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Te Whare Wānanga o Waitaha

ENMG 680 ENGINEERING MANAGEMENT PROJECT

PRODUCT QUALITY IMPROVEMENT INTERNSHIP WITH AUCKLAND BIOSCIENCES

AUCKLAND BioSciences
excellence

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Document Control

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Disclaimer

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Redaction of Information

Some information in this report has been redacted due to being deemed commercially sensitive to Auckland BioSciences. This content was removed after the report had been duly examined.

Executive Summary

Auckland BioSciences (ABS) produces adult bovine serum for use in cell culture. Adult bovine serum is the liquid fraction of clotted blood collected from adult cattle. ABS produce this by collecting whole blood directly from the animals, allowing it to clot naturally, and extracting the serum via a centrifugation process.

One of the quality parameters for serum is haemoglobin. Haemoglobin is a protein that exists inside red blood cells, and is released into the surrounding blood fluid when blood cells are ruptured, a phenomenon known as haemolysis. Haemolysis increases the amount of haemoglobin measured in the end product. High levels of haemoglobin are considered to be undesirable in serum.

Current Situation

ABS currently produces serum with a haemoglobin specification of < 45 mg/dL. This is relatively high compared to the standard industry expectations for adult serum, which range from 25-45 mg/dL. ABS occasionally have issues with producing serum batches that exceed the specification of 45 mg/dL, which has occurred in 10 batches over the past 12 months, resulting in product that must be discarded or sold at less than cost price.

ABS is seeking to lower the haemoglobin levels measured in the end product, which will prevent production batches from exceeding 45 mg/mL, and also potentially meet the higher market haemoglobin specification of 30 mg/dL. This would enable ABS to sell serum at a higher average price.

This project was carried out using a DMAIC quality improvement framework. A literature review was carried out on this framework, which confirmed its suitability, due to its data driven, iterative approach that enables a person with limited subject knowledge to effectively carry out a quality improvement project.

Scope of Project

The scope of this project includes:

- Identifying significant causes of haemolysis in ABS' production process.
- Identifying ways to eliminate or reduce haemolysis in a commercially feasible manner.

Summary of Findings

It was determined that almost all significant haemolysis occurs when cutting and damaging the blood once it has clotted. ABS' production process involves cutting the blood after it has clotted in order to increase the surface area, which in turn increases the rate at which blood is drained from the clot, and therefore increasing the total volume of serum produced.

The relationship between clot damage and haemoglobin was quantified by expressing clot damage as *% damaged surface area*. For a fixed total volume, the following relationships between key parameters were identified:

$$Serum\ Volume\ Output \propto Total\ Clot\ Surface\ Area$$

$$Haemoglobin\ Level \propto Damaged\ Surface\ Area\ (\%)$$

where: **Serum Volume Output** = Total volume of serum produced
Total Clot Surface Area = Amount of surface area created naturally via clotting
Damaged Surface Area = The proportion of surface area that is created by cutting

By quantifying these relationships, it was possible to identify the clot surface area and allowable damaged surface area that will enable ABS to produce the targeted low-haemoglobin serum in adequate volumes.

ABS' current production process (for an average blood collection of 3000L) was benchmarked as shown below:

ABS' Current Production Process: Key Parameters

Input Variables		Output Variables	
Total clot surface area	240 m^2	Volume of serum produced	1000 L
% damaged surface area	73%	Average haemoglobin level	40 mg/dL

It is estimated that if ABS are to meet the higher-end market haemoglobin specification of 30 mg/dL, the average selling price could be increased by $__ per litre. In order for ABS to consistently meet this specification, an average haemoglobin level of 25 mg/dL needs to be achieved (to allow for variance).

ABS' current average selling price is $__ per litre. If haemoglobin levels are reduced to 25 mg/dL, thereby increasing the average price to $__ per litre, then volume recoveries must remain at least 90% of current levels in order to realise a financial benefit from the increase in quality.

By analysing the relationships between total surface area, damaged surface area, serum output volume and haemoglobin level, it was determined that any potential solution that will add sale value must satisfy the following requirements:

- Total clot surface area must be more than 208 m^2
- Less than 18% of the total surface area is allowed to be damaged (created by cutting)

Recommendations

- Modify the production process to allow for blood to be collected and clotted in stainless steel grids. This will maximise the surface and eliminate the need to perform cuts. It is estimated that this solution can yield more than 1000 L of serum with an average haemoglobin level of 20 mg/dL.
- Carry out further investigation into the other potential causes of haemolysis (listed in Appendix E), allowing ABS to further lower haemoglobin levels, adding more value to their product.

Impacts of Recommended Solution

The identified solution involves collecting whole blood directly into a grid that fits into ABS' current processing equipment. This causes no significant clot damage, and enables ABS to increase the total clot surface area of a normal production day by approximately 40%; potentially increasing the total production output while simultaneously improving product quality.

A feasibility study for this solution showed that it would require an additional clotting vat (ABS currently use two) and 12 steel grids (21x11), with an estimated total cost of $5,900.

By lowering haemoglobin levels to an average of 20 mg/dL, ABS can increase its average selling price by $__ per litre. Neglecting any resulting increase in serum output, and any labour savings that would come as a result of this solution, ABS would increase its average daily profit by $__. It is estimated that it would take approximately 6 months to increase the average sale price by $__ per litre, as ABS

would need time to expand into the higher-end market. Therefore, once the benefits of this solution had been realised (i.e. haemoglobin is reduced and the average sale price increased), it would take [redacted] production days to cover the capital costs required to implement the solution. It would then add approximately $[redacted] to each day's profit thereafter.

The benefit for ABS is not limited to increasing the average sale price: low haemoglobin serum is also an indication of 'carefully processed', high-quality serum. ABS' goal is to produce 'premium quality' adult bovine serum, and to clearly demonstrate this to customers it must be better in almost every specification in comparison to 'average' adult bovine serum. Therefore, by improving haemoglobin levels to an average of 20 mg/dL, ABS would truly be able to market themselves as a producer of 'premium adult bovine serum'. This impact, although difficult to define in terms of increased revenue, will improve ABS' brand image and is, therefore, an important part of realising ABS' strategic vision.

Contents

1. Introduction

1.1 Company background

Auckland BioSciences is a small, early-stage company in the life sciences industry that produce animal derivatives for use in pharmaceutical manufacture and research. Their core product is adult bovine serum, which accounts for 95% of revenues. This is manufactured from the blood produced as a by-product of NZ's meat industry.

Auckland BioSciences was founded in October 2013, when they acquired the assets of an existing serum manufacturer. Production is carried out at a single site, with an output capacity of approximately 250,000 liters of serum per year. The company is relatively small, with 10 full-time employees.

The initial target market for this product is the manufacture of veterinary vaccines, particularly those for Foot & Mouth Disease — they use bovine serum extensively in their cell-culture production processes. These organisations are largely found in developing economies, with ABS' key markets including China, India, Russia, and Southeast Asia.

The business is currently growing into higher-end markets, including Europe & South America, but this is partially constrained by current levels of haemoglobin in the product, which are slightly higher than standard specifications.

Due to the young age of the company, and the constraints associated with running a start-up, there is very limited internal R&D capacity. This means there is currently little formal understanding of how to manage end-product quality parameters through modification of the serum production process. Key product parameters are not fully understood, limiting the effectiveness of the current quality systems.

2. Project Overview

2.1 Purpose:

To improve the quality of serum produced by Auckland BioSciences by lowering the level of haemoglobin present in the end product.

2.2 Aims:

- Understand why haemolysis is occurring in the production process.
- Determine a way to eliminate or reduce haemolysis in a commercially feasible manner.

2.3 Background and significance

Haemoglobin is a protein found inside red blood cells that exists to carry oxygen. Red blood cells make up about 45% of total blood volume, and are one of the main components removed during the serum production process. When red blood cells are ruptured, haemoglobin is released into the surrounding fluid (blood plasma). This phenomenon is known as haemolysis. Once haemolysis occurs, it is almost impossible to extract the haemoglobin out of the plasma through a mechanical process, and it therefore remains in the end product. Haemoglobin is an undesirable substance in serum as it affects visibility when used in cell culture.

Haemoglobin levels are indicated by the colour of the serum. Serum that is low in haemoglobin is a straw yellow colour, while serum that is high in haemoglobin will appear dark orange/red. Haemoglobin levels are typically measured by calculating the light absorption of the serum at various wavelengths using a spectrophotometer.

By reducing serum haemoglobin levels, ABS will be able to sell a better quality product to a higher-end markets e.g. European countries.

3. Development of a course of action

3.1 DMAIC Approach

A brief literature review was carried out to determine the applicability of a DMAIC framework to the project. This review is attached in Appendix A.

DMAIC is an abbreviation for *Define, Measure, Analyse, Improve,* and *Control*. It is a tool used in Six Sigma quality improvement projects, although the use of its framework is not limited to Six Sigma.

Steps in the DMAIC framework are as follows:

Table 1: Steps in the DMAIC Framework

1. Define	• Define the problem and the process improvement opportunity • Define customer requirements • Define and document current process
2. Measure	• Identify what to measure • Understand variation in the process • Determine baseline
3. Analyse	• Analyse data and process • Develop hypotheses around root causes • Validated root causes through data analysis
4. Improve	• Generate improvement ideas addressing root causes • Evaluate and select optimal solution • Pilot changes
5. Control	• Review pilot results • Modify solution if required • Roll out solution • Closure and celebrate success

4. Define

4.1 Identifying customer requirements

ABS has a good understanding of customer requirements after a year of being active in the market. Customers order serum based on product specifications, allowing ABS to directly understand their requirements. Product specifications for adult bovine serum include: haemoglobin, protein, pH, osmolality, endotoxin, and bioburden.

Haemoglobin is an undesirable substance in serum as it affects visibility when used in cell culture. In terms of performance, there is no evidence to suggest that haemoglobin levels less than 100 mg/dL have any effect on cell culture (Atlas Biologicals, 2014).

