noted that in only 33 or 52% of occasions was the spurious MCV result also accompanied by a low MCHC. This demonstrates that a low MCHC may be an indicator of a spurious result but cannot be relied upon to detect all spurious MCV results.

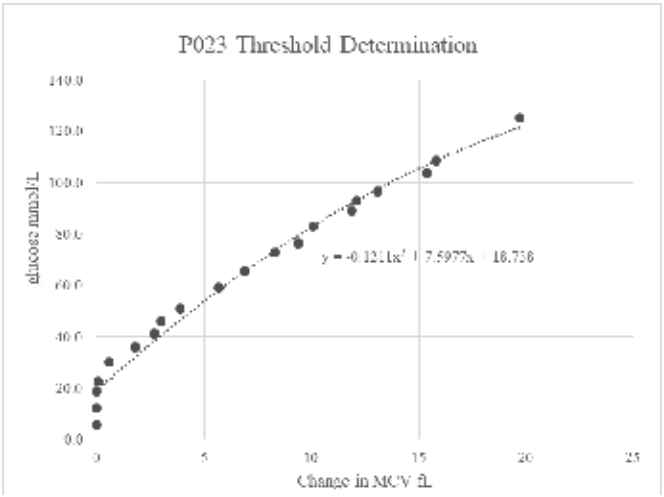

Figure 1. *Participant 23 threshold (y) determined to be 33mmol/L when the MCV > 2 fL(x).*

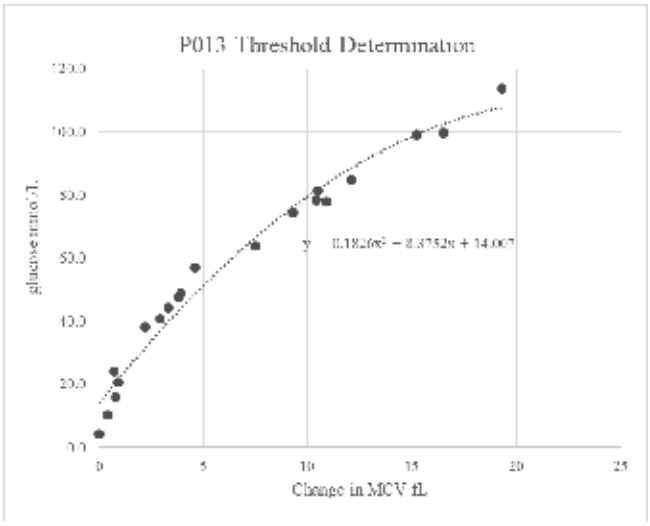

Figure 2. *Participant 13 threshold (y) determined to be 26 mmol/L when the MCV > 2 fL (x).*

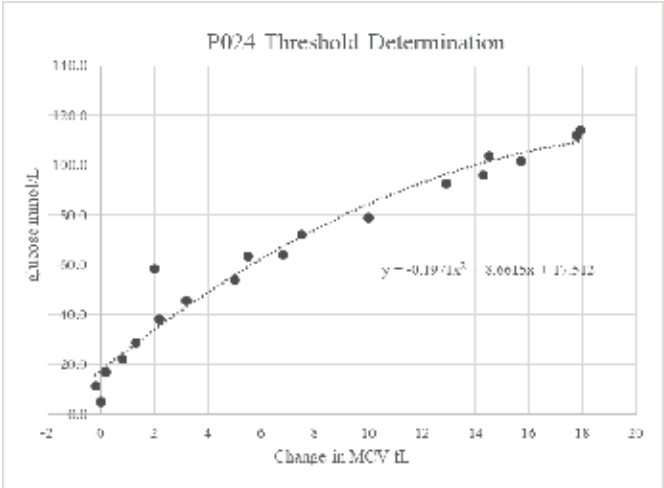

Figure 3. *Participant 24 threshold (y) determined to be 39 mmol/L when the MCV > 2 fL (x).*

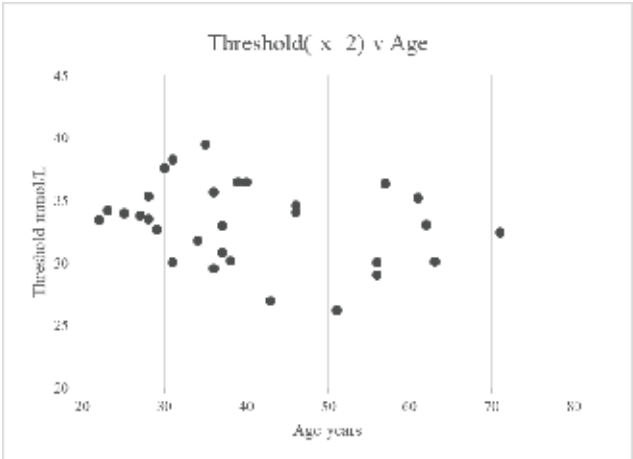

Figure 4. *Relationship between age of participant and threshold shows no correlation. Number of participants 30. Age range 22-71 yr.*

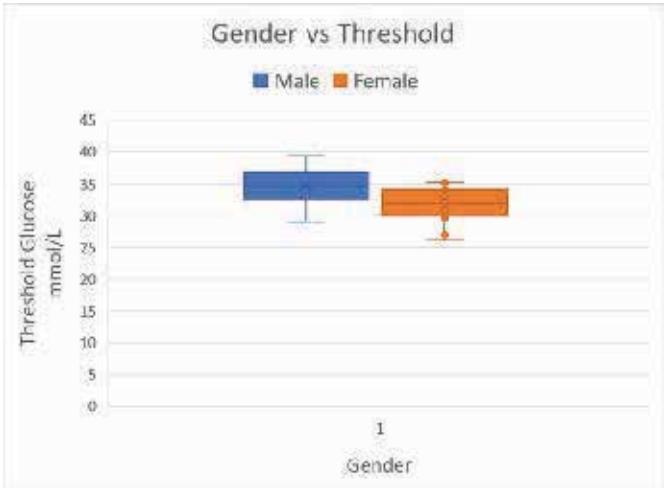

Figure 5. *Relationship between gender of participant and threshold shows slight difference in threshold between the 14 male (average threshold 35 mmol/L) and 16 female (average threshold 32 mmol/L).*

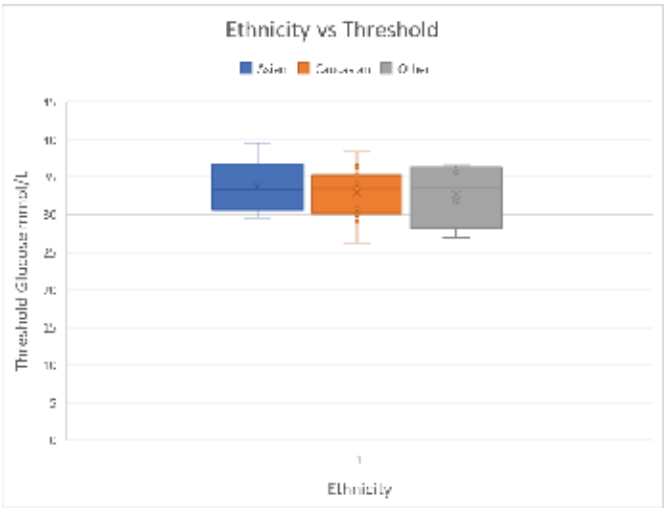

Figure 6. *Relationship between ethnicity of participant and threshold shows no correlation with ethnic origin (Asian n=8, Caucasian n=18, Other n=4).*

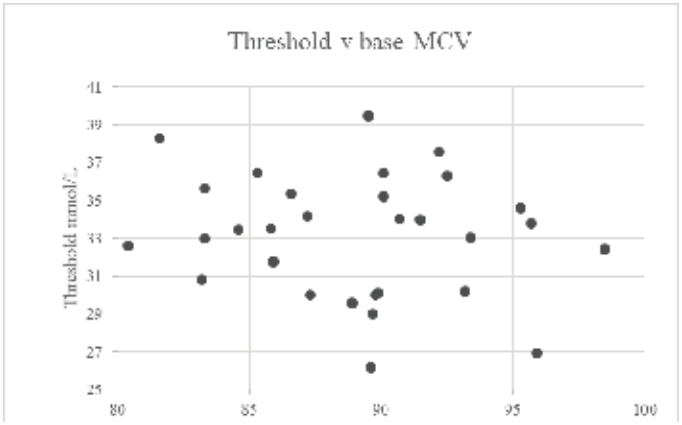

Figure 7. *Relationship between base MCV of participant and threshold over the 30 participants shows no correlation. Baseline MCV range 82-99 fL.*

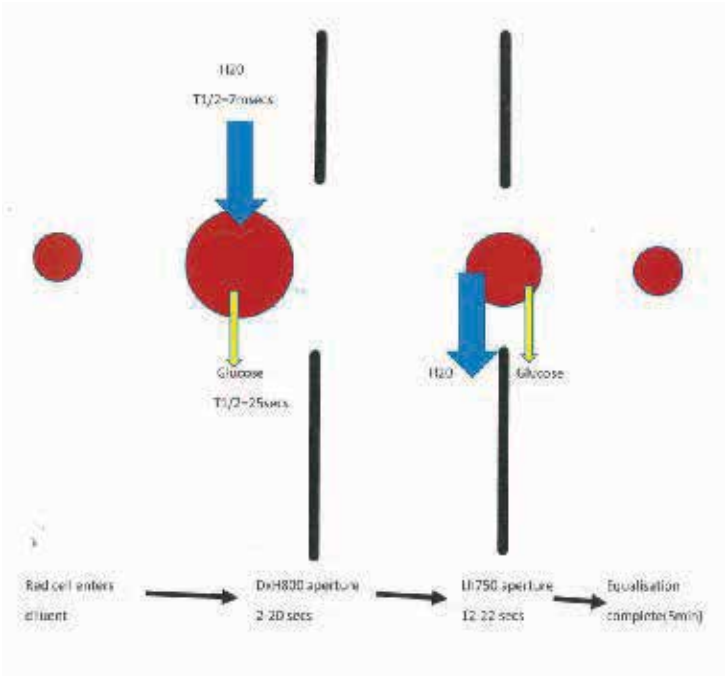

Figure 8. *Schematic representation of red cell size at key time point.*

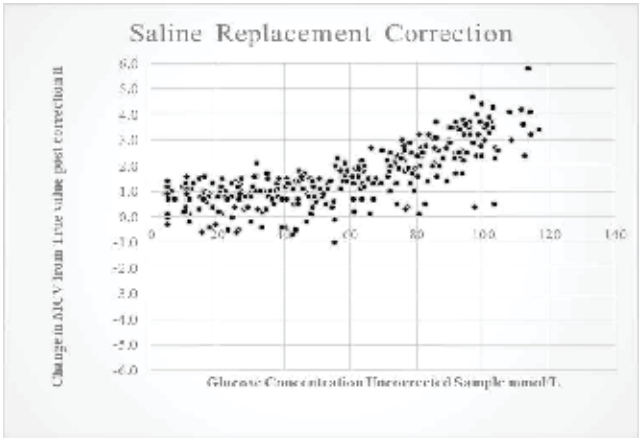

Figure 9 . *Correction of spurious MCV by saline replacement method. Corrections performed on 20 samples each (glucose concentrations adjusted incrementally) from 15 participants. Correction effective up to 60 mmol/L where MCV post corrective measure increases above 2 fL from baseline.*