ABS is currently selling to lower-end markets such as those in developing countries. The customers in these countries prioritize on low-cost, and therefore do not have typically strict limitations on haemoglobin levels. Customers in these lower end markets will purchase serum with haemoglobin levels less than 45 mg/dL. ABS has a current specification limit for haemoglobin of 45 mg/dL. Any batch that exceeds this amount is either discarded or sold at a significantly reduced price.

Higher end markets, such as those in most European countries, require serum of higher quality since they are using it in higher-grade pharmaceuticals. These customers do not generally purchase serum that has haemoglobin levels greater than 30 mg/dL.

By improving haemoglobin levels, ABS will:

a) not have to discard production batches (due to haemoglobin levels that exceed 45 mg/dL).
b) be able to sell to higher end markets, potentially at a higher selling price.

5. Measure & Analyse

In order to improve haemoglobin levels in the end product, the process and its implications on product quality need to be better understood. Due to the complexity of analysing this process, this stage was broken into three phases:

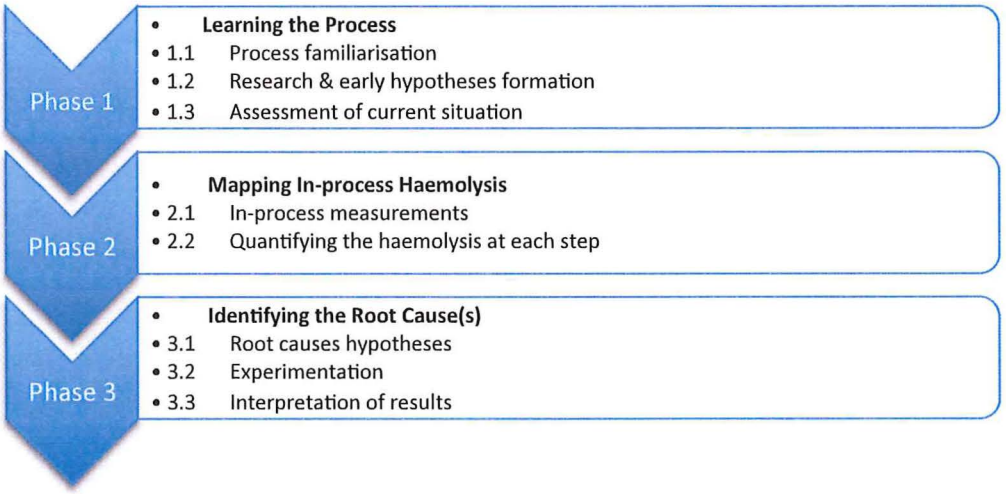

Figure 1: Phases of Measurement and Analysis

5.1 Phase 1 – Learning the Process

5.1.1 Process Familiarisation

To be able to improve the process requires a detailed understanding of how the process is carried out.

In order to develop this understanding, a week was spent in production, learning each step, and understanding the resources required to manage the process.

A map of the process showing each step is shown below.

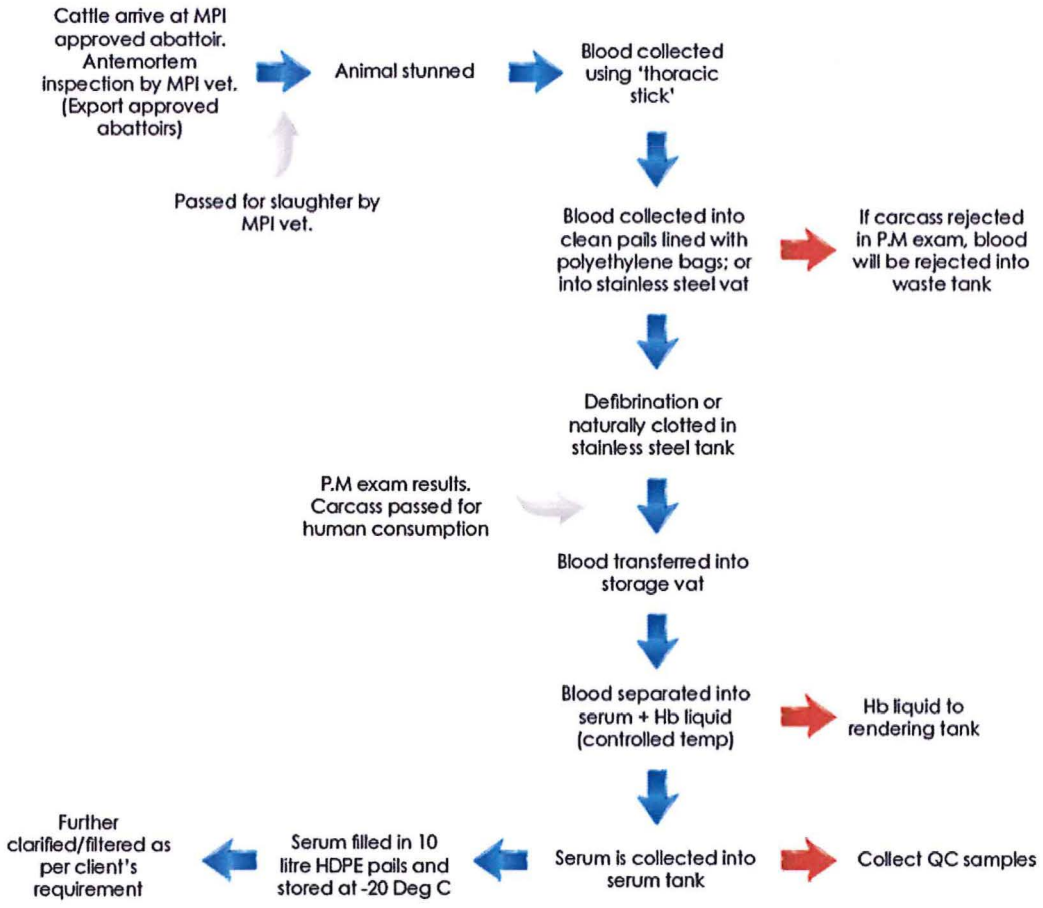

Figure 2: Production process map

The following defined terms will be referred to throughout this report from here onwards:

- **Whole blood** – Blood that has not yet clotted.
- **Clot** – Coagulated blood.
- **Pre-serum** – The liquid that is collected when drained from the clots.

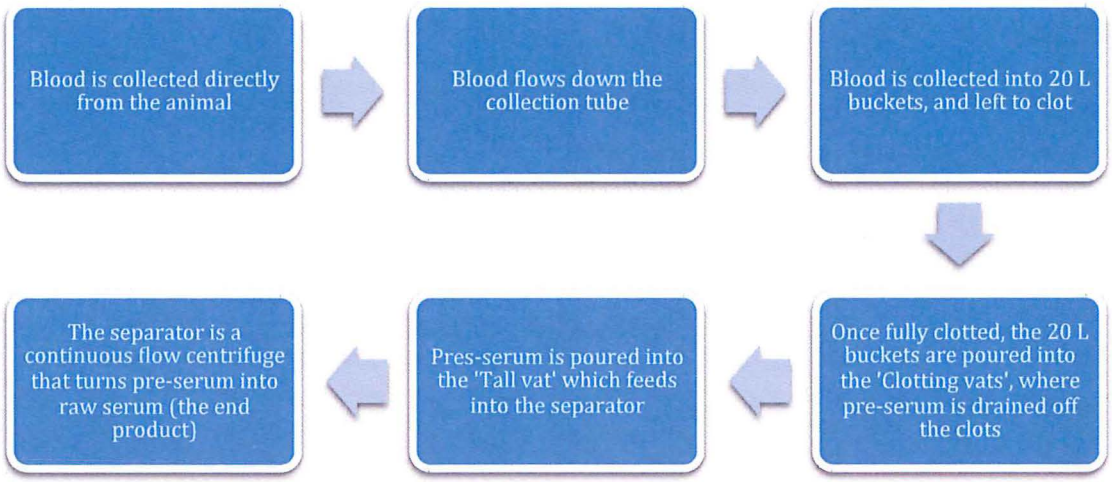

Figure 3: Simplified Production Process Steps

On average, approximately 3000L of blood is collected each day. This volume will typically vary from 2500L to 3500L, which is largely due to the number of animals slaughtered each day. Other significant variables that impact the volume of blood collected include the size of the cows, the efficiency of the process technicians, and also the slightly unpredictable flow rate for each animal.

The typical serum yield is 1 litre of serum per 3 litres of blood i.e. the typical serum production is 800-1200 L per day.

Required Resources for the Production Process (General production only)

Labour:

- Two process technicians are required throughout the day to collect blood from the animals.
- For the first 3 hours of production, two technicians are required in the production room – filling buckets, checking for notification of condemned animals, pouring buckets into vats, and draining from the vats.
- For the remainder of production, two additional technicians are required to perform – starting the separator, emptying the vats, draining serum, and bucketing and storing serum.

Equipment:

- 36 × 20L buckets each with a plastic liner – these 36 buckets are used for blood collection, and are cycled throughout the day. As there is approximately 3000L of blood collected each day, each bucket is cycled 4-5 times.
- Two clotting vats are used hold the clots while pre-serum is being drained. Each vat contains four perforated baskets, approximately 100L in volume. Due to the volume of un-clotted blood that drains straight through the baskets, 6-7 buckets (120-140L) of blood is poured into each basket.
- One continuous flow separator is used to separate the pre-serum.
- Raw serum (final product) is packaged in 10L buckets.

5.1.2 Assessment of Current Situation

Using the DMAIC framework, the purpose of the 'Measure' step is to establish a baseline for the quality parameter as the basis for improvement. ABS gets each production batch tested for various parameters, one of which haemoglobin. Therefore, this baseline was available from ABS' historic lab results.

ABS' haemoglobin levels for each production day since November 2013 is shown in the following figure.