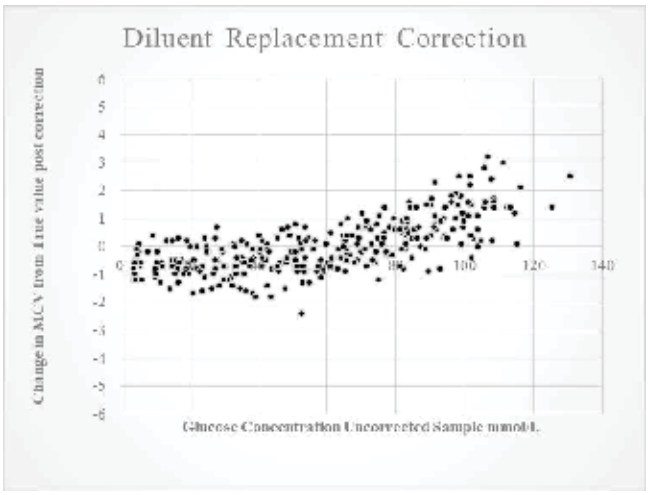

Figure 10. *Correction of spurious MCV by DxH diluent replacement method. Corrections performed on 20 samples each (glucose concentrations adjusted incrementally) from 15 participants. Correction effective up to 80 mmol/L where MCV post corrective measure increases above 2 fL from baseline.*

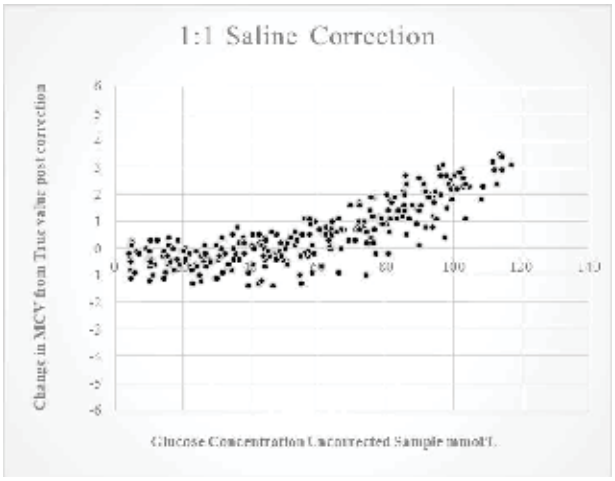

Figure 11. *Correction of spurious MCV by 1:1 dilution with saline. Corrections performed on 20 samples each (glucose concentrations adjusted incrementally) from 15 participants. Correction effective up to 80 mmol/L where MCV post corrective measure increases above 2 fL from baseline.*

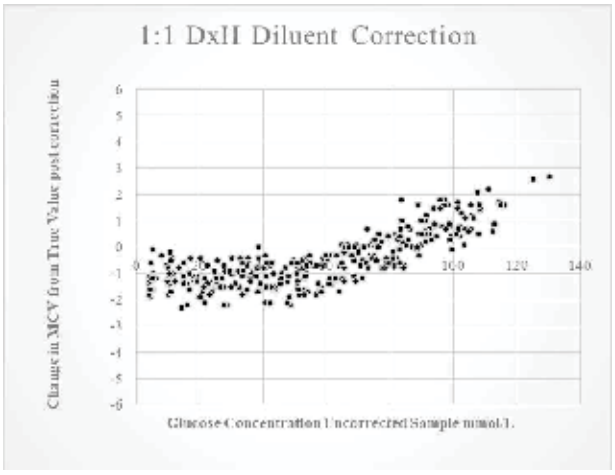

Figure 12. *Correction of spurious MCV by 1:1 dilution with DxH800 diluent. Corrections performed on 20 samples each (glucose concentrations adjusted incrementally) from 15 participants. Correction effective up to 80 mmol/L where MCV post corrective measure increases above 2 fL from baseline.*

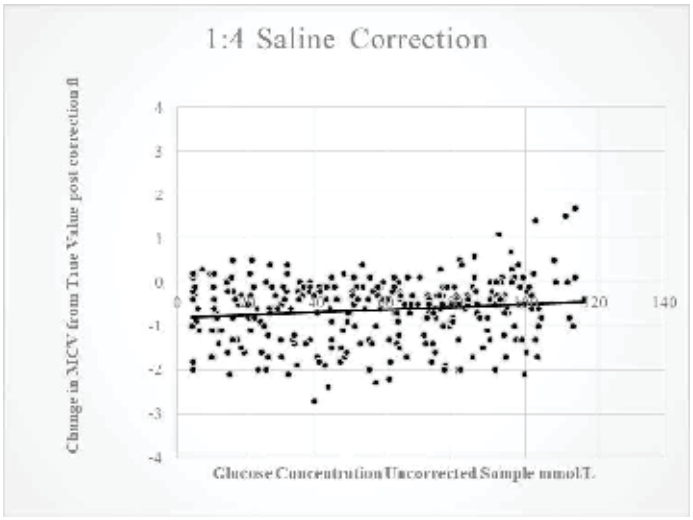

Figure 13. *Correction of spurious MCV by 1:4 dilution with saline. Corrections performed on 20 samples each (glucose concentrations adjusted incrementally) from 15 participants. Correction effective up to 120 mmol/L. Slight but insignificant over correction of -0.5 fL.*

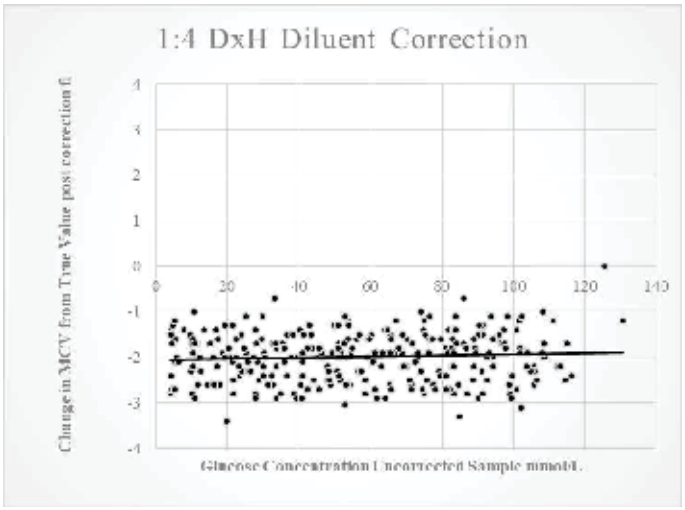

Figure 14. *Correction of spurious MCV by 1:4 dilution with DxH800 diluent. Corrections performed on 20 samples each (glucose concentrations adjusted incrementally) from 15 participants. Correction effective up to 120 mmol/L. Significant over correction of -2.0 fL. This is accompanied by a spuriously high MCHC.*

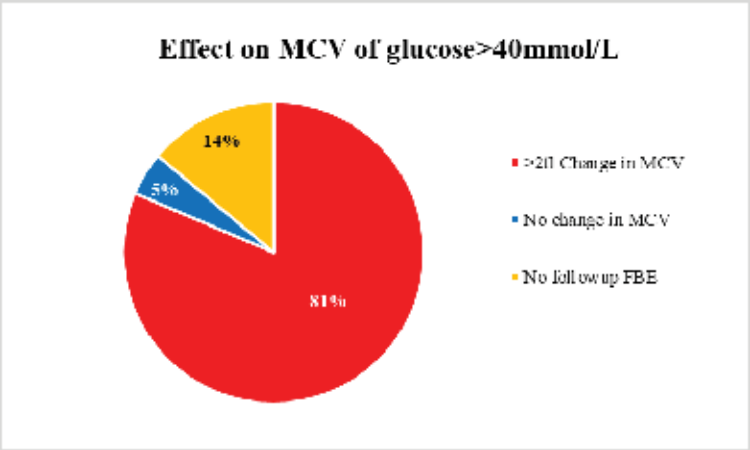

Figure 15. *Incidence of spurious MCV results when glucose concentration >40 mmol/L. Total (48 episodes), >2 fL change in MCV (43 confirmed episodes), no change in MCV (2 episodes), 6 episodes with no follow up FBE Shows significant numbers of MCV results affected in this range (81 %).*

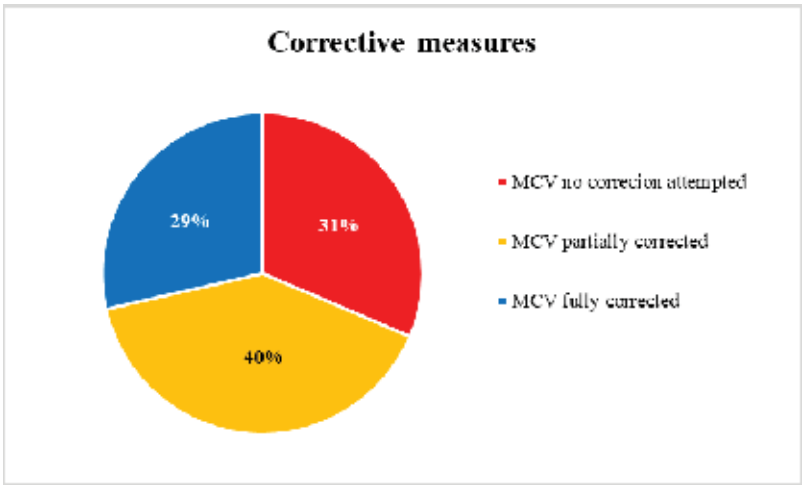

Figure 16. *Performance of corrective measures when glucose >40 mmol/L. 24 spurious MCV results detected with corrective actions applied. Full correction achieved for 10 (29%) episodes. Partial correction achieved for 14 (40%) episodes. 11 (31%) episodes were not detected and no corrective measures applied.*

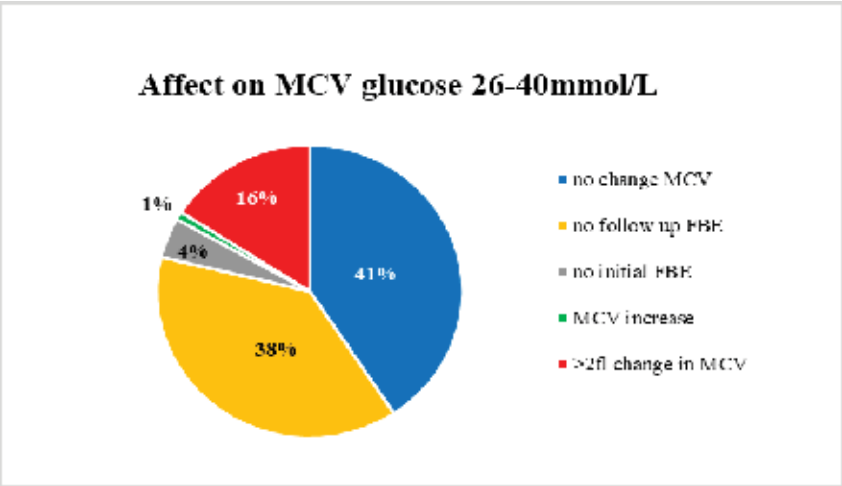

Figure 17. *Incidence of spurious MCV when glucose 26-40 mmol/L. Total 112 episodes in this range. 15 (16%) confirmed spurious MCV (>2 fL change), 43 (38%) episodes with no follow up FBC (affect on MCV not determined), 59 (41%) episodes showed no change in MCV, 4 episodes with no initial FBC (affect on MCV not determined). 1 episode with an increase in MCV due to transfusion.*

Table 1. *Analysis of DxH800 diluent, LH750 diluent and 0.9% Saline. Comparison of osmolality and levels of osmotically active ions and glucose.*

ANALYTE	DxH800 DILUENT	LH750 diluent	SALINE
Na^+ mmol/L	228	233	151
K^+ mmol/L	0.89	0.94	0.75
osmolality	332	336	285
pH	6.8	6.8	7.1
Glucose mmol/L	0.0	0.0	0.0

Discussion

Spurious MCV results have been demonstrated at high glucose levels. The effect varies from individual to individual, each having a unique threshold at which the error can occur. This study set out to find a threshold which could be used as a trigger or guide for the investigation of a possible spurious MCV result. The threshold used must reduce the incidence of error to an acceptable rate. The recommendation is to use the threshold of 26 mmol/L glucose as a trigger for further investigation as this was the lowest threshold determined in this study. Retrospective examination of patient results detected some spurious results below this level so the threshold should be used as a guide and not an absolute cut off. This threshold is specific to the DxH800 analyser. This study, as have previous studies (Holt *et al* 1981; Planas *et al* 1985 and Van Doijhoven *et al* 1996), demonstrated that the effect of hyperglycaemia on the MCV is time dependent and varies from model to model. With new generation instruments measuring FBC in shorter time frames, it would be worthwhile for haematology laboratories to establish their own threshold specific for their particular analyser that can be used to investigate possible spurious MCV results.

Measures applied to correct the spurious MCV should aim to correct the result to within 2 fL of the true or normalized value. The method that best achieves this is the 1:4 dilution with saline or the 1:2 dilution with saline up to glucose level of approximately 60 mmol/L. The threshold varies from individual to individual and the corrective measure should aim to reduce the glucose level to below the threshold and to ensure this happens the dilution should reduce the concentration of glucose to <26 mmol/L. The use of analyser diluent should be avoided as this is prone to causing overcorrection of the MCV and introduction of spurious high MCHC results.

The advantage of the 1:4 dilution with saline method is the reduction in time taken to resolve the spurious result and the elimination of calculation errors. The results can also be transmitted directly to the LIS for validation if the analyser has a 1:5 pre-dilute mode. One drawback is the lack of a white cell differential obtained by this method. It would be helpful for the Beckman Coulter Company to find a solution to this issue. The question as to whether 5 min incubation with a 1:1 dilution with saline would be adequate was beyond the scope of this project but may be investigated further if the 1:1 dilution option was to be considered. Another possible solution that could be explored by Beckman Coulter would be to increase the mixing time before measurement of the MCV commences to greater than 25 sec or to similar time to the LH750 thus increasing the time for equalization and reducing the incidence of this error.

Review of previous investigations of spurious results showed that the majority above the old threshold of 40 mmol/L were corrected, however there is room for improvement. Attention to detail and cooperation between biochemistry and haematology is vital for the threshold method to work. An alert placed in the computer system to prompt the biochemistry scientist to inform the haematology scientist to investigate could be one way to improve the process. Education of not only the haematology staff but also the biochemistry staff is important to foster cooperation between disciplines.

Spurious results may impede investigation of anaemia. The MCV or size of the red cell is a guide to further investigation. A macrocytic anaemia may indicate megaloblastosis as a cause while in a microcytic anaemia, iron deficiency or haemoglobinopathy are the focus of investigation. Whilst a blood film may raise questions as to the validity of a result, standard comments recommending B12/folate/LFT or conversely iron deficiency/haemoglobinopathy are commonly used based on MCV results. As shown by the data, hyperglycaemia can result in spurious MCV results, which in a significant number of cases, can be misleading. A high proportion of cases with high glucose levels above the threshold have only one FBC tested and so the laboratory often has only one opportunity to release correct results. The implementation of a glucose threshold to trigger further investigation of a possible spurious MCV is a step towards achieving this.

Acnowledgements

Clark Rachel, Applications Specialist, Haematology, Beckman Coulter

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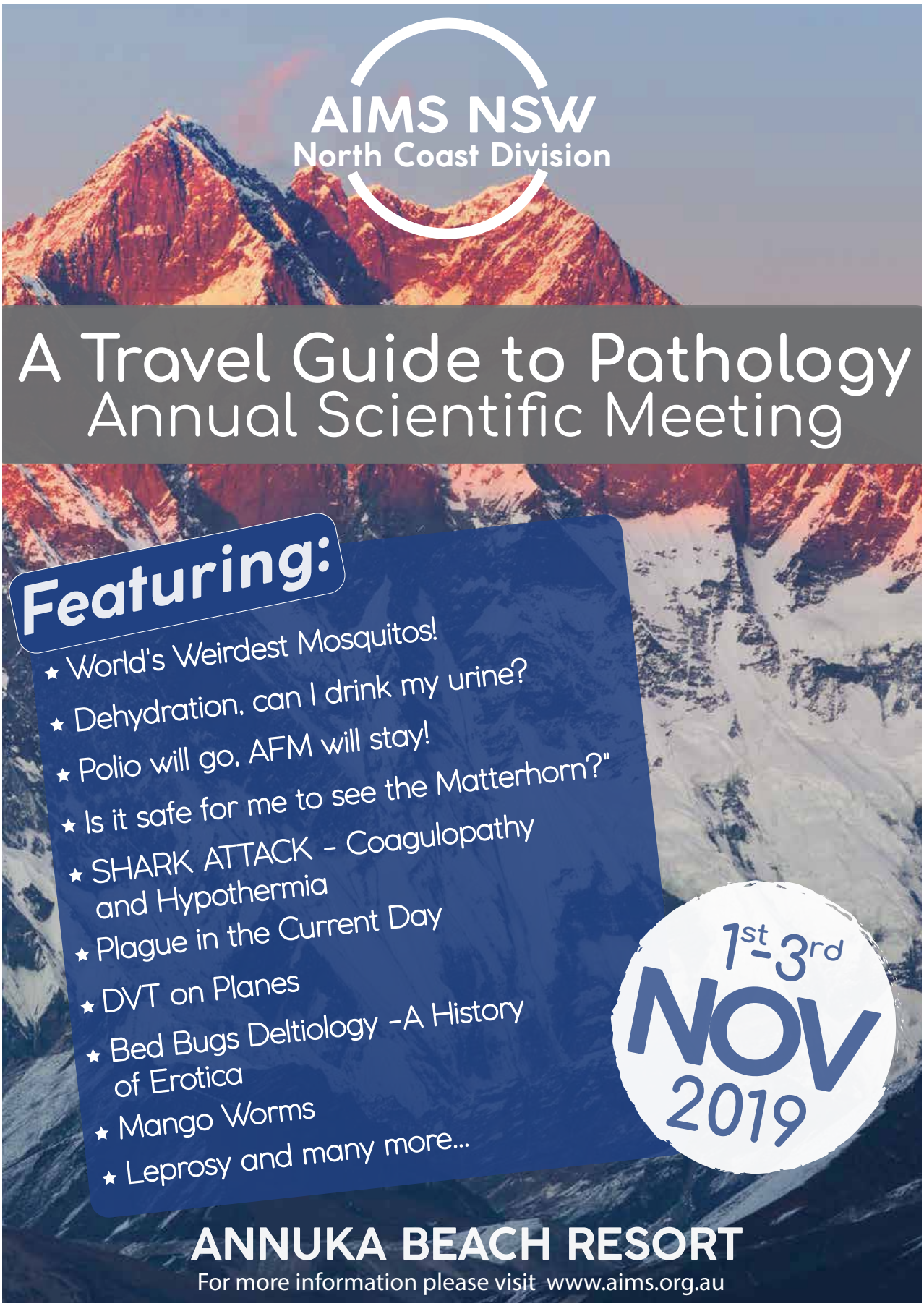
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Fibrinogen replacement in trauma patients: effect on anticoagulant proteins antithrombin, protein C and protein S

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Abstract

Fibrinogen replacement in severe trauma has been shown to improve patient outcomes. Hypofibrinogenaemia is a side effect of severe trauma and fibrinogen replacement has become an important form of treatment. Major haemorrhage protocols are increasingly replacing fresh frozen plasma and cryoprecipitate with fibrinogen concentrate, largely due to convenience and speed. This study investigated the plasma levels of anticoagulant proteins following trauma in patients randomly assigned to receive either fibrinogen concentrate or cryoprecipitate. Antithrombin, protein C and protein S levels were assessed prior to fibrinogen replacement, on admission, and 6, 12, 24 and 72 hours post admission. Levels of anticoagulant proteins were low at presentation for all patients who met the clinical requirements for fibrinogen replacement. Following treatment, the cryoprecipitate group demonstrated rapid recovery, with plasma levels of anticoagulant proteins returning to the normal range by 24 hours but significant differences were seen in protein S concentrations between the treatment groups at 12 hours post ICU admission. This may suggest that fibrinogen replacement products have different effects on anticoagulant protein levels post trauma and disruptions to this balance may cause consequences for recovering patients. As fibrinogen concentrate use increases, anticoagulant protein function and risk of thromboembolic events in the longer term needs to be considered as thromboembolic events occurred in 27% of patients, demonstrating that post treatment complications are common in this cohort. The implications of this are unclear. There may be a role for combined fibrinogen replacement therapy in major haemorrhage protocols but these results suggest more research is required.

Keywords: antithrombin, protein C, protein s, fibrinogen concentrate, coagulopathy, trauma, cryoprecipitate

Introduction

Trauma is a leading cause of mortality worldwide with an annual death toll greater than 5 million and expected to exceed 8 million by 2020 (Spahn *et al* 2013). Emerging evidence suggests that rapid treatment of trauma patients with fibrinogen replacement therapy leads to improved outcomes by helping to correct coagulopathy and reduce the number of blood products used (Mitra *et al* 2007; Winearls *et al* 2017a; Nardi *et al* 2015). It is starting to become standard practice for major haemorrhage protocols (MHP) to include early fibrinogen replacement using either cryoprecipitate (Cryo) or fibrinogen concentrate (FC) (Spahn *et al* 2013). However there is no international consensus and a lack of robust data, leading to variation in protocols amongst institutions (Rossaint *et al* 2016; Stanworth *et al* 2016). FC and Cryo are often considered to be equivalent as a source of fibrinogen and are used interchangeably in many MHPs (Jensen *et al* 2016). A Cochrane systematic review into the use of fibrinogen concentrate in bleeding patients concluded that there is a lack of evidence to support the superiority of one protocol over another but more research is urgently required in this area (Wikkelsø *et al* 2013).

The aim of this study is to examine plasma levels of natural anticoagulant proteins over time in severe trauma patients before and after treatment with FC or Cryo. A comparison of samples from patients receiving different fibrinogen replacement products will be conducted and assessed for differences. Plasma samples were collected from trauma patients recruited to the Fibrinogen Early in Severe Trauma Study (FEISTY). It may suggest that one treatment protocol is superior to another if a difference between the treatment groups is shown.

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The consequences of acute trauma

Acute trauma involves a rapid reduction in blood volume leading to hypovolaemic shock which is a major cause of mortality and morbidity in trauma patients. Volume replacement is essential to reduce mortality in these patients, however replacement therapy may result in unintended consequences and complications (Hess *et al* 2008; Levy *et al* 2012). It is now well established that levels of essential blood constituents may be diluted to pathologically low levels during MHPs. Despite surgical correction and volume replacement, patients still succumb to post trauma coagulopathy (Winearls *et al* 2017a). Coagulopathy occurs when disruptions to the finely balanced blood coagulation system cause excessive bleeding or clotting, resulting in adverse clinical outcomes (Cohen *et al* 2013).

Trauma induced coagulopathy (TIC) is complex and poorly defined, with the exact mechanism still not identified (Simmons and Powell 2016). Factors such as dysfunction of coagulation proteases following hypothermia, acidosis, hypoxia and tissue damage have been implicated in TIC. The past ten years have seen a paradigm shift in our understanding of TIC (Kushimoto *et al* 2017). Patients categorised as having TIC have a mortality rate approaching 50%, however this can be reduced by targeted administration of blood products (Davenport 2013). It has been reported that 20-47% of trauma patients present with a coagulopathy, even prior to intervention (Cohen *et al* 2013). There are some suggestions that this coagulopathy is actually disseminated intravascular coagulation (DIC) with a fibrinolytic phenotype, however a clear consensus has not been reached (Lier *et al* 2011). The primary mechanisms involved seem to be tissue hypoperfusion, leading to acidosis and activation of protein C (PC), which in-turn mediates TIC by increasing fibrinolysis (Cohen *et al* 2012). Acidosis, measured by base deficit, impairs coagulation factor activity leading to a haemostatic imbalance (Frith *et al* 2010). Previous studies have examined levels of PC in trauma patients, however a comparison of PC levels in different fibrinogen replacement treatment groups is yet to be performed (Jensen *et al* 2016). Many other mechanisms contribute to coagulopathy including tissue and endothelial damage, platelet dysfunction, and hyperfibrinolysis and these need to be defined more completely (Meledeo *et al* 2017; Cadroy *et al* 1997).