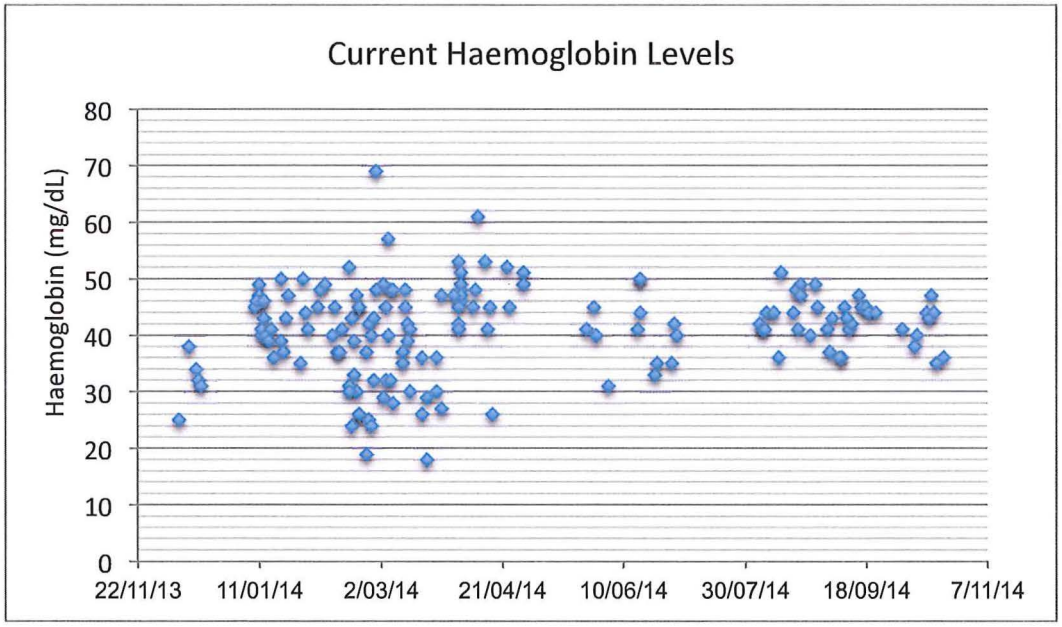

Figure 4: Current Haemoglobin Levels

Table 2: Statistical data for ABS' haemoglobin levels

Average	41 mg/dL
Maximum	63 mg/dL
Minimum	19 mg/dL
Standard deviation	7.87
Batches exceeding 45mg/dL	10

The average haemoglobin level from this data is 41.03. This is very close to ABS' upper limit of 45 mg/dL. Note that 10 batches exceeded ABS' specification limit. These batches were either discarded, or sold at cost price.

It is important to also note the accuracy and consistency of these measurements. ABS gets its haemoglobin measurements from testing carried out by a third party. These measurements have been known to vary up to 15% for samples that are re-tested.

5.1.3 Research & hypotheses formation

Independent research was carried out in order to gain an understanding into the properties of blood/serum, the general serum production procedures, and known causes of haemolysis.

A summary of findings is attached in Appendix B.

5.2 Phase 2 – In-Process Haemolysis

Due to the number of potential causes, it was determined that the best approach would be to measure the amount of haemolysis occurring throughout production process. This can be achieved by taking samples of blood/pre-serum/serum at various stages in the process, completing the separation in a desktop centrifuge, and measuring the haemoglobin levels using a spectrophotometer.

5.2.1 In-process measurements

The goal of the in-process testing is to determine which steps/stages in the process comparably cause the most haemolysis.

Five locations in the process were identified as suitable stages for collection, mainly due to the convenience of being able to take samples without interrupting or hindering general production. These locations were:

1. Directly from the animal
2. Bottom of collection tube
3. Clotting vat(s) outlet
4. Tall vat outlet
5. Separator outlet

Difficulties were encountered with collecting and separating whole blood (i.e. blood collected directly from the animal and from the bottom of the collection tube). The issue was regarding separating serum from the blood clot. It was found that during centrifugation, whole blood samples were frequently observed to produce a solid serum-gel, rather than the expected liquid serum. This made it practically impossible to measure the haemoglobin levels for such samples. After much trial and error, independent research and consultation with experts in this field, it was determined that an anti-coagulant was required to prevent this from happening, and enable a proper separation, allowing measurements for haemoglobin levels to be made.

111 samples were taken in total. In order to reduce the risk of un-intended biased sample collections, it was ensured that at each stage samples taken included a range of:

- Collection times (start to end of day)
- Uncontrollable flow rates (e.g. from collection tube)

5.2.2 Quantifying the haemolysis occurring at each stage

Results from the in-process testing are shown in figure 5 below:

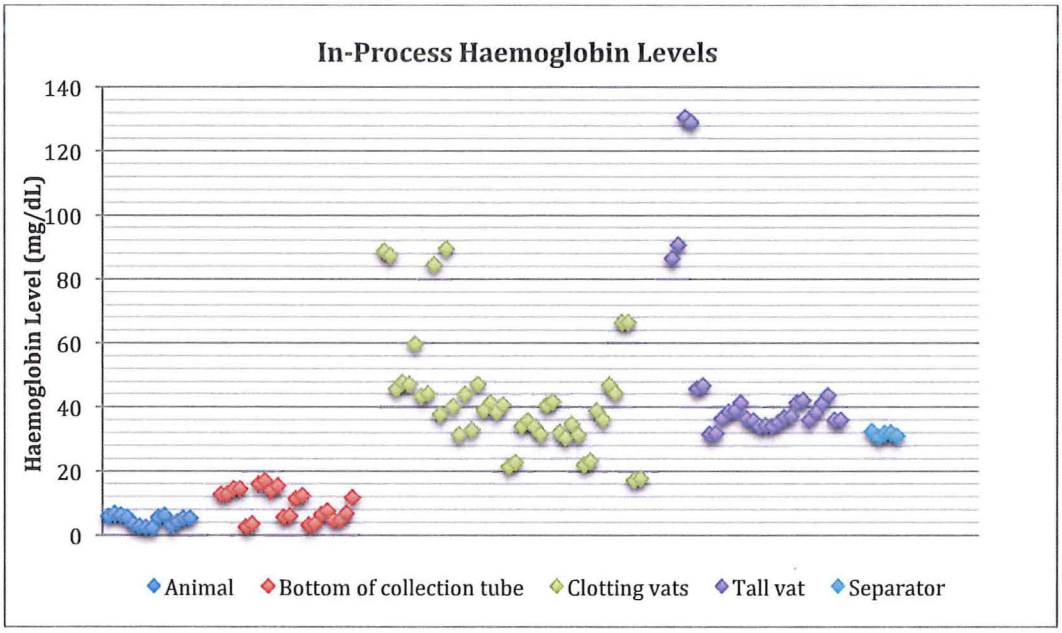

Figure 5: Haemoglobin levels of samples taken from various stages in production

Table 3: Analysis of in-process haemoglobin levels

Location	No.# samples	No.# measurements	Max. (mg/dL)	Min. (mg/dL)	Std. Dev.	Average (mg/dL)
Animal	7	14	6.62	2.35	1.53	4.68
Bottom of collection tube	11	22	16.99	2.63	4.48	9.49
Clotting vats	21	42	89.49	17.32	18.24	42.94
Tall vat	14	28	130.52	31.57	26.87	48.11
Separator	5	5	32.41	30.75	0.68	31.62

Analysis of Results

Figure 5 effectively demonstrates the varying haemoglobin measurements of samples taken at each step in the process. From figure 5, the following conclusions can be drawn:

- Significant variation of haemoglobin levels occurs in samples taken from both the clotting vats and the tall vat.
- The majority of haemolysis occurs between the bottom of the collection tube and leaving the clotting vats.

It was noted that the average haemoglobin level for samples taken from the separator was much lower than the averages from the clotting vats and the tall vat. In theory, this should not occur, as haemoglobin levels can only increase over the process. However, this identifies some limitations of the method used to take samples. This result may indicate the following:

- The separation method used with the desktop centrifuge causes more haemolysis than the continuous flow separator used in production.

- The collection/draining technique causes more haemolysis than the normal 'continuous flow' collection used when collecting higher volumes (as per production). The techniques that may have led to non-representative samples include:
  - Semi-opened tap valves
  - On/off flow

If the measured outliers from the clotting vats and the tall vat are ignored, then their respective average measurements are much closer to what would be expected. The table below shows the new averages for each of these stages, with values higher than 60 mg/dL discarded.

Table 4: Average haemoglobin levels for clotting vats and tall vat (ignoring outlier measurements)

Location	Original No.# samples	Original Average (mg/dL)	No.# samples discarded	New Average (mg/dL)
Clotting vats	21	42.94	3	36.67
Tall vat	14	48.11	2	37.93
Separator	5	31.62	0	31.62

These averages are much closer to that of the separator, although they are still slightly higher (5.05 mg/dL and 6.31 mg/dL, respectively). This discrepancy indicates that the limitations discussed previously (and possible others) are still valid.

Using this adjusted data, the haemolysis occurring between each step was quantified.

Table 5: Quantified in-process haemolysis

From		To	Haemolysis (mg/dL)
Animal	→	Bottom of tube	4.81
Bottom of tube	→	Exiting clotting vats	27.18
Exiting clotting vats	→	Exiting tall vat	1.26

Conclusions from results

- Most of the haemolysis is occurring between collecting from the bottom of the tube and being drained from the clotting vats.
- A small amount of haemolysis occurs between collecting from the animal and reaching the bottom of the collection tube.
- Negligible haemolysis is occurring between exiting the clotting vats and exiting the tall vat.

➢ **Determined next action**: Investigate in detail the process between the bottom of the tube and draining from the clotting vats.

5.3 Phase 3 – Identifying the Root Cause

To purpose of this phase is diagnose the cause of the significant haemolysis - observed to be occurring between the point of collecting blood from the bottom of the tube and draining the from the clotting vats.