Detecting trauma induced coagulopathy

Currently, clinicians rely on standard laboratory tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen assays (FibC) to monitor coagulopathy. Characterisation of coagulopathy is difficult and often impedes optimisation of treatment, however emerging technologies such as thromboelastometry may improve this process (Davenport 2013; Davenport *et al* 2011). Thromboelastometry provides rapid whole blood coagulation testing at the point of care and the FibTEM result reflects the circulating fibrinogen levels. In this trial, fibrinogen dosing was guided by FibTEM A5 results, as per the MHP for this site using the ROTEM® instrument (Winearls *et al* 2017b). Coagulation factor levels additional to fibrinogen are not routinely monitored in the trauma patient, so the effect of different treatment protocols on the haemostatic balance is not known and requires more research.

TIC has been defined as an International Normalisation Ratio (INR) >1.2 on presentation post trauma (Frith *et al* 2010; Davenport *et al* 2011) however there is growing suspicion that standard laboratory tests fail to detect TIC (Cohen *et al* 2013; Lier *et al* 2011). Twenty-five studies failed to reach consensus on the definition of TIC, with INR lower limits ranging from 1.2 to 1.6. This suggests that INR is not an adequate measure of TIC and does not fully reflect the underlying coagulopathy (Peltan *et al* 2015).

Measurement of fibrinogen as a marker of TIC has also been discussed in the literature. Low fibrinogen concentration on admission has been associated with increased trauma severity, hypoperfusion and increased transfusion requirements (Rourke *et al* 2012; Brohi *et al* 2008). Pre-operative low fibrinogen levels can in fact be used as a predictor for complications following cardiac surgery (Guan *et al* 2018) but during acute trauma, it may be more complicated. Low fibrinogen levels have been associated with severe trauma prior to administration of fluids and these levels deplete rapidly upon the onset of shock through a mechanism of consumption, fibrinolysis and fibrinogenesis (Rourke *et al* 2012; Kushimoto *et al* 2017). This has been well covered in other reviews (Hayakawa 2017b).

As PC has been shown to be an important mediator of TIC, it is possible that its measurement may be a useful indicator. Although the PC assay can be run in a similar timeframe to an aPTT, standard laboratory testing is often too slow for clinical use. An advantage of using thromboelastometry is the relatively rapid test speed as the instrument is often available at the point of care. Other approaches involve rapid testing panels for actively bleeding patients (Chandler *et al* 2010) however, these approaches may not fully define the underlying coagulopathy. Currently, there is no consensus on the best way to monitor haemostasis in the trauma patient.

Fibrinogen and its contribution to trauma induced coagulopathy

Fibrinogen, the most abundant blood coagulation factor, accounts for 4-7% of total plasma proteins. Integral to the blood coagulation system, fibrinogen is converted to fibrin by thrombin in the final stages of blood clot formation, thus

preventing further bleeding. Fibrinogen also plays a role in platelet aggregation. There is now increasing evidence that fibrinogen is important in TIC, which is often associated with lower levels of fibrinogen and higher levels of fibrinogen break down products (He *et al* 2018). Following treatment for severe trauma, fibrinogen is the first coagulation factor to become critically depleted and hypofibrinogenemia has been shown to be a major risk factor in the trauma patient due to the development of a coagulopathy (Spahn *et al* 2013; Meledeo *et al* 2017). This appears to be associated with the dilutional effect of product replacement, which may be further complicated by consumption, acidosis and hypothermia (Wikkelsø *et al* 2013).

Prevention of coagulopathy has been shown to be more effective than treatment and this can be achieved either through replacement of fibrinogen or reduction of fluids (Wikkelsø *et al* 2013). The use of MHPs has contributed to lower mortality rates through the systematic use of blood products (Malone *et al* 2006). However, MHPs are not available in every institution, nor are they consistent. Significant mortality rates may still be observed when coagulopathy is present and plasma fibrinogen levels are normal, indicating that the mechanisms involved are complex and poorly understood (Brohi *et al* 2007a). Treatment of coagulopathy and the resulting imbalance of coagulant and anticoagulant proteins is inconsistent and robust evidence supporting particular therapies are lacking (Meledeo *et al* 2017).

Fibrinogen replacement protocols

Historically, fibrinogen replacement has been in the form of fresh frozen plasma (FFP) (Nascimento *et al* 2010). FFP is whole blood plasma, separated and frozen soon after collection and contains all coagulation factors. Specific targeted products such as prothrombin complex concentrates (PCC), FC and Cryo only contain a fraction of the coagulation factors. FC contains only plasma derived fibrinogen in a lyophilised form, and its primary benefit is the speed of administration (Wikkelsø *et al* 2013). Cryo is prepared by recovering the cold-insoluble precipitate of FFP, and contains most of the FVIII, fibrinogen, FXIII, von Willebrand's Factor and fibronectin (Callum *et al* 2009). FC and Cryo are used in trauma situations to replace fibrinogen without the excessive volume of FFP. PCC contains vitamin K dependent factors FII, FVII, FIX, FX, antithrombin (AT), PC and protein S (PS). As protocols move further away from FFP, ancillary blood components that are ordinarily replaced in trauma will no longer be routinely replenished. There is no consensus as to whether or not Cryo is superior to fibrinogen concentrate for rapid fibrinogen replacement, and major haemorrhage protocols often use the two products interchangeably (Wikkelsø *et al* 2013; Jensen *et al* 2016). It is likely that one fibrinogen replacement product is superior to the others, and one of the aims of this study is to determine if there is a detectable difference between the treatment options. The FEISTY study will compare time to administer fibrinogen replacements, FC and Cryo, as it is generally accepted that FC is faster (Wikkelsø *et al* 2013; Winearls, *et al* 2017b). While fibrinogen levels are monitored quite closely, other coagulation factor levels are not routinely tested on trauma patients and due to difficulties recruiting patients and collecting samples in acute trauma cases, data is limited. While fibrinogen replacement studies are abundant they have been restricted largely to elective cardiac surgery patients (Meledeo *et al* 2017). There is an urgent need for data to describe appropriate coagulation testing and product replacement to guide treatment for acute trauma patients.

Protein C, protein S and antithrombin

Resuscitation of trauma patients is centred around replacing blood volume, correcting coagulopathy, and restoration of haemostasis. The priority is to administer products that prevent blood loss, such as clotting factors, antifibrinolytic products and platelets to improve short term survival. More research is required to investigate longer term outcomes of the haemostatic imbalances that arise in trauma. Often, these imbalances will manifest as thromboembolic events days or weeks post trauma (Peltan *et al* 2015). Outcomes after the administration of large volumes of blood products includes lung injury, sepsis and multiorgan failure and these are often associated with haemostatic imbalances (Hess *et al* 2008). AT, PC and PS have complicated interactions with other proteins and may have cytoprotective functions in the trauma patient (Cohen *et al* 2012). This study will focus on the levels of anticoagulant proteins and their potential effects on haemostasis in the trauma patient.

PC is likely the most important anticoagulant protein in TIC (Cohen *et al* 2012; Brohi *et al* 2007). Its primary function is to regulate factors V and VIII by inactivating them, along with cofactor PS and therefore the activity of these proteins is linked. In this study, PS was measured in parallel with PC to establish trends in the data as the proteins should be present in similar amounts. Trauma patients often switch to a hypercoagulable state post treatment, and this can be correlated with a depletion of PC and in fact it has been shown that the hypercoagulable state may exist prior to treatment, and not because of it (Hess *et al* 2008; Brohi *et al* 2008; Kushimoto *et al* 2017). The cytoprotective effect of PC is thought to play a significant role in the trauma patient (Cohen and Christie 2016) and studies in mouse models have shown that blocking PC function results in 100% mortality after severe trauma (Chesebro *et al* 2009). Many studies have outlined the important role of PC in mediating TIC, and it is likely to be a focus of future research.

AT is an anticoagulant protein that interferes with clot formation by inhibiting pro-coagulant factors thrombin and activated factor X, as well as other proteases. It is most well known for being the target of heparin, a common anticoagulant drug. Heparin increases the activity of antithrombin up to 1000 times. Many studies show decreased AT following severe trauma, leading to dysregulation of coagulation (Hayakawa 2017a) but the implications of this observed decrease are unclear. AT exhibits independent anti-inflammatory properties in addition to its anticoagulant effects similar to the cytoprotective effect of PC (Afshari *et al* 2008). Blood products such as Thrombotrol®-VF contain recombinant AT and are used in congenital AT deficiencies. Several studies have examined the off-label use of these products as a potential treatment in severe illness, but the efficacy is still debated (Tagami *et al* 2015; Allingstrup *et al* 2016). The inflammation process may be modified by AT, independent of coagulopathy (Afshari *et al* 2008). AT, along with PC and PS, is present in FFP, but not Cryo or FC. If replacement is beneficial, then exclusion of FFP from MHPs may have a significant effect on TIC. More research is required to investigate the role of AT in severe trauma, and the potential therapeutic use of recombinant AT in trauma patients.

Materials and methods

Patient recruitment and the FEISTY study

This study is a prospective randomised controlled trial involving trauma patients at The Townsville Hospital. The patient cohort comprised those presenting to the emergency department with severe trauma. Samples were collected between April 2017 and March 2019. Eligible patients were affected by trauma, judged to have significant haemorrhage or predicted to require significant transfusion with ABC Score ≥ 2 or by treating clinician judgement (Winearls *et al* 2017b). The ABC score is an indicator of massive transfusion requirement (Nunez T C *et al* 2009). During the trial period, 15 patients presented to the emergency department in Townsville. Patients were randomised into two treatment groups, one receiving FC and one receiving Cryo. The FC group received RiaSTAP®, which was reconstituted and administered at the point of care. The Cryo group received frozen blood products from the laboratory which had been requested, thawed at 37°C and dispatched by staff as per routine protocol. The control group consisted of patients that qualified for the study but did not meet the requirements for fibrinogen replacement. These patients only received packed red blood cells or no intervention. This study is a sub-study of the FEISTY trial (clinical trial ID NCT02745041, ethics approval HREC/16/QGC/128).

Blood sample collection

Blood samples were collected for analysis at presentation to emergency and prior to fibrinogen replacement (0 h) or at ICU admission, and 6, 12, 24 and 72 h post admission. Blood samples were not collected at every target time point due to the technical difficulties involved with sampling from trauma patients.

The patients were randomly assigned to treatment groups by the treating clinician to receive either FC (n=7) or Cryo (n=4). The control group (n=4) did not receive fibrinogen replacement and consisted of patients with an average injury severity score (ISS) on presentation of 20 ± 12.2. Of these patients, three received RBC only and 1 no intervention. There was a wide variation in the number of blood products each patient received, as expected in trauma, and detailed analysis of products is beyond the scope of this study. Average total blood products and units of fibrinogen received by each treatment group are listed in Table 1. The patients were grouped based on the type of treatment received, regardless of the number of products. The average standard laboratory test results at presentation, ISS and thromboembolic complications for each treatment group were also recorded (Table 1).

Measurement of anticoagulant proteins

Anticoagulant proteins PC, PS and AT were measured at all time points on citrated platelet free plasma, stored at -80°C within 2 hours of collection. Results are expressed as a percentage, which is the convention for these proteins, assuming 100% is a normal level. Reference ranges were sourced from Pathology Queensland standard ranges. Analyses were performed on the ACL-TOP 500 instrument (Instrumentation Laboratories, Bedford, MA). AT and PC were both assessed using HaemosIL chromogenic assays (Instrumentation Laboratories, Bedford, MA). Free PS antigen was measured using an HaemosIL immunoturbidometric assay. All analyses were conducted in accordance with the manufacturer's recommendations and performance was assessed using commercial quality control material sourced from HaemosIL. Normal and abnormal controls were run with each batch of samples to ensure quality of results, as is standard commercial laboratory practice. Citrated plasma was thawed at 37°C for 5 min prior to analysis.

Statistical analysis

Statistical analysis of results was performed to determine differences between treatment groups FC and Cryo using a 2 tailed t-test with unequal variance and a significance level of 0.05. Both treatment groups were also compared to the control group (Tables 1 and 2).