5.3.1 Hypothesise potential causes

Actions & conditions that impact blood/pre-serum between these two identified locations were identified, evaluated, and prioritised in terms of their likelihood for being the root cause. This evaluation was based on the Student's knowledge and experience with the process thus far. The following table shows the list of these potential causes, and their associated likelihood estimate (ranked relatively from 1 to 5 (max)).

The purpose of ranking the likelihood of each is to prioritise which causes should be tested first, as testing them all would be a very long process.

Table 6: Potential causes and estimated likelihood

Step in process	Possible cause	Likelihood
1. Collection into 20L bucket with plastic liner.	Impact onto bottom of bucket when collecting from tube	2
2. Liner is 'twist-sealed' and allowed to clot for 40-50 minutes in the production room.	Extended contact with plastic liner	1
	Clotting time	3
	Clotting temperature	4
3. After clotting for 40-50 minutes, the plastic liner is opened and the clot is cut into quarters using the cutting knife.	Cutting the clot into quarters	5
4. The blood/clot is then poured into the perforated baskets in the clotting vat.	Pouring the clot into perforated baskets	4
5. The clots are then cut again (3x3) using the knife.	Cutting the clots in the baskets	5
6. Clots sit in perforated baskets for 90-120 minutes, as pre-serum is collected at the bottom of the vat.	Time bleeding off the clot	4
	Bleeding through the perforated basket	3
	Dripping to the bottom of the vat	3
	Sitting in the bottom of the vat (sitting temperature, extended contact with cells, dissolving clots etc.)	4
7. Pre-serum is drained through the tap valve at the bottom of a vat, through a sieve.	Draining through the tap	3
	Flowing through a sieve	3

High priority causes to test were identified as:

- Cutting the clots (into quarters & when in baskets)
- Clotting temperature
- Pouring the clots into perforated baskets
- Time bleeding off the clot

5.3.2 Re-creating the serum production process

It was determined that the simplest way to test each of these causes was to recreate a simplified serum production process, with as few uncontrolled variables as possible.

This process includes bleeding a single clot (600-1000mL) that has clotted for 30 minutes in a sieve.

The first variable to measure, due to convenience was bleed time, i.e. how the haemoglobin level varies over time for pre-serum draining off the clot.

Pre-serum was continuously collected into 15mL tubes, isolating each tube with a corresponding bleed time. This also made it possible measure the bleed rate for small volume clots.

Figure 6 below is a representative result for the haemoglobin levels of pre-serum bled over time.

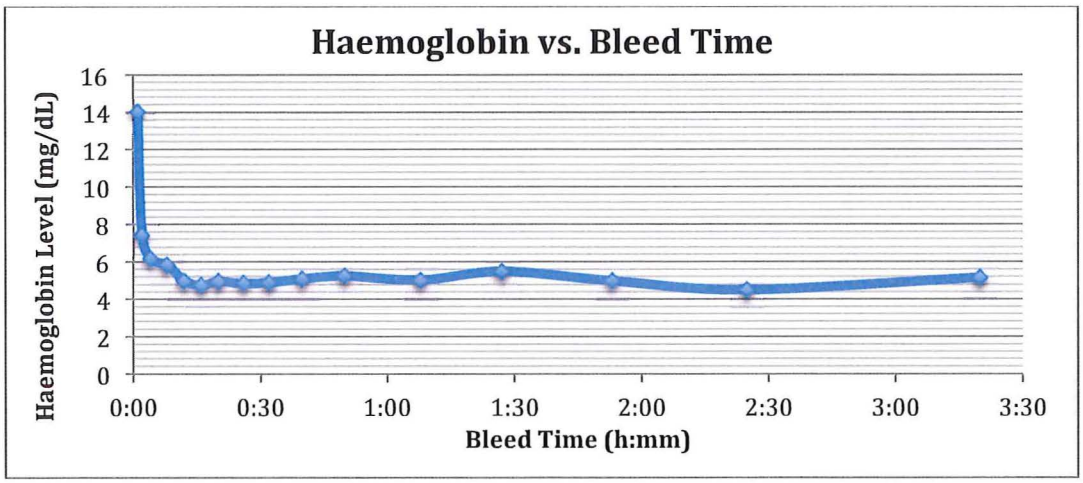

Figure 6: Representative result of haemoglobin vs. bleed time

The bleed rate (in terms of total recovery) was measured as follows:

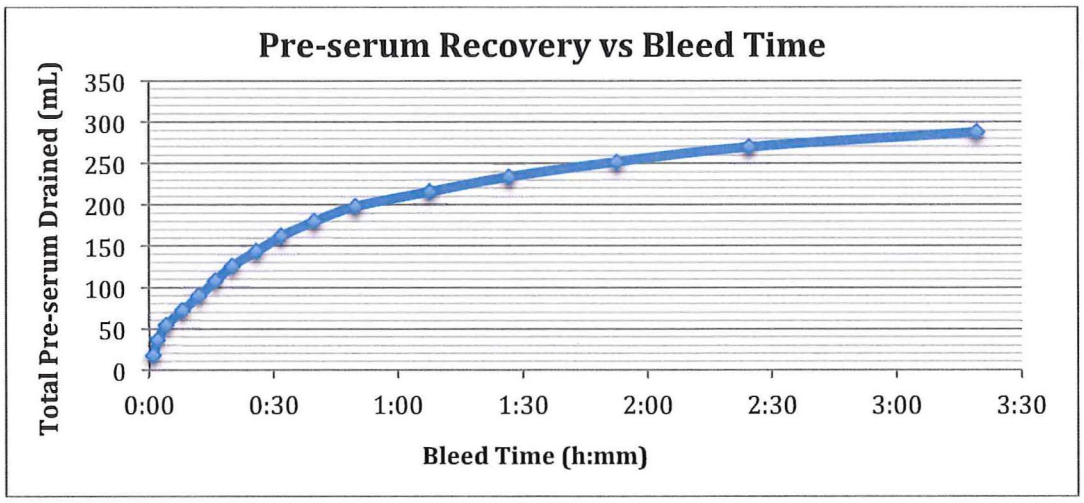

Figure 7: Representative result of pre-serum recovery over time

Note: These results are representative of what was measured during testing. All tests that were carried out displayed similar relationships.

Conclusions

From these results, the following conclusions were made:

- Haemoglobin does not increase over time (within 3 hours)
- Haemoglobin levels as low as 4 mg/dL can be obtained for off-the-clot blood
- Of the total volume drained in 3 hours (270mL):
    - 60% was drained in the first 30 minutes
    - 78% was drained after 1 hour
    - 96% was drained after 2 hours

5.3.3 Re-introducing variables

The first potential cause investigated was 'clot cutting'.

Clot Cutting

The simplified procedure specified above was carried out with the addition of using a knife to cut the clot once it had begun draining. The number of cuts were varied, and the impact on haemoglobin and pre-serum recovery were measured continuously.

Figure 8 below shows a representative result of what happens to haemoglobin when the clot is cut.

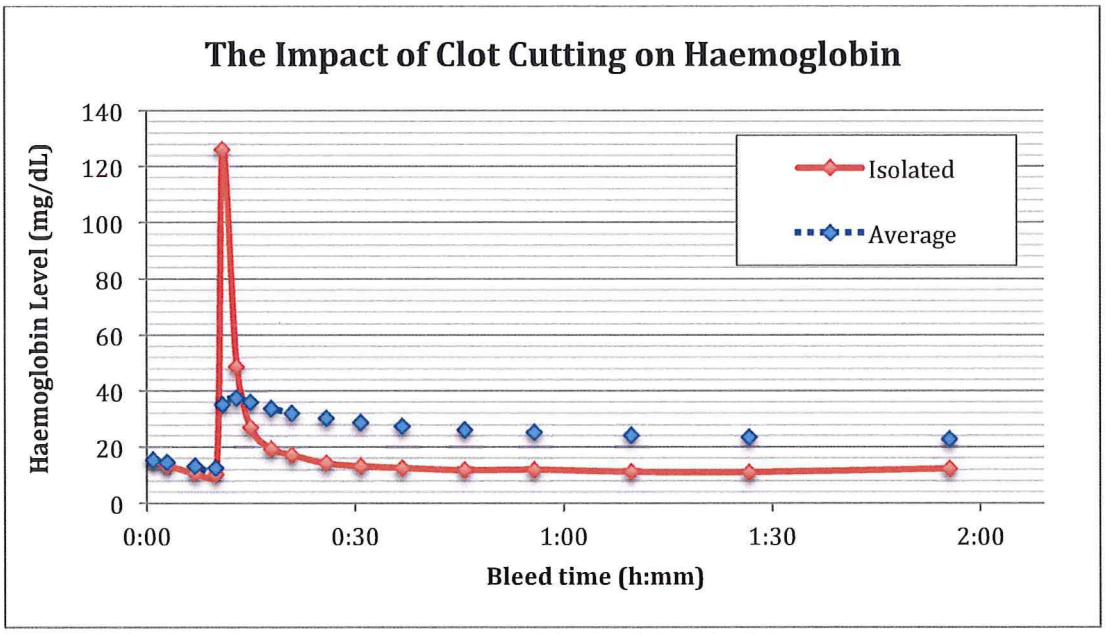

Figure 8: Haemoglobin levels for a 'cut' clot

Note: the 'Isolated' line indicates samples that were collected into 15mL tubes and measured individually. The 'Average' line is the mean value for all of the pre-serum that has drained.

In this case, the clot was cut once down the middle after 10 minutes of draining, indicated by the sudden 'spike' in haemoglobin.

Observations:

- Haemoglobin levels spike significantly after cutting.
- Within 15 minutes of cutting, haemoglobin levels for pre-serum being drained had returned to its original level.
- Due to the reduced bleed rate over time, the average haemoglobin of the total pre-serum bled remained approximately 100% higher than the steady state level.

The impact on bleed rate is shown below in figure 9.