Due to the small sample size and uneven patient groups, effect sizes were also calculated (Table 3). The absolute

effect size is the difference between the average, or mean, outcomes in two different intervention groups. In studies that compare different interventions, effect size is the magnitude of the difference between groups (Sullivan and Feinn 2012). To measure effect size between the FC and Cryo groups, Hedges' g formula was used. Hedges g is a score used when comparing uneven populations using their mean, standard deviation and number of data points. The resulting effect size is a measure of difference between groups, with a g score equal to 1 indicating that the two groups differ by 1 standard deviation. It has been established that a small effect size (which cannot be detected by the naked eye) is 0.2g, a medium effect is 0.5g, a large effect is 0.8g and a very large effect is 1.3g (Hedges 1981; Sullivan and Feinn 2012). Standard error from the mean (SEM) is represented in all charts as error bars (Figures 1 and 2).

Results

The aim of this study was to compare plasma levels of anticoagulant proteins AT, PC and PS following trauma and treatment with either FC or Cryo on presentation to emergency and post fibrinogen replacement. A summary of patient interventions and outcomes is shown in Table 1. There was no significant difference between total blood products received during the event or plasma fibrinogen at presentation between treatment groups or control group. The control group had a lower average ISS score and combined with the stable fibrinogen levels did not meet the requirement for fibrinogen replacement. The FC and Cryo treatment groups were well matched for ISS, with the FC groups having an average of 31.2 ± 14.3 and the Cryo group 31.0 ± 4.2 (Table 1).

Patients receiving fibrinogen replacement (FC or Cryo) may have additionally received RBC, platelet products or FFP. Patients receiving Cryo required significantly more units (12.5 vs 2.9) of product than those receiving FC and this is due to the concentrated nature of the FC.

There was insufficient data in this study to determine if total thromboembolic events (TE) differed between treatment groups. In this study the overall TE complication rate was 27% and the TE occurrence for the FC and Cryo treatment groups and control group was 14%, 50% and 25% respectively.

The control group had an average INR of 1.1 ± 0.1, which did not meet the criteria for TIC (INR>1.2). Both the FC group (1.4 ± 0.2) and the Cryo group (1.4 ± 0.1) had average INRs which were significantly higher than controls ($p<0.05$) meeting the criteria for TIC (Table 1). Overall, 66% of patients had TIC, and all were allocated to receive fibrinogen replacement.

These data show that the method for fibrinogen treatment selection (FibTEM A5 level) identified TIC in accordance with an INR threshold of 1.2.

Plasma levels of natural anti-coagulant proteins

There was no significant difference in the anticoagulant protein levels between the two treatment groups on presentation (Figure 1 and Table 2). There was no significant difference between the treatment and control groups for AT and PS on presentation, however, the PC level for the FC group was significantly lower than the control group ($p<0.05$).

The control group showed little change in anticoagulant protein levels over time (Figures 1 and 2). Both treatment groups showed plasma protein levels that steadily declined until 12 h post ICU admission (Figure 1). Protein plasma levels then gradually recovered to normal levels over a period of several days (Figure 1).

Change of anticoagulant protein level over time

Levels of anticoagulant proteins at 0, 6 and 12 h post admission are displayed in Table 2. By 12 h post ICU admission the anticoagulant protein levels for the control and Cryo groups remained within the reference range, while, the AT and PC levels for the FC group had dropped below the level of the reference range and were significantly lower than the control group ($p<0.05$). The level of PS was significantly lower in the FC group at 12 h post ICU admission than for the Cryo group. Figure 2 depicts the percent change in anticoagulant protein levels between the 0 and 12 h timepoints after ICU admission. Data has been represented as a proportional change (%) from the initial protein level. PC remained constant over time, while the greatest variation was seen in PS (41% decrease ± 5%). In the Cryo group, PC showed the greatest increase (15% ± 10%) and PS a decrease of only 17% ± 2%.

Effect size

The greatest difference in plasma anticoagulant protein levels between treatment groups was seen at 12 h post ICU admission. The effect size scores for these changes are listed in Table 3, demonstrating the anticoagulant protein levels following treatment of hypofibrinogenemia with fibrinogen products from different sources. Effect size was calculated for the FC vs Cryo groups at 12 h post ICU admission for each anticoagulant protein (Table 3). From these scores, it may be determined there was a medium effect of treatment on PC, a large effect on AT levels and a very large effect on PS (Sullivan and Feinn 2012). To control for effect size, presentation protein levels were compared between FC and Cryo groups. At the point of presentation, no fibrinogen had been administered. There was a small effect of allocation on PC and an undetectable effect on AT and PS. Effect sizes were also then calculated for levels

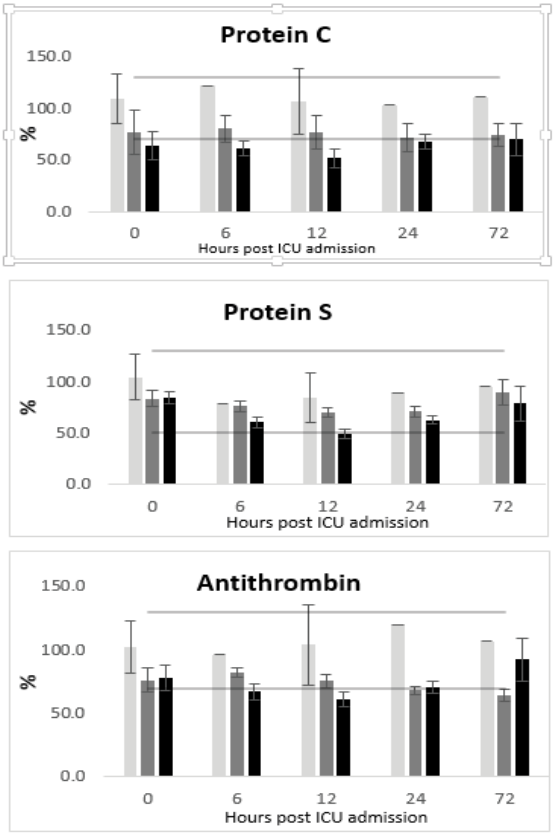

Figure 1: *Antithrombin (AT), Protein C and Protein S levels (%) displayed as mean +/- SEM for Control: light grey, Cryoprecipitate (Cryo): dark grey, and Fibrinogen Concentrate (FC): black. Samples were collected upon admission 0 hours and 6, 12, 24 and 72 hours post ICU admission. *Indicates significant difference between treatment groups ($p< 0.05$).*

24 h post ICU admission. It is noted that while AT and PC had small and undetectable differences 24 h post, the PS effect was still large due to the very large effect seen at 12 h post ICU admission. In summary, a small difference between FC and Cryo treatment groups was seen for AT and PC at presentation, a large difference 12 h post ICU, then a small difference 24 h post ICU. A small difference between the treatment groups was seen for PS, then a very large difference at 12 h post ICU admission which translated to a medium effect at 24 h post. This effect can be seen to continue through to 3 days post ICU admission (Figure 1).

Discussion

The aim of the study was to determine if choice of fibrinogen product for treatment following trauma resulted in changes to plasma anticoagulant protein levels in patients 6, 12, 24, 72 h post ICU admission. There was no significant difference in plasma AT and PS levels between the control and treatment groups on presentation. There was no significant difference between PC levels of the treatment groups on presentation, however, the PC for the FC group was significantly lower than the control group. This may be a limitation of this study or be representative of the severity of the trauma and onset of TIC.

Plasma levels of anti-coagulant proteins protein C, protein S and antithrombin

A decrease in anticoagulant proteins post severe trauma is a reproducible phenomenon and was expected in this study. The protein levels in the control group remained stable over time. When compared to the control group, both treatment groups demonstrated lower anticoagulant protein levels across all time points. This effect has been described in a number of studies (Yanagida *et al* 2013; Cohen *et al* 2012; Hayakawa 2017a; Johansson *et al* 2011) however what is yet to be described is the levels of these proteins following different intervention therapies (Jensen *et al* 2016).

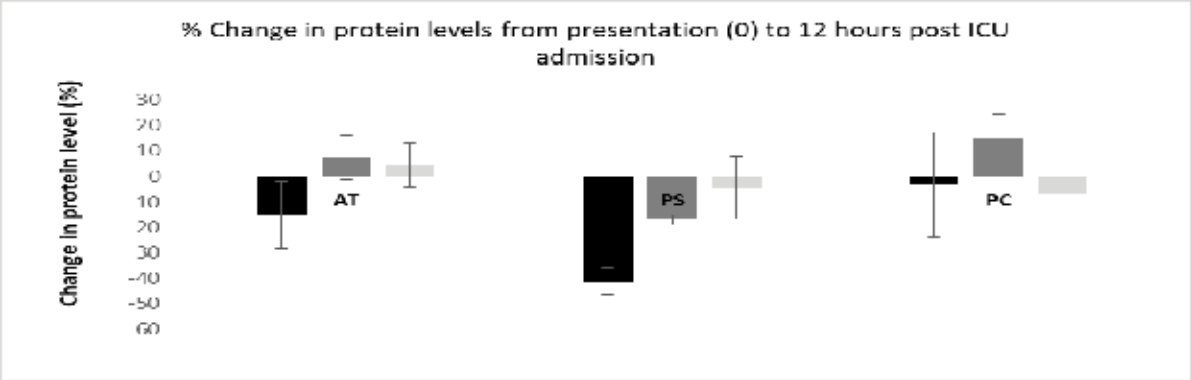

Figure 2. *Variation of protein levels as a percentage of presentation protein levels 12 hours post ICU admission displayed as mean +/- SEM for each treatment group. Fibrinogen Concentrate (FC) group: black, Cryoprecipitate (Cryo) group: dark grey, Control group: light grey.*

Table 1. *Summary of patient interventions and outcomes.*

Variable	Groups (fibrinogen treatment post severe trauma)		
	FC	Cryo	Control
Units of fibrinogen received	2.9 ± 1.4 †	12.5 ± 3.5†	0 *
Total units of other blood products	8.4 ± 6.5	14.5 ± 15.2	3.0 ± 2.9
Injury Severity Score (ISS)	31.2 ± 14.3	31 ± 4.2	20 ± 12.2
Thromboembolic events	1 (7)	2 (4)	1 (4)
FibC level at presentation (g/L)	2.4 ± 2.0	1.6 ± 0.7	2.3 ± 0.3
PT at presentation(s)	15.1 ± 1.9 †	15.0 ± 0.8 †	11.7 ± 0.6
INR at presentation	1.4 ± 0.2 †	1.4 ± 0.1 †	1.1 ± 0.1
aPTT at presentation(s)	35.4 ± 13.6†	42.5 ± 14.7	22 ± 1.0

Outcomes for patients receiving Fibrinogen Concentrate (FC), Cryoprecipitate (Cryo) or blood products alone (Control). Values are displayed as means +/- SEM. †Denotes significant difference ($p<0.05$) from control group, *Denotes significant difference between treatment groups ($p<0.05$).

Table 2. *Anticoagulant proteins on presentation (0) and 6 (6) and 12 (12) Hours post ICU admission.*

Variable	Groups			
	FC	CRYO	Control	Reference Range (%)
AT (%) presentation	78.2 ± 10.1	76.3 ± 9.6	103.0 ± 3.0	70 - 130
AT (%) 6 hours	67.4 ± 6.2	82.5 ± 3.8	96.0 ± 0	
AT (%) 12 hours	61.0 ± 5.6†	75.5 ± 5.5	104.0 ± 11.0	
PC (%) presentation	63.8 ± 14.0†	77.3 ± 21.5	109.8 ± 12.7	70 - 130
PC (%) 6 hours	62.0 ± 7.0	80.5 ± 13.3	122.0 ± 0	
PC (%) 12 hours	51.8 ± 9.0†	77.0 ± 15.8	106.5 ± 8.5	
PS (%) presentation	84.8 ± 5.8	83.7 ± 8.0	104.5 ± 10.2	50 - 130
PS (%) 6 hours	60.6 ± 5.2	76.0 ± 5.5	78.0 ± 0	
PS (%) 12 hours	49.2 ± 4.5*	69.8 ± 4.5	84.0 ± 1.0	

Antithrombin (AT), Protein C (PC) and Protein S (PS). Results are presented as means +/- SEM. Reference ranges from Pathology Queensland 2019. *Denotes significant difference between treatment groups ($p<0.05$), †Denotes significant difference ($p<0.05$) from control group.

Table 3. *Effect Size of different fibrinogen replacement treatments.*

Anticoagulant protein	Hedges' score of effect size for FC vs Cryo (g)			
	Presentation (0)	12	24	Effect size* at 12 hours post
Antithrombin	0.096	0.868	0.295	Large
Protein C	0.389	0.472	0.188	Small to medium
Protein S	0.075	2.447	0.806	Very large

*Small effect size (which cannot be detected by the naked eye) is 0.2g, a medium effect is 0.5g a large effect is 0.8g , a very large effect is 1.3g (Hedges 1981; Sullivan and Feinn 2012).

By 12 h post admission to ICU the AT and PS levels for the Cryo group were not significantly different to those in either the control or FC group, however, the FC group had anticoagulant protein levels significantly lower than the control group and PS levels significantly lower than the Cryo group (Figure 1, Table 2). As this difference was not seen at presentation before fibrinogen replacement therapy, it can be concluded that the differences were primarily due to the source of fibrinogen selected. Not only was this effect discernible visually, but an analysis of effect size showed a very large effect between the treatment groups was seen for PS, a large effect for AT and a medium effect for PC (Table 2).

After the 12 h timepoint the PS and PC levels returned to within normal range. While the AT levels for the treatment groups remained low, the AT levels for the FC group returned to within the reference range and Cryo group remained below the reference range. This suggests that there may be an effect on anticoagulant protein levels post fibrinogen replacement therapy dependent on treatment type. The sample size for this study was small, however the results suggest a difference in the anticoagulant protein levels between the fibrinogen treatment types, and an increased recovery of anticoagulation proteins with Cryo treatment as compared to FC.

Choice of fibrinogen product

In many major haemorrhage protocols, FC and Cryo are used interchangeably and they are widely considered to be equivalent products (Rossaint *et al* 2016; Wikkelsø *et al* 2013). The difference in treatment groups is significant for PS, and the data trend is reproducible across all three proteins tested. This would indicate that there may be a benefit to using one treatment over the other for maintenance of anticoagulant protein levels. If this is true, it would also apply to coagulation factor levels across the board. More research in this area is required with a larger sample size to adequately assess FC versus Cryo as a treatment for hypofibrinogenaemia and the effect on haemostatic balance. If additional factors were tested in a future study, a difference between fibrinogen replacement products and their effects on coagulation may be further highlighted.

Findings from this study support the use of Cryo for fibrinogen replacement and for maintaining anticoagulant protein levels. This finding was unexpected. Anticoagulant proteins are known to be low in trauma patients (Cohen *et al* 2012; Jesmin *et al* 2016) therefore the effect of fibrinogen replacement is important. It is generally accepted that FC is a superior source of fibrinogen to manage bleeding and for the treatment of TIC (Wikkelsø *et al* 2013; Jensen *et al* 2016). FC is often preferred due to speed of administration, standardised fibrinogen concentration, clinical convenience, and reduced risk of pathogen transmission and immune-mediated complications (Winearls *et al* 2017a; Rossaint *et al* 2016; Sørensen and Bevan 2010). What is less clear is the effect of removing Cryo from a protocol with regards to thromboembolic complications (Wikkelsø *et al* 2013). Although this study determined Cryo to be superior for conserving anticoagulant protein levels, the group receiving this treatment did have a higher thromboembolic complication rate. As this is a novel pilot study, the results are unusual enough to suggest that further research is required. The mechanism behind the better maintenance and recovery of anticoagulant proteins and clotting factors following treatment using Cryo rather than FC, may involve several pathways.

The vitamin K dependant clotting and anticoagulant factors, which include AT, PC and PS, are only present in FFP (Ben-Tal *et al* 2003). They are not listed as constituents of Cryo or FC. As most of the patients in this study did not receive FFP, it can be assumed that the anticoagulant factors were minimally replaced in all severe trauma patients. This being the case, it is more difficult to explain why there is a difference between the fibrinogen treatment groups. One explanation might be that if anticoagulant proteins are not being replaced, they may be being consumed at different rates. Efforts were made to standardise the fibrinogen content of the products used in the FEISTY trial (Winearls *et al* 2017b), therefore, differences in fibrinogen concentration would not be considered to be a contributing factor. Therefore, additional clotting factors found in Cryo may be important for the conservation, maintenance and recovery of AT, PC and PS in the severe trauma patient.

The fibrinolytic activity of PC and PS has been shown to be important in preventing organ failure in the trauma patient (Chesebro *et al* 2009; Cohen and Christie 2016). Control of PC levels has also been shown to slow or prevent the progression of TIC (Jesmin *et al* 2016). There is an important protective role for anticoagulant proteins, especially PC and AT, which may have cytoprotective and anti-inflammatory effects that may have been underestimated in many studies (Davenport 2013; Chesebro *et al* 2009). This may also explain why the largest effect was seen at 12 h post admission, when systemic inflammation would likely be high and this would also be consistent with the reduced effect after several days in ICU. Further studies are required to tease apart the importance of including Cryo, as well as FC, in an optimal MHP. While FC is more convenient, faster and can be administered more easily, Cryo contains factors that are likely to be important in the severe trauma patient, although the exact mechanism for this remains unknown.

Limitations of this study

There are a number of limitations with this study apart from the small sample size. The FC group had a significantly lower

PC level at presentation when compared to the control group. This may be a factor of the more severe ISS for this group when compared to the control group. There is also an assumption made that the rate of replenishment of proteins is equivalent across all treatment groups, although this was not measured specifically.

Conclusion

Investigation of coagulopathy in trauma patients is an area of research that requires attention. Little data are available, and it is difficult to draw meaningful conclusions in the face of such limited information. As new therapies, such as fibrinogen concentrate, and new point of care testing devices, such as thromboelastometry, become available, new protocols are being established quickly (Rossaint *et al* 2016; Wikkelsø *et al* 2013). Inevitably, the burden of evidence will lag behind the creation of optimised treatment protocols that are proving to be superior to standard practice (Rourke *et al* 2012).Further studies are likely to show that there is a role for Cryo, as well as FC, in an optimal MHP.

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Journal-based CPD No. 62
Page 1 of 1

Questions relating to the article *'Spurious mean cell volumes in hyperglycaemia on the UniCel DxH800 analyzer'* at page 44 of this issue.

1.	Hyperglycaemia is known to cause spurious MCV results on impedence analysers.	True/False
2.	The mean cell volume (MCV) is a key white cell parameter in the full blood count (FBC) that doctors use to determine the condition of a patient.	True/False
3.	Using the DxH800 diluent to perform the dilutions was shown to over correct the MCV and cause spuriously high MCHC results.	True/False
4.	A raised MCV in anaemia may indicate megaloblastosis as a cause.	True/False
5.	A raised MCV may lead to investigation of iron deficiency or haemoglobinopathy.	True/False
6.	A normocytic normochromic anaemia does not require investigation of possible blood loss or haemolysis..	True/False
7.	High serum glucose levels have been known to cause spuriously high MCV measurements on impedance cell counters.	True/False
8.	Glucose is a non-polar osmotically active substance which readily crosses the red cell membrane.	True/False
9.	Two methods of correction were evaluated, each method was evaluated using both DxH800 diluent and 0.9% saline (15 participants each).	True/False
10.	The method of correction 'Replacement of plasma with saline or diluent' is not currently in use at Monash health.	True/False

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Journal-based CPD No. 63
Page 1 of 1

Questions relating to the article *'Fibrinogen replacement in trauma patients: Effect on anticoagulant proteins antithrombin, protein C and protein S'* at page 62 of this issue.

1.	Fibrinogen replacement in severe trauma has been shown to improve patient outcomes.	True/False
2.	Thromboembolic events occurred in 27% of patients, demonstrating that post treatment complications are common in this cohort.	True/False
3.	Trauma is a leading cause of mortality worldwide with an annual death toll greater than 6 million et and expected to exceed 11 million by 2020 (Spahn ***et al*** 2013).	True/False
4.	FC and Cryo are often considered to be equivalent as a source of fibrinogen and are used interchangeably in many MHPs (Jensen ***et al*** 2016).	True/False
5.	Coagulation factor levels additional to fibrinogen are routinely monitored in the trauma patient.	True/False
6.	Twenty studies failed to reach consensus on the definition of TIC, with INR lower limits ranging from 1.2 to 1.6.	True/False
7.	INR is not an adequate measure of TIC and does not fully reflect the underlying coagulopathy.	True/False
8.	Low fibrinogen concentration on admission has not been associated with increased trauma severity, hypoperfusion and increased transfusion requirements.	True/False
9.	Fibrinogen, the most abundant blood coagulation factor, accounts for 4-7% of total plasma proteins.	True/False
10.	Prevention of coagulopathy has been shown to be more effective than treatment and this can be achieved either through replacement of fibrinogen or reduction of fluids.	True/False

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OPINION ARTICLE

Medical science - a forgotten profession in Australia?

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Abstract

Over the last decade the Australian Institute of Medical Scientists (AIMS) has highlighted many times the lack of professional recognition of its members. In 2010 the Australian Health Practitioners Regulation Agency (AHPRA) was established but they decided that medical scientists were sufficiently controlled by a registered pathologist and National Australian Testing Authority (NATA) accreditation and suggested that they develop a process of self-regulation. In many other countries around the world, medical scientists are required to hold a practicing license in order to be employed in clinical laboratories. This license provides recognition to the profession and protection to the public. The governmental regulatory bodies in these countries maintain a register of practitioners, monitor continuing professional development (CPD) and provide disciplinary measures if required. However, with the increase use of technology and the changing role of the scientist, clinical oversight is increasingly not required or provided. Is it time to recognize the contribution that medical scientists make to the Australian Health Workforce and provide them the same professional status as other developed nations?

Keywords: medical science, professional regulation

Introduction

Historically, the only professions recognized prior to the Industrial revolution were: Divinity, Medicine and Law; these were called the "learned professions" (Hare 2000). During the 19th century, the increasing global industrialization led to an expansion of the skilled working population. This expansion provided an opportunity for like-minded individuals to begin meeting locally and to discuss the merits of their occupations. These meetings and their discussions led to the formation of the first professional societies, with a rudimentary form of self-regulation. By doing so they ensured that full legal incorporation and government recognition could be achieved.

The articles of professional membership afforded some protection for its members and recognition of their contribution to the community at large. Some of the major milestones that mark an occupation as a profession include:

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- a defined scope stating the profession's purpose and goals;
- qualifications for education, experience and professional development;
- a code of professional conduct to guide what should or should not be done under given circumstances;
- recognized certification that requires maintenance;
- standards that are consistent with other peer groups (Williams 1998).

In Australia, this definition has been applied to many professions and is not restricted to healthcare. Surveyors, teachers, builders, plumbers and electricians all meet the above criteria. Members of these occupations are required to maintain a practicing license, which is recorded on an independently monitored register backed by legislation to protect the public and the worker. For example, the minimum requirement for renewing an electrician's license is by approved continuing professional development (CPD) for skill maintenance and evidence of annual basic life support (BLS) training (Queensland Government 2017).

In June 2004, the Council of Australian Governments (COAG) commissioned a study of the health workforce in Australia. By the end of the following year a 400 page report was tabled by the Australian Government (Productivity

Commission 2005). This extensive document mentions "medical scientist" four times, in two tables, a paragraph about Medicare charges and recording the submission made by The Australian Institute of Medical Scientists (AIMS). This despite the fact that medical scientists account for more of the Australian workforce than dentists, medical imaging workers and ambulance officers/paramedics.