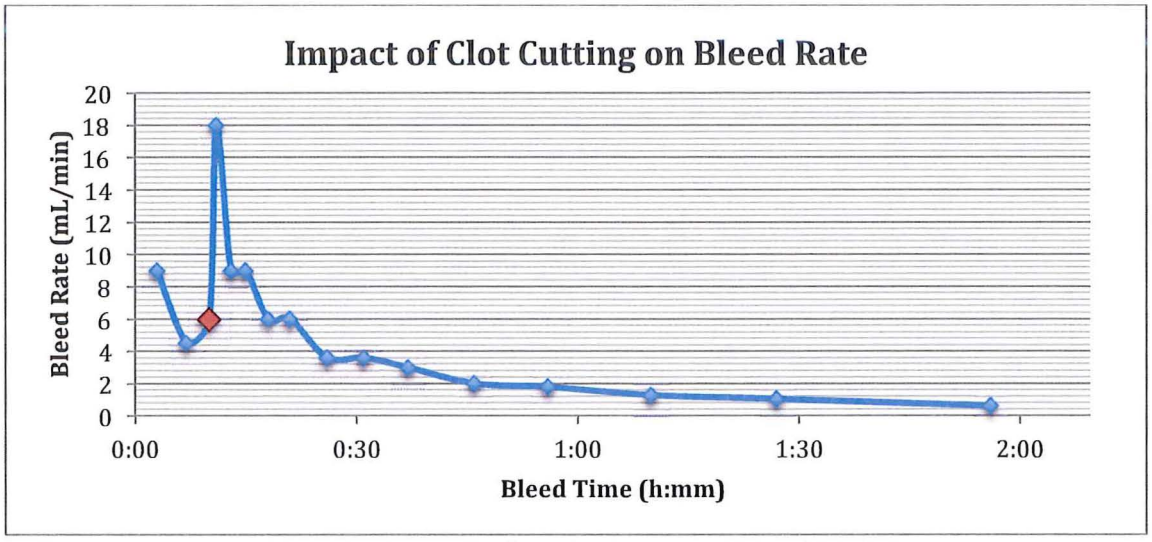

Figure 9: Bleed rate over time for a clot that was cut once after 10 minutes

The red data point indicates the time of the cut. Note that these measurements were recorded to the nearest minute, which limits their tolerance, and explains the staggered behaviour of the data points – the actual behaviour of the bleed rate curve is expected to be much smoother.

Observations:

- Cutting the clots has an immediate effect on the bleed rate – in this case increasing it by 200%.
- After 10 minutes the bleed rate had decreased to the rate it was before cutting.
- The bleed rate then continues to exponentially decay.

5.3.4 Conclusions

Due to the time constraints of the project, and the evidence indicating that a significant cause of haemolysis in ABS' process had been determined, the remaining potential variables were not investigated.

Further testing to determine the impacts of these variables is recommended (see Appendix E)

From the investigation into the impact of clot cutting, the following conclusions were determined:

- **Significant haemolysis occurs due to cutting the blood clots**
- **Cutting the blood clots increases the surface area, which increases the bleed rate**

6. Improve and Control

Having determined the significant cause of haemolysis, the next step was to understand how much it affects ABS' production process, and determine if its impact can be eliminated or reduced in a manner that is both technically and commercially feasible.

6.1 Full-scale Validation

It was observed that cutting the clot momentarily increased both haemoglobin levels and the bleed rate. Clot cutting can be defined as literally cutting the clot, or any action that causes the clot to

break, exposing additional surface area. It is believed that breaking the bonds within the clot matrix ruptures the cell membranes of the red blood cells at the exposed surface, releasing haemoglobin.

The following terms are defined for reference use in the remainder of this report:

- *Clot breaking* – any action that separates a blood clot, exposing new surface area.
- *Damaged surface area* – the new surface area that is exposed due to clot breaking.

Benchmarking ABS' Production Process

If the impact of clot breaking is to be reduced, it is necessary to first quantify its presence in ABS' production. The following assessment involves estimating the following variables for a typical production day:

- Blood volume collected
- Natural surface area of clotted blood
- Damaged surface area created (quantified clot breaking)
- Pre-serum drained
- Total serum produced

The assessment of ABS' production process is attached in Appendix C .

A summary of the calculated variables is shown below:

Table 7: Calculating the surface area in ABS' normal production

Blood volume collected	3000 L
Pre-serum drained	1500 L
Serum produced	1000 L

Natural surface area of clotted blood	64.42 m^2
Damaged surface area created	
Cutting in buckets	54.60 m^2
Pouring into vats	54.60 m^2
Cutting in vats	66.15 m^2
Total damaged surface area	175.35 m^2
Total surface area	239.77 m^2
Percentage of damaged surface area	73%

Limitations:

- This estimation assumes that all cutting is creating new surface area (i.e. the blade does not pass between clots).
- It was difficult to estimate the amount of damaged surface area created when pouring into the vats. It was observed that the clots break-up considerably, and was therefore estimated to be a 100% surface area increase when pouring after being cut into quarters. It is assumed the actual amount could be in the range of 50-200%.

Validating improvements at full-scale

The next identified step was to determine what happens to the haemoglobin level if ABS were to eliminate damaged surface area from the process.

Trial Test:

A short trial was performed in production where no clots were cut, and buckets were emptied delicately into the vat baskets (to prevent damage when breaking up). The result of this test was:

- Significantly less pre-serum was drained from the clots (approximately 50%)
- The resulting haemoglobin level was approximately 20 mg/dL

From the results of this trial, it was assumed that 20 mg/dL is the minimum haemoglobin level that can be obtained by removing clot damage alone.

Conclusions

The short trial carried out in production validated the conclusions that had been made from earlier testing, which are:

- Haemoglobin levels are proportional to damaged surface area.
- Pre-serum recovery is proportional to total surface area.

These results indicate that if ABS is to improve haemoglobin levels without any **significant** process changes, a trade-off will have to be made between quality and quantity (haemoglobin level vs. serum produced).

It is therefore necessary to understand what the value of low-haemoglobin serum is to ABS.

6.2 The Value of Low Haemoglobin Serum

Direct Impacts

ABS have a product specification of haemoglobin levels < 45 mg/dL. This sells for an average price of $__ per litre. As mentioned in section 4.1, haemoglobin has no affect on the performance of serum when used in cell culture. However it is still specified by customers to ensure the serum will be satisfactory in terms of cell visibility, and also because it is an indication of the quality and care taken in producing the serum.

By improving haemoglobin levels to less than 30 mg/dL, ABS will be able to sell serum to higher-end markets. It is believed that this would increase the selling price by $__ per litre. Many higher-end customers have a requirement of < 30 mg/dL, so therefore the impact this quality parameter has on price is a step-change at the 30 mg/dL point. Any further improvements will involve no price increases. Likewise, any improvement that does not reach below 30 mg/dL will have no impact on selling price.

Indirect Impacts

Aside from being able to sell for $__ more per litre, low haemoglobin is an indication of product quality, and therefore an important factor in creating a positive perception of ABS' brand. If ABS' strategy involves being branded as a manufacturer of 'premium adult bovine serum' then the importance of low haemoglobin levels become much more than just 'increased selling-price'.

6.3 Identifying Potential Solutions

An ideal solution for lowering haemoglobin involves maximising the surface area without the need to cut or damage the clot.

6.3.1 Formulating Tools for Assessment

Haemoglobin vs. Damaged Surface Area

Test results showed the following relationship between damaged surface area and the average haemoglobin for 1.5 - 3 hours of draining.

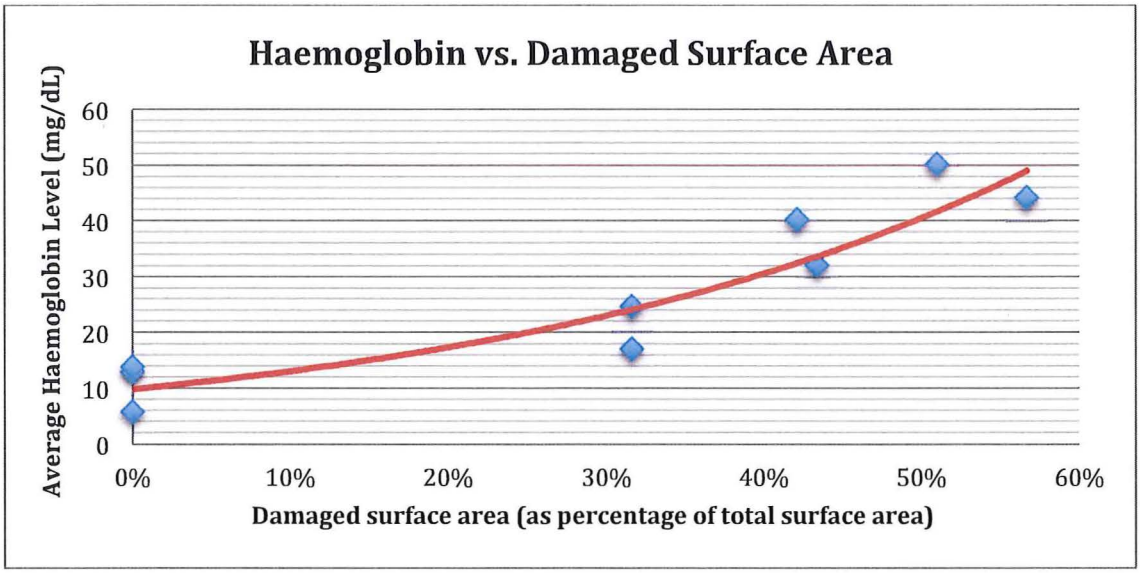

Figure 10: Relationship between damaged surface area and average haemoglobin level

From previous testing, it is known that on a production scale:

- 0% damaged surface area results in 20 mg/dL
- 73% damaged surface area results in 40 mg/dL

In order to model this at a production scale, it was assumed that the relationship between the average haemoglobin level and damaged surface area is linear. Although Figure 10 demonstrates this to be slightly incorrect, its usefulness in modelling the behaviour of clots in production makes it an extremely useful tool.