One of the recommendations of the report was for the formation of a national accrediting body for Healthcare Professions. This recommendation was based on the need for control of a profession that exposed an increased risk of harm to the public. As a consequence, the Australian Health Practitioner Regulation Agency (AHPRA) was established in 2010 to centralize this function within the federal government (Australian Health Workforce Advisory Council 2008).

In 2016 the Accreditation Liaison Group (ALG) of AHPRA, published a "*Comparison of international accreditation systems for registered health systems*". They identified the United Kingdom, Ireland, America, Canada and New Zealand as examples of developed western countries. Those nations were considered Australia's closest philosophical neighbors "*chosen as they have comparable health standards*" with "*well established regulatory structures and comparable standards of education in their health professions*" (AHPRA Acceditation Liaison Group 2016).

Despite the consistency in regulating the medical science profession among the comparable countries, this requirement for regulation does not extend to Australia. This point of difference is due to apparent legal constraints governing AHPRA, which is laid down in the 2008 document "*Intergovernmental Agreement for a National Registration and Accreditation Scheme for the Health Professions*"(Australian Health Workforce Advisory Council 2008) signed by the Premiers of each state and the Prime Minister.

In this document six criteria were defined to assess whether a profession required regulation. The initial query is if it is possible or practical to regulate the occupation, with further conditions around the nature of the work including potential harm to the public and existing controls. It would be beneficial to take a closer look at those countries identified in the report and understand why the medical scientist profession in Australia was, and is, considered different and why this profession doesn't meet the requirements set by AHPRA.

The United Kingdom

With the rise of technology and occupational specialization, many groups in the UK began to claim professional status. Occupations such as pharmacists, psychologists, nurses, and teachers could claim to have become professions by 1900. While laboratory workers at the time had varied duties and skills that assisted the medical profession, their professional status took longer to evolve.

In 1912 the Pathological and Bacteriological Laboratory Assistants Association (PBLAA) was founded in Liverpool as an initial step towards professional recognition. The members continued to be self-regulated up until the beginning of the Second World War when the Emergency War Committee took over functions of the PBLAA (Petts 2012). Reinvention of the PBLAA as the Institute of Medical Laboratory Technology (IMLT) in 1942 led to the development of education frameworks for night school students and in 1945 a "Fellowship by Thesis" (Petts 2012) was introduced. The first PBLAA examinations were held in 1921 and the IMLT revived these with their inaugural "Intermediate and Final" examinations in 1947. The "Fellowship by Dissertation" followed in 1953 (Petts 2012).

By 1961 the first members were placed on the Council for Professions Supplementary to Medicine register (CPSM). The first Bachelor of Medical Laboratory Science was offered by Portsmouth Polytechnic in 1974. Four years later the IMLT was rebranded to become the Institute of Medical Laboratory Science (IMLS) and phased out the "final" examinations which had been in use for thirty years up to this point.

The Institute of Biomedical Science (IBMS) was established in 1994 (IBMS 2018), and along with the Health and Care Professions Council (HCPC) provides the model for regulation of medical scientists globally. This model comprises two critical parts, the first, the IBMS, provides education, training support and assessment of its member's competencies. The second, the HCPC, is an independent regulatory authority and was introduced to protect the public by defining standards of professional care and behavior for the health professions. While medical scientists in the UK are able to be employed in laboratories without registration there is considerable financial and professional recognition to attaining it (HCPC 2018).

Ireland

The story of medical laboratory science in Ireland began in 1922 with the formation of the Irish Free State. Initially, the profession was governed as a branch of the IBMS. In 1961 the formation of the Medical Laboratory Technology section of the Workers' Union of Ireland provided the impetus for establishment of the occupation. However, the early years were not easy with the union calling a number of strikes in order to gain some respect for their members.

The pressure brought by the union paid off and in 1974 the Academy of Medical Laboratory Science was established to develop a framework of acceptable qualifications. By 1981 the Academy mandated that a degree course replace the

diploma as the core requirement for employment in an Irish laboratory. The following year the Fellowship qualification was revived, completing the domestic qualifications framework and removing the last vestiges of the old system.

In 1984 the Union Executive "i*ndicated the desirability of some form of membership of a single body*", and the Academy was added to the new regulations. This removed the final barrier to recognition and in 1986 the Irish Government approved the Academy as the professional body to represent medical laboratory scientists (Devine 2012). To be employed in an Irish pathology laboratory today, an employee must be a member of the Academy of Clinical Science and Laboratory Medicine.

An employee is responsible for maintaining their license by completing the required CPD. A member can apply for Fellowship once they have completed a relevant post-graduate qualification and has been a member for two years. All senior scientists in Ireland must be a Fellow of the Academy (ACSLM 2018). By the end of 2018, statutory registration of medical scientists will be required of the Irish Regulation body, *An Chomhairle um Ghairmithe Sláinte agus Cúraim Shóisialaigh* (CORU) (CORU 2018) which will provide legal support for the Academy and this mirrors the framework in the United Kingdom.

The United States of America

The American Society of Clinical Pathologists (ASCP) was established in 1922. Its aims are "*to achieve greater scientific proficiency in clinical pathology and to maintain the status of Clinical Pathologists on an equal plane with other medical specialties*"(ASCP 1971). Dr Philip Hillkowitz led the establishment of the Registry of Medical Technologists in 1928. This was a significant step in allowing the formation of the American Society for Medical Technology (ASMT) to represent the interests of laboratory personnel (ASCP 1971).

By 1953 ASCP made provisions to include the Board of Schools of Medical Technology, which approved education programs and for three medical technologists to sit on its Board. ACSP updated its constitution in 1966 to include standards for the performance of various laboratory procedures, standards of training, maintenance of a voluntary program of certification and examination of medical technologists (ASCP 1971).

It now has more than 100,000 members and holding a current registration is a requirement for employment in twelve states. Most of the other states require a nationally recognized certification in order to practice. The ASCP is politically active at the highest levels of government and ensures what is considered to be some of the highest laboratory standards and practice in the world. While the system of licensing is still complex due to the various state regulations, the ASCP is the most common certification available to laboratory workers and provides considerable professional recognition worldwide (ASCP 2018).

Canada

Canada began the path towards a robust provincial and federal registration framework in the early part of the last century. In a paper from 1966 they mention "*The interests of the Canadian Medical Association in medical laboratory technology have been evident since the establishment in the early 1930s of the Committee on Approval of Training Programs for Medical Laboratory Technologists*" (*unknown* 1966). The Canadian Society for Medical Laboratory Science (CSMLS) is now the federal body which governs licensing countrywide with each state maintaining an internal register of medical scientists.

The CSMLS offers the first level of certification through a national examination only after credentials have been verified and English fluency has been ascertained (CSMLS 2018). Only then may a scientist apply to the individual State or Provincial College to be added to the register. Scientists must participate in a considerable level of CPD to retain their practicing license and this is mandatory in order to continue employment.

The New Zealand

The history of the New Zealand Institute of Medical Laboratory Scientists (NZIMLS) started in 1923, but the formation of the New Zealand Association of Bacteriologists on the 9th of April 1946 marked the true beginning. The association was renamed the New Zealand Institute of Medical Laboratory Technology (NZIMLT) in 1960 and finally became the NZIMLS in 1990 (Paterson 1996). Initially membership was voluntary with its members held to a code of ethics.

This all changed in 2000 following a highly publicized case involving a lone pathologist working in Gisborne, which was documented in the Annual report of the Medical Council of New Zealand (MCNZ) (Ineson 2000). The subsequent enquiry made forty-six recommendations to government. In 2003 the Health Practitioners Competence Act (NZ Government 2003) required all medical scientists to register with the MSCNZ.

The NZIMLS and MSCNZ now provide a two-tier registration platform in New Zealand that aligns with the UK model for medical scientists. The NZIMLS is responsible for providing and monitoring education, offering a CPD program and for the assessing the qualifications of technicians, pre-analytical technicians and scientists (NZIMLS 2018). The MSCNZ issues annual practicing licenses for laboratory workers, monitors competency assessment and conducts disciplinary matters (MSCNZ 2018).

Australia

The story of the Australian Institute of Medical Scientists (AIMS) began in 1913, with the formation of a branch of the Pathological and Bacteriological Laboratory Assistants Association UK (PBLAA) in New South Wales. In 1932 it was renamed the Society of Laboratory Technicians of Australasia and it was incorporated on the 2nd of March 1937 at the University of Sydney. Their first order of business was to publish the "Laboratory Journal of Australasia" and to design examination criteria modeled on the British system.

A training platform was discussed and was in its infancy when the onset of hostilities in Europe halted proceedings until 1944. The history of AIMS mentions that "*blackout restrictions at the University of Sydney proved an interference to the society's night classes*" (Stanger et al 2014). 1950 was a landmark year, with the first federal meeting of the Executive Council held in Melbourne. The result of this meeting led to each state establishing its own Examining Council and issuing a Federal Diploma based on a countrywide minimum educational standard.

The diploma was adopted by every state except New South Wales which insisted on maintaining the original Certificate-level qualification (Stanger et al 2014). The training of medical scientists continued through the newly formed Colleges of Advanced Education (CAEs) and from 1966 onwards students received "Professional Diplomas", but the Society recognized that a degree was more appropriate. It was not until a 1988 review of higher education that the CAEs were granted university status that the first Bachelor degrees were offered.

To date eleven undergraduate and three postgraduate degrees at 10 Australian Universities (AIMS, 2018). At this point the history of the profession in Australia has followed a similar path to those of the other comparable countries identified by the ALG in the "*Comparison of international accreditation systems for registered health systems*" (AHPRA Acceditation Liaison Group 2016).

In 2003, a survey was undertaken of the public and other health professions in Australia in an effort to ascertain their perception of medical scientists. The study showed that 46% of the medical scientists working in Australia had a low perception of their professional status and 28% experienced low occupational satisfaction (McGregor and Moriarty 2003). The main reasons cited were a lack of respect or recognition of their skills and opportunities for CPD.

In a 2016 report "*A Snapshot of the Australian workplace*" (McMillan 2016), 72% of workers sought purpose and meaning through their work. Only a third believed they were supported toward professional development by their employers. However, of note are the survey results of the general public and other health professionals including doctors, nurses, radiographers and physiotherapists. Among the general public, only 3% were aware of the role of medical scientists, with 19% believing that pathologists performed laboratory tests. When it came to other health professions, 11% were aware of scientist's scope of practice and 48% thought that pathologists were responsible for conducting testing within a clinical laboratory (McGregor and Moriarty 2003).

The leadership of AIMS decided that this was not an acceptable position considering Australia's standing as a developed nation like England, America and New Zealand, all with recognized medical scientists contributing to their healthcare systems. Therefore, they began a lengthy campaign to address this discrepancy.

In 2006 AIMS made a Submission to the Productivity Commission study position paper "*Economic Impacts of Migration and Population Growth*" which informed the commission; "T*here is no registration of medical scientists in Australia*" (Noble 2006). The submission also stated that, "*there is no gap between migration assessment and employment assessment in the case of medical scientists*" (Noble 2006). AIMS has the federal authority in Australia to assess competency for migrant medical laboratory workers in the country, but membership is voluntary for domestic scientists.

In 2007 Badrick described the difficulty in making strategic decisions regarding the profession. Highlighting the lack of a register "*there is little accurate information on numbers and demographics of medical scientists*" or an up to date Scope of Practice for medical scientists "*there is no clear definition what medical scientists actually do in laboratories*" (Badrick 2007).

The issue was raised again in 2008 when AIMS informed the National Health and Hospitals Reform Commission "*there is no statutory registration for medical scientists" and "that on whose professional judgment so much of the health care system depends*" (Noble 2008). This agrees with The Royal College of Pathologists of Australasia (RCPA) public information leaflets which state that "*Pathology test results influence about 70% of healthcare decisions*" (Royal College of Pathologists 2018). However, the basis of the 70% claim is disputed, as there is a limited amount of evidence, but data from the Mayo Clinic Electronic Result Enquiry system published in 2000 indicates that pathology results could affect up to 94% of all clinical diagnoses (Hallworth 2011).

AHPRA was established to provide services to a group of fourteen professions which does not include medical scientists, a position which is inconsistent with comparable

best practice in overseas jurisdictions. AHPRA did not consider medical scientists' work of high enough risk to monitor, citing that the National Australian Testing Authority (NATA) accreditation for laboratories and registration of the supervising pathologist to be sufficient (Human Capital Alliance 2017).

In March 2011, the Pathology Association Council highlighted the lack of licensing of medical scientists again, but this time with a possible solution. The group proposed a system of self-governance with "*the creation of a Certification Board and ongoing Certification of medical scientists.*"(Pathology Associations Council 2011). The proposal identified the need for CPD and the definition of competencies, suggesting that AIMS administer these duties.

This initiative was promising and in 2012, a letter published in Clinical Chemistry referred to "*poor retention of scientists in the workforce and lack of a career structure are inextricably linked to role definition*" (Badrick and St John 2012). It was stated that this was being addressed by the Pathology Associations Council through the following initiatives:

- identification of roles and functions linked to skills and competencies;
- recognition of qualifications/skills/competencies/ experience across all states of Australia;
- removal of barriers to career progression leading to a better developed career path for medical scientists in Australia (Badrick and St John 2012.)

The following year South Australia Health appeared to have recognized that medical scientists were unregistered and sought to develop measures to protect the public by providing guidance for health practitioners who are not members of AHPRA. These measures required the application of a Code of Conduct for unregistered health practitioners to apply to all laboratory workers in South Australia. It was stated that from March 13, 2013, the Code must be displayed together with information on the compliant process and evidence of staff qualifications (Swan 2013).

Paradoxically, a laboratory operating in any private or public hospital, licensed health service, ambulance service or aged care facility is not required to display the above documents. These exclusions encompass the majority of places in which a laboratory would be ideally established. South Australia Health's argument to support the exclusions was to assert that "*The vast majority of unregistered health practitioners will already be practicing in a manner which is consistent with the requirements of the Code of Conduct because they are committed to the provision of safe and ethical health services*" (Swan 2013).

This serves to highlight the level of risk that politicians seem to be willing to assume for a group of workers that the government has no oversight over and which relies on the good intentions of its individuals. This approach devalues a formal registration process to that of an informal acknowledgement that the act of practicing is considered necessary and sufficient to be classed as a practitioner.

AIMS is still concerned about this situation and in a submission on their website in May 2015 they informed members that "*our case was rejected as medical laboratory scientists do not directly influence patient outcomes*" (AIMS 2015). Government officials recommend that the profession should "*develop a system for self-regulation*" (AIMS 2015). In 2016, Badrick and Wilson, wrote of these recurring themes saying "*If further consideration is taken for certification in Australia, prior due diligence would include comparisons with other countries and professions*" (Badrick and Wilson 2016).

In an effort to address these issues, AIMS commissioned Human Capital Alliance (HCA) consultancy to research, conduct analysis and develop a plan for the "*Establishment of a national model for professional certification of medical scientists and technicians working in Australian pathology laboratories*" (HCA 2018). The project commenced with the first discussion paper in November 2017, followed by stakeholder consultations in November 2017 and February 2018 and the development of a "Position Paper" in April 2018. Delphi conferences followed and a final workshop in late 2018. The project has now completed with final position paper published in November 2018 and the final report submitted to the Department of Health in April 2019.

Discussion

Over the last decade AIMS has unsuccessfully tried to establish formal registration of the medical scientist profession in Australia as occurs in the international community. Countries with similar developed health systems and historical partners all recognize the importance of laboratory workers. They have enshrined this in law with protected titles, standards of conduct, performance, proficiency and ethics.

The decision by AHPRA to exclude medical scientists from the national regulatory framework in favor of clinical oversight and accreditation has limited AIMS' role in the profession. AIMS' role in accrediting domestic degrees and credentialing of overseas degrees only means that it has some of the functions of a professional regulatory body but not all. As there is no formal requirement for medical scientists to be members of AIMS and, critically, no mandatory registration requirement for employment within a laboratory, then the profession is voluntarily self-determined.

In practical terms, anybody is able to work in an Australian clinical laboratory as there is no legal requirement for any particular qualification. This, combined with voluntary membership of the only group that can provide any meaningful judgement of a person's skills and qualifications leaves the Australian public open to unnecessary risk. Given the significance of pathology testing in healthcare and the expanding role of medical scientists, a critical evaluation is required of the decision by AHPRA that medical scientists do not require registration because they do not sufficiently influence patient outcomes.

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The AIMS Research Engagement Grant Scheme aspires to recognise, support and engage with research-oriented medical scientists through annual bursary and grant opportunities to encourage pathology-related research and CPD. For enquiries and expressions of interest please email contact@aims.org.au.

AIMS will be accepting applications after the 23rd of September 2019.

FUNDING OPPORTUNITIES	ELIGIBILITY	VALUE (AUD)
Scientific Communication Bursary	Graduate or final year student from a university offering an AIMS accredited program	2 x $500
Honours Research Bursary	Candidates enrolled at a university offering an AIMS accredited program	3 x $1000
MPhil or Master by Research Seeding Grant	Candidates enrolled at a university offering an AIMS accredited program	2 x $2,500 or 1 x $5,000
Doctor of Philosophy (PhD) Research Seeding Grant	Candidates enrolled at a university offering an AIMS accredited program	2 x $5,000 or 1 x $10,000
AIMS Fellowship (Stage 4) Bursary	Candidates enrolling in or undertaking Stage 4 of the AIMS Fellowship	3 x $2000
Clinical or Laboratory Research Grant	Medical scientists with an acceptable* degree	2 x $5,000 or 1 x $10,000
Education or Leadership & Management Research Grant	Medical scientists with an acceptable* degree	2 x $5,000 or 1 x $10,000

https://www.aims.org.au/cpd/research-engagement-grant-scheme#

https://www.aims.org.au/documents/item/1216

www.apace.org.au

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

APACE offers an online user-friendly diary to record your professional activities. The APACE website features a step-by-step guide and our National Office staff are on hand to help. If you are an AIMS member, when you register for an AIMS event, your CPD points are automatically added to your diary.

APACE has been approved by the New Zealand Medical Laboratory Science Board as a re-certification programme for New Zealand Medical Laboratory Scientists and by the Royal College of Pathologists Australia (RCPA) as a continuing professional development recognition programme for Fellows of the Faculty of Science. APACE is also a great way to ensure compliance with NPAAC requirements for Medical Pathology Services.

AUSTRALIAN INSTITUTE
OF MEDICAL SCIENTISTS

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.

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: programs@aims.org.au

1. **Bifidobacteria: Genomics & Molecular Aspects** edited by B. Mayo, & D. Van Sinderen. Caister Academic Press. xii + 260 pages.

2. **Medicine and Sport Science Volume 55: Cytokines, Growth Mediators & Physical Activity in Children during Puberty** edited by J. Jurimae, A.P. Hills & T. Jurimae. Karger. viii+178 pages.

3. **Digestive Diseases The Keys to IBD 2010: Treatment, Diagnosis & Pathophysiology.** Edited by G. Rogler & W. Sandborn. Karger. 188 pages.

4. **Else Kröner-Fresenuis Symposia Volume 1: Molecular Mechanisms of Adult Stem Cell Aging** edited by K.L. Rudolph. Karger. xii+108 pages**.**

5. **Endocrine Development Volume 24: Hormone Resistance and Hypersensitivity** edited by M. Maghnie, S. Loche, M. Cappa, L. Ghizzoni & R. Lorini. Karger. viii + 160 pages.

6. **Frontiers of Hormone Research Volume 41: Endocrine Tumor Syndromes and Their Genetics** edited by C.A Stratakis. Karger. xii + 187 pages.

7. **Frontiers of Hormone Research Volume 39: Kallmann Syndrome & Hypogonadotropic Hypogonadism** edited by R. Quinton. Karger. x+174 pages.

8. **Frontiers of Neurology & Neuroscience Volume 27: Neurological Disorders in Famous Artists: Part 3** edited by J Bogousslavsky, MG Hennerici, H Bäzner, C Bassetti. Karger. 240 pages.

9. **Generic: The Unbranding of Modern Medicine** by Jeremy A. Greene. John Hopkins University Press. 368 pages.

10. **Human Pathogenic Fungi: Molecular Biology and Pathogenic Mechanisms** edited by Derek J. Sullivan & Gary P. Moran, Caister Academic Press. x + 342 pages.

11. **Internal Medicine: A Doctor's Stories** by Terrence Holt. Black Inc. 273 pages.

12. **Intolerant Bodies: A Short History of Autoimmunity** by Warwick Anderson and & Ian R. Mackay. John Hopkins University Press. 250 pages.

13. **More Than Hot: A Short History of Fever** by Christopher Hamlin. John Hopkins University Press. 400 pages.

14. **Pediatric and Adolescent Medicine Volume 19: Metabolic Syndrome and Obsesity in Childhood and Adolescence** edited by W. Kiess, M. Wabitsch, C. Maffeis, A.M. Sharma. Karger. x + 202 pages.

15. **Phage Therapy - Current Research and Applications** edited by Jan Borysowski, Ryszard Miedzybrodzki & Andrzej Gorski. Caister Academic Press. 368 pages.

16. **Shigella: Molecular and Cellular Biology** edited by William D. Picking & Wendy L. Picking. Caister Academic Press. 280 pages

Australian Institute of Medical Scientists

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For enrolment enquiries please contact the AIMS National Office:

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Phone: (07) 3876 2988

Postal Address: PO Box 1911, MILTON QLD 4064, AUSTALIA

Or contact Steve Mackay:

E-mail: aimsqap@dspl.com.au

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 programs@aims.org.au. If an article is too large to be submitted by email, it should be submitted on an or USB stick.

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. Do not include a separate section for conclusions, these should be given in the discussion.

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/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:

Journal Reference:

Stein MK, Downing RW, Rickels K 1978. Self-estimates in anxious and depressed outpatients treated with pharmacotherapy. *Psychol Rep* 43: 487-492.

Personal Author(s) of a book:

Osler AG 1976. *Complement: mechanisms and functions*. Englewood Cliffs: Prentice-Hall.

Editor, Compiler, Chairman as Author:

Rhodes AJ, Van Rooyen CE, comps. 1968. *Textbook of virology: for students and practitioners of medicine and the other health sciences*. 5th ed. Baltimore: Williams and Wilkins.

Chapter in Book:

Weinstein L, Swartz MM 1974. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. *Pathologic physiology: mechanisms of disease*. Philadelphia: WB Saunders; 457-472.

Online documents:

National Center for Biotechnology Information. OMIM: online Mendelian inheritance in man. http://www.ncbi.nlm.nih.gov/omim. Accessed February 25, 2007.

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 (Figure 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).

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

Abbreviation or Symbol	Standard Units of Measurement
g	gram
g	gravity
Hz	hertz
h	hour
IU	international unit
K	kelvin
kg	kilogram
L	liter, litre
m	meter, metre
min	min
M	molar
mL	millilitre
mol	mole
N	newton
nm	nanometre
p	probability
rpm	revolutions per min
s	second
wk	week
yr	year

Additional information

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

Style Manual Committee. Council of Biology Editors. *Scientific style and format: the CBE manual for authors, editors, and publishers.* 6th ed. Cambridge University Press, 1994.

Style manual for authors, editors and printers. 6th ed. John Wiley & Sons Australia Ltd, 2002.

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

Day RA. *How to write and publish a scientific paper.* Philadelphia, Institute for Scientific Information Press, 1979.

Zeiger M. *Essentials of writing biomedical research papers*. 2nd ed. New York, McGraw-Hill, 2000.

Matthews JR, Matthews RW. *Successful scientific writing: a step-by-step guide for the biological and medical sciences*. 3rd ed. Cambridge, Cambridge University Press, 2007 [Also available in eBook format.]

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

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To enrol in the Fellowship program or for further information please contact the AIMS National Programs Manager:

Ph: +61 7 3876 2988

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