Figure 11 demonstrates the estimated relationship between haemoglobin and damaged surface area in production.

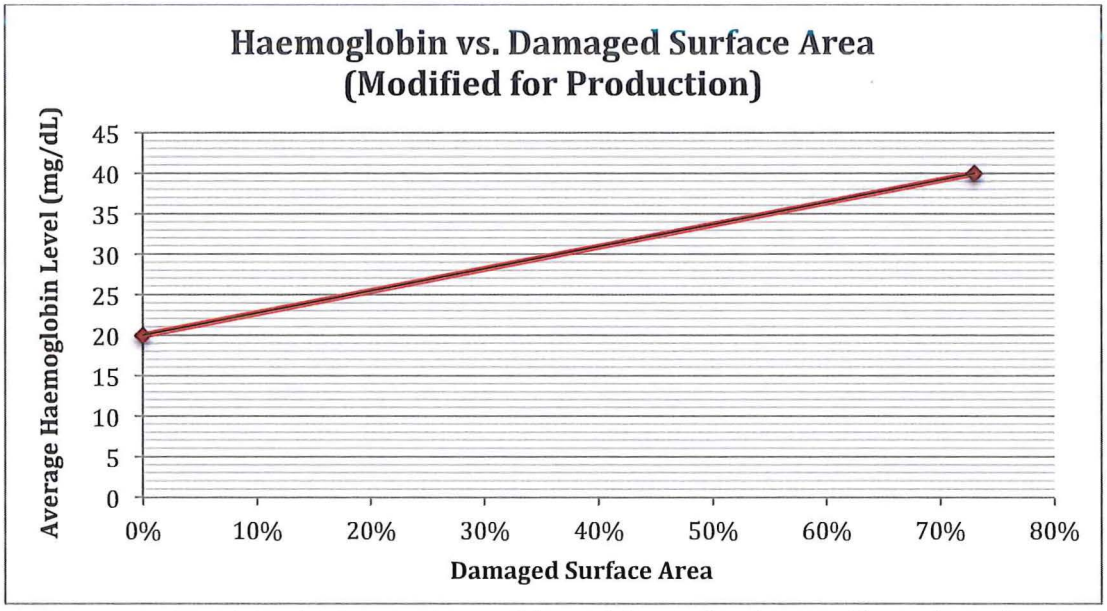

Figure 11: Relationship between haemoglobin and damaged surface area (for production)

Serum Recovery vs. Surface Area

It is was estimated that ABS' process produces a total surface area of 239.77 m^2. This yields, on average, an estimated 1000 L of serum. The production trial that was carried out (see section 6.1) produced 83 m^2 total surface area, and yielded approximately 500 L of serum. This enables the following linear relationship to be determined.

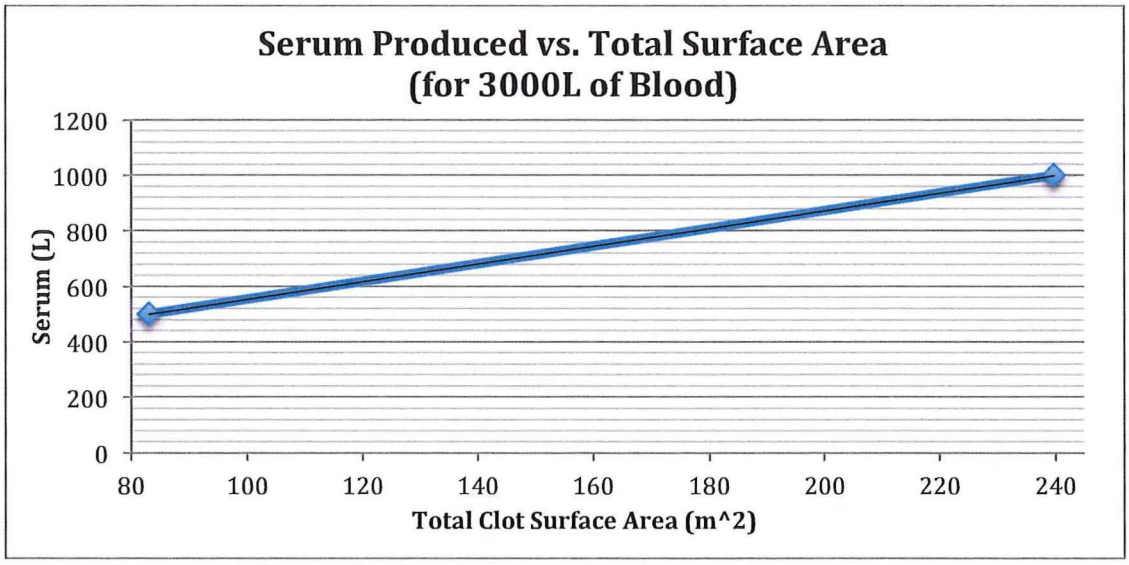

Figure 12: Relationship between serum produced and total surface area (on production scale)

6.3.2 Potential Solutions

The following ideas were identified as potential solutions for lowering haemoglobin levels while producing adequate volumes of serum:

1. Less clot cutting (no other process changes)
2. Clotting in smaller containers
3. Clotting in a grid

6.4 Feasibility of Solutions

A feasibility study was carried out for each of the potential solutions. This included an assessment of both their technical and commercial feasibility.

If ABS is to improve haemoglobin levels to meet higher-end market specifications, levels must consistently be less than 30 mg/dL. Figure 11 shows that in order to obtain haemoglobin levels below 30 mg/dL, damaged surface area must be no greater than 36%. To ensure that the solution consistently produces lower than 30 mg/dL, a target of 25 mg/dL was set, corresponding to a damaged surface area of 18%.

ABS averages 1000 L of serum output per production day. At an average of $[redacted] per litre, this has a sale value of $[redacted]. It was determined that by lowering haemoglobin levels below 30 mg/dL, selling price would be increased to an average of $[redacted] per litre. If ABS are to improve haemoglobin to meet this specification, in order to increase the total sale value of each production day, they must produce 900 L of serum per day. This corresponds to a required total surface area of 208 m^2.

Therefore, in order for ABS' solution to add value, the following two conditions must be met:

- Total surface area greater than 208m^2
- Damaged surface area is less than 18%

6.4.1 Less Clot Cutting

Without any clot breaking, normal production creates a total surface area of 64.42 m^2. In order to get the required volume of serum (>900L), surface area would have to be increased by 144 m^2, resulting in 69% damaged surface area (corresponds to 39 mg/dL – figure 11).

Therefore, this method is not technically feasible. **There has to be more surface area created naturally.**

6.4.2 Clotting in Smaller Containers

Assuming 25 mg/dL for average haemoglobin and 900 L of serum output is set as a target, the minimum size/shape for a collection container was determined as:

- 170m^2 of naturally created surface area
- 38m^2 of damaged (cut) surface area

An assessment was carried out that showed it is not feasible to collect into round buckets, as it would require minimum of 143 buckets to be used (or on stand-by) in production at any one time, and that is for a bucket 40 cm in diameter, and only filled 4 cm high.

The use of square buckets (more surface area) was also assessed, and deemed not feasible due to also needing over 100 buckets in production.

6.4.3 Clotting in a Grid

This solution involves collecting blood directly into the clotting vats. Note: in normal production, each vat comprises of four 100L perforated baskets that are used to hold and drain the clots.

Description of solution:

1. Large plastic liners are placed in each basket in order to prevent the blood being drained while still liquid.
2. The vats are arranged such that blood from the collection tube can be delivered directly into the baskets.
3. Once each basket is full, a grid is placed in the basket before it is allowed to clot.
4. Once the blood has fully clotted, the grid is removed and the plastic liner is pulled out from underneath the clots.
5. The clots are then drained as per normal procedure.

The grid would be similar to what is shown in figure 13, shown below.

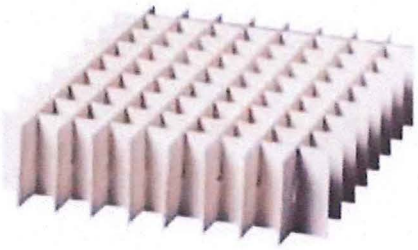

Figure 13: Grid used to maximise surface area of clotted blood

Technical Feasibility

The amount of surface area is easily controllable as it is defined by the grid matrix. 208 m^2 was determined as the minimum total surface area required for a solution to add value over the current procedure.

Each basket can hold 100 L of blood. Therefore, a cycle of 30 baskets will be required for clotting and draining the daily blood collection of 3000 L.

The following table shows the various total surface areas that are obtainable with varying grid sizes.

Table 8: Total surface area created (m^2) with varying grid sizes

Grid	x	1	2	3	4	5	6	7	8	9	10	11
x	Spacing (mm)	425	213	142	106	85	71	61	53	47	43	39
1	800	42	57	71	86	100	114	129	143	158	172	186
2	400	50	65	79	93	108	122	137	151	165	180	194
3	267	58	72	87	101	115	130	144	159	173	187	202
4	200	65	80	94	109	123	137	152	166	181	195	209
5	160	73	87	102	116	131	145	159	174	188	203	217
6	133	81	95	110	124	138	153	167	182	196	210	225
7	114	88	103	117	132	146	160	175	189	204	218	232
8	100	96	110	125	139	154	168	182	197	211	226	240
9	89	104	118	132	147	161	176	190	204	219	233	248
10	80	111	126	140	155	169	183	198	212	227	241	255
11	73	119	133	148	162	177	191	205	220	234	249	263
12	67	127	141	155	170	184	199	213	227	242	256	271
13	62	134	149	163	177	192	206	221	235	249	264	278
14	57	142	156	171	185	200	214	228	243	257	272	286
15	53	150	164	178	193	207	222	236	250	265	279	294
16	50	157	172	186	200	215	229	244	258	272	287	301
17	47	165	179	194	208	222	237	251	266	280	294	309
18	44	173	187	201	216	230	245	259	273	288	302	317
19	42	180	195	209	223	238	252	267	281	295	310	324
20	40	188	202	217	231	245	260	274	289	303	317	332
21	38	195	210	224	239	253	267	282	296	311	325	339

Note: the light green/yellow highlighted cells indicate that the minimum surface area requirement ($208m^2$) has been met. The darker green indicates greater surface area – more serum output and therefore more desirable.

By using this solution it is possible to create more surface area than normal production level ($240\ m^2$), without the need to damage clots. For example, a 21x11 grid produces $339\ m^2$ of surface area, 41% more than the normal production level. This has potential to both improve the average haemoglobin level to 20 mg/dL, and yield more than 1000 L of serum per day.

Potential issues that were identified & their resolutions are listed below:

- **Inability to pull out the plastic liner from underneath the clots** – to be confirmed.
- **Not having enough vat capacity to collect, clot and drain** – see below.
- **Not being able to remove the grid from the basket** – either getting stuck or removing it with clots stuck in it – to be confirmed.
- **Blood may clot before inserting the grid** – to be confirmed. If this is an issue, the grid can be placed in the basket before filling, and a process technician evenly distributes the blood between the grids by moving the tube.

An analysis of the production capacity determined that in order to drain the blood for at least 90 minutes, an additional vat would be required. This would be possible in terms of production space, as there would no longer be any need to hold 36 buckets, as well as the large bench that holds the buckets.

Commercial Feasibility

Commercial impacts of implementing this solution include:

- The purchase of an additional vat
- The purchase of 12 custom-made steel grids
- The labour required to setup and clean the additional vat each day
- The plastic liners that are required for each basket

It was determined that the impact of labour requirements would be neutral. The time taken to setup and clean one extra vat is similar to the time taken to prepare and clean 36 plastic lined buckets.
The need to purchase liners for each basket was also determined to have a neutral impact, as 36 plastic liners for the collection buckets is no longer required (assuming 30 large liners = 36 medium - sized liners).

There is potential to reduce labour requirements with this solution, as demonstrated in the following table.

Table 9: Impacts on Labour Requirements

Activity	Time taken to perform	Number of times required	Total Time
Labour required			
Transferring tube between baskets	10 seconds	30	5 minutes
Labour no longer required			
Interchanging buckets when full	10 seconds	150	25 minutes
'Twist-sealing' and storing on bench	10 seconds	150	25 minutes
Opening liner and cutting the clots	1-minute	25	25 minutes
Pouring into baskets	1-minute	25	25 minutes
Net Change			Less 1hr 35 min

At an average pay rate of $17 per hour, this is a potential labour saving of $27 per day, and an annual saving of approximately $7,000.

The following commercial feasibility study was carried out, assuming:

- the low haemoglobin levels allowed ABS to sell for an extra $__ per litre
- No changes in labour costs
- No changes in serum output volume

Item	Unit Cost	Number required	Total Cost
Clotting vat (400L capacity)	$5,000	1	$5,000
Steel grid	$80	12	$960
Total			$5,960

Value added	Daily Production Volume	Profit Added
$__ per L	1000 L	$__ (per production day)

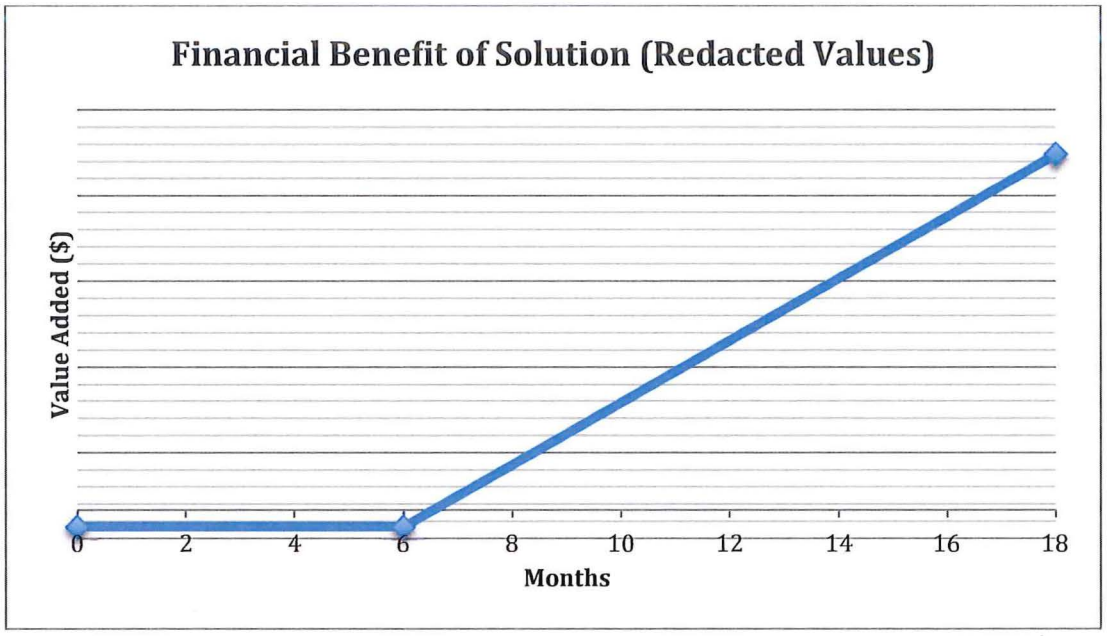

Figure 14: Financial Benefit – Assuming it takes 6 months to realise benefit

It is assumed that once implemented, it will take 6 months to increase the average sale price by $__/L, due to the time needed to expand into a new market. Once the benefits have been realised, this solution has the potential to return $__ of additional profit within the next 12 months. It could then go on to add $__ of additional profit each year.

Note: Figure 14 assumes an immediate step change in the average selling price. It should be noted that this would not occur in reality. However, the model is representative over an 18 month period.

7. Conclusions

The aims of this project were to:

- Understand why haemolysis is occurring in the production process.
- Determine a way to eliminate or reduce haemolysis in a commercially feasible manner.

The first aim has been met.
It was determined that the most significant haemolysis was occurring in ABS' production process due to cutting the blood clots. The blood clots are cut/broken up to increase the amount of surface area, which increases the rate at which pre-serum is bled off the clots. It was determined that this also caused red blood cells to rupture, releasing significant amounts of haemoglobin, and tainting the end-product.

A potential solution has been identified that is expected to meet the second aim.
As well as reducing haemolysis, this solution has the potential to increase the total volume of serum produced, and is less labour intensive than the current process.

ABS processes an average of 3000L of blood each production day. By clotting this blood in 20L buckets, 64 m^2 of surface area is created naturally. Cutting the clots and pouring them into the vat baskets (breaking up the clots) was measured to increase the surface area by a total of 175 m^2, meaning the resulting total surface area of 239 m^2 was comprised of 73% *damaged* surface area.

By preventing clots from breaking, ABS were able to lower haemoglobin levels to 20 mg/dL. However, due to the significantly reduced surface area, serum out-put was decreased by 50%.

A solution was identified that would allow blood to be clotted with more surface area without the need to cut or damage the clots. This solution involves collecting whole blood directly into the vat baskets, and clotting them in a steel grid (21x11). This causes no significant clot damage, and enables ABS to increase the total clot surface area of a normal production day by approximately 40%, potentially increasing the total production output.

A feasibility study for this solution showed that it would require an additional clotting vat and 12 grids (21x11), with an estimated total cost of $5,900.

The direct impact of this solution is that it will add at $__ to ABS' daily profit. This neglects any other potential benefits that would come as a result of higher volumes and saved labour costs. It is estimated that it would take 6 months after implementation for the benefits to be realised. Therefore, a return on the initial capital investment would be made after 6 months and 2 weeks.

It is equally important to consider the indirect benefits that will come as a result of producing low-haemoglobin serum. ABS markets itself as a 'premium' adult bovine serum producer. Relative to the competition in the industry, ABS' current haemoglobin levels are by no means 'premium'. This improvement is highly influential to ABS' strategic growth - ABS aims to compete on quality not cost, and therefore the benefits of this solution go deeper than just an increased selling price.

8. Recommendations

- **Action the implementation plan for modifying the process to clot blood in grids** (outlined in Appendix F) – This involves an investigation of the full-scale proof-of-concept before making the required major process changes. If successful, ABS will be able be able to produce a minimum of 1000 L of serum with haemoglobin levels averaging 20 mg/dL.

- **Complete further testing for additional causes of haemolysis** (outlined in Appendix E) – Due to time constraints with the project, only the impact of clotting was investigated in detail. Eliminating clot damage from the process has a limitation of lowering haemoglobin levels to 20 mg/dL. This is due to other factors in the process which cause haemolysis (listed in appendix E). While any further improvements in haemoglobin levels will not allow ABS to further increase its average selling price, by lowering the average level it will ensure that fewer batches are produced over specification (30 mg/dL).

9. Project Review - Personal Reflection

What were some of the significant challenges faced during this internship?

No specialist knowledge – Coming into this project I had no experience in microbiology or anything remotely similar. I was granted the task of lowering haemoglobin levels in serum, without knowing what serum is, let alone haemoglobin. What I lacked in specialist knowledge I was able to make up for in persistent hard work. The DMAIC framework used allowed me to logically solve this complex problem by iterating my methods until I converged on a solution. Ultimately, this took much longer than it would have if I had had specialist knowledge in this area, and although a significant amount of research was carried out, I believe I took the best approach for the limited time that was available.

Novel subject area – Not only was this area unfamiliar to me, but there was very little relevant research available that was specific to my needs. There is lots of published research regarding the processing of human blood and its properties, including potential causes of haemolysis, but almost nothing on bovine serum, particularly when processing in high volumes. Even after finding the main root cause of haemolysis, I haven't been able to find supporting literature that relates to cutting blood clots.

Ultimately, these two aspects of the project resulted in me underestimating the scope, which led to delays, and placed me under significant time pressure in the final weeks of the project. I thought this was an excellent example of how the four dimensions of project management (knowledge, novelty, complexity and pace) can lead to significant over-runs for both time and cost.

What were some of the key skills I have developed that can be applied in the future?

This internship has developed my ability to acquire and apply knowledge in an unfamiliar area, and in a way that will actually realise a commercial benefit. The analytical approach I have used to solve such a complex problem is a broad skill that can be applied to any industry.

I have developed the ability to self-manage my own projects under time-pressure. As mentioned, I found it particularly difficult to plan this project having no experience in this field. The resulting issues and delays, and how I managed these, are lessons I will take into future project planning.

How has this internship experience impacted my career plans?

This internship has made me realise that I am not limited to a narrow choice of industries. I have realised that the skills I have developed throughout my university experience, particularly in MEM, are skills that enable me to adapt and learn in a new environment. Businesses solve problems, and the problem solving skills developed through studying engineering enable me to solve these problems in an analytical manner, a skill that is applicable to any industry.

I have really enjoyed working for a small business. I was able to see the importance of my project and the direct impact it has on the business. This was extremely motivating. If I were to carry out exactly the same tasks in a larger organisation, where the impact had much less significance, I don't think I would have enjoyed it. It wasn't what I was doing that made me enjoy this internship, it was why I was doing it. This has made me realise the importance of being able to make an impact, and this is a key factor I will consider when evaluating career opportunities in the future.

Would I recommend this internship to future MEM students?

Yes, definitely. This internship has given me real-world exposure to almost every subject covered in MEM. I believe it has confirmed the relevance MEM has to the real world.

Being part of a small business has enabled me to get exposure to almost every aspect of running a business – from operations to strategy. This has developed my learning in these areas considerably. Also, in such a small company, it is added motivation doing something that can actually have a significant impact and add real value.

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Appendix A – DMAIC Review

DMIAC is an abbreviation for *Define, Measure, Improve, Analyse, and Control.* It is a data-driven life-cycle approach to Six Sigma projects used for improving, optimizing and stabilizing business processes and designs (Sokovic, Pavletic, & Pipan, 2010). It is the core tool used to drive Six Sigma projects. However, its use is not limited to Six Sigma, as it can be used as a framework for other improvement applications (Lynch, Bertolino, & Cloutier, 2003).

The following flow chart shows how DMAIC is applied to quality improvement projects.

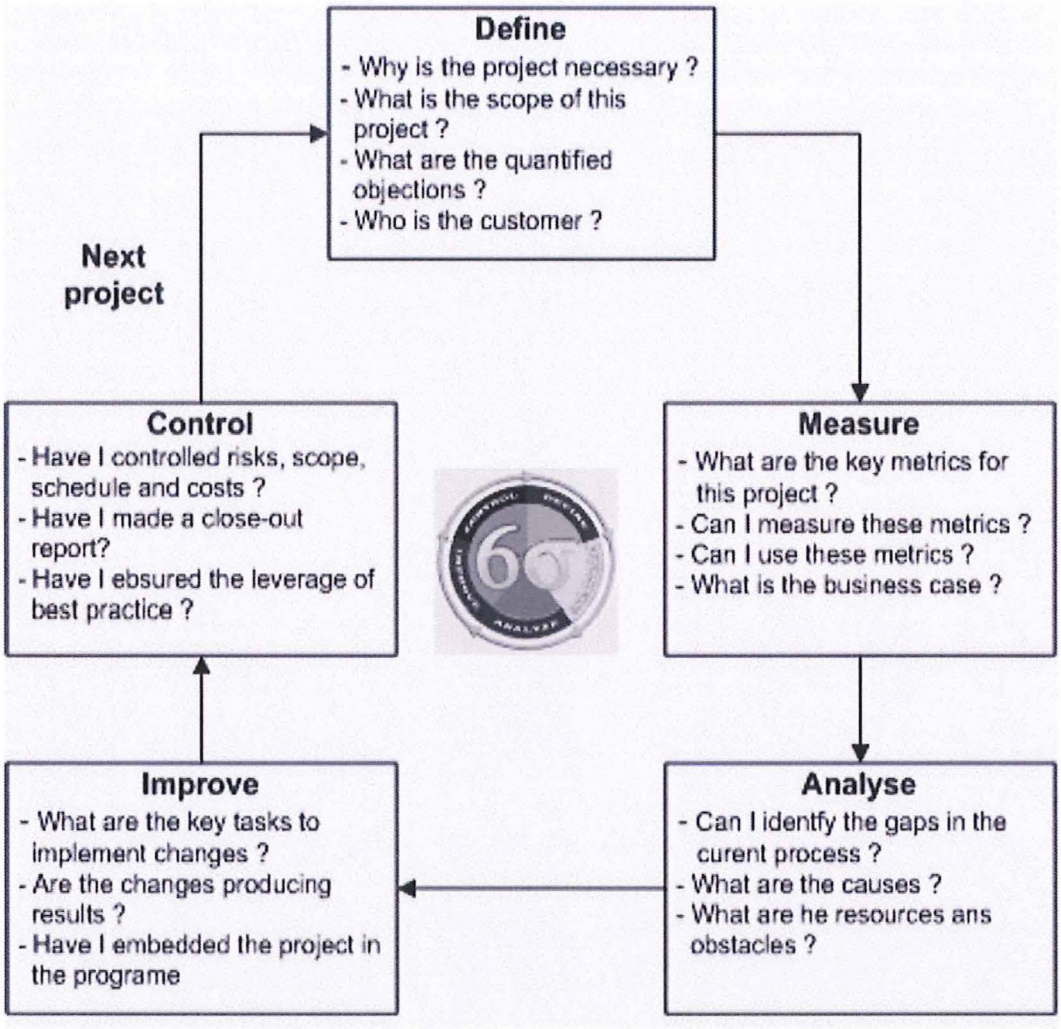

Figure: The DMAIC Approach (Sokovic et al., 2010)

DMAIC is systematic and fact-based, providing a rigorous, result-oriented framework. Despite the methodology appearing to be a linear, waterfall model, but it should be noted that the most effective use of DMAIC is when the process is flexible and iterative. This is particularly applicable to projects where persons involved are new to the tools, techniques or the subject area in general (Sokovic et al., 2010).

DMAIC is a suitable tool for improving processes via a logical, data-driven methodology. Its application to this project was identified as highly suitable due to the lack of the Student's prior knowledge in this

area. Despite the extremely complex field, and the Student's inexperience, a DMAIC framework allows the problem to be broken down into a logical, simple step process. This essentially eliminates the need for a high level of detailed knowledge, as the methodology allows for iterative progress, and the result-oriented approach ensures these iterations converge towards an identified improvement.

Conclusion

- DMAIC is a rigorous, logical framework for a 'common-sense' approach to quality management. Despite the negative associations with having a framework for 'common-sense', in complex projects, or novel subject areas, often such a framework is necessary. It allows the person implementing to review and iterate their approach, to ensure that unproductive time is not wasted, and a proper project plan can be followed.

Appendix B – Research

Secondary research was conducted in order to gain an understanding of haemolysis and its potential causes. While the DMAIC framework allows for an iterative solution to converge on an eventual root cause, improving subject knowledge in this area enables hypotheses to be formed. This not only speeds up the process, but it also enables the Student to consider the impact of sample collection methods and testing procedures, which could determine whether these methods were placing any unintended bias on the results.

Summary of findings

The following were found to be potential causes for haemolysis:

- Improper specimen collection and handling techniques (Arzoumanian, 2003)
- Vigorous mixing or shaking of a specimen (Arzoumanian, 2003)
- Prolonged contact of serum or plasma with cells (Boyanton & Blick, 2002)
- Exposure to excessive heat or cold (Lemery, 1998)
- Any mechanical trauma (Burns & Yoshikawa, 2002)
- Forceful flow through a needle (Lemery, 1998; Savory & Bill, 1996).
- An improper choice in the venepuncture site (Burns & Yoshikawa, 2002)
- Prolonged tourniquet time causes the interstitial fluid to leak into the tissue and cause haemolysis (Burns & Yoshikawa, 2002)
- Contact with alcohol (P. A. Wayne, 1998)
- An improper venepuncture, indicated by a slow blood flow, may indicate occlusion due to the lumen of the needle being too close to the inner wall of the vein, causing haemolysis (Bush & Mangan, 2003)
- The use of a small-bore needle, resulting in a large vacuum force applied to the blood, may cause shear stress on the red blood cells, causing them to rupture (Lemery, 1998; Sharp & Mohammad, 1998; P. A. Wayne, 1998).
- Centrifugation an incorrect times and/or speeds (P. Wayne, 2004)

Appendix C – Assessment of Clot Damage in ABS' Procedure

Natural surface area created in normal production

Surface area of 20L bucket:

Diameter = 280mm
Height = 325mm
Surface area = $0.41m^2$
Due to sitting in a plastic liner, it is assumed that there is an additional 5% surface area, as it does not clot in the shape of a perfect cylinder.
Surface area = $0.43m^2$ (per bucket)

Total surface area for an average day's production:

Blood collected = 3000L
Number of buckets = (3000L) ÷ (20L) = 150 buckets
Total surface area = (150 buckets) × ($0.43m^2$) = $64.42m^2$

Surface area per unit volume:

(Surface area of bucket) ÷ (Volume of bucket)
($0.43m^2$) ÷ ($20\times10^{-3}m^3$) = 21.47 m^2/m^3

Damaged Surface area

Surface area is created (non-natural) by the following actions:

- Cutting into quarters
- Pouring into the vats
- Cutting in the vat baskets (3x3)

Below shows the calculations for determining how much area is 'created' by each action.

Cutting into quarters in the buckets

Created area per cut = 2 × (Diameter) × (Height)
= 2 × (280mm) × (325mm)
= $0.182m^2$ per bucket
Assuming two cuts are made:
Created surface area = $0.364m^2$ (per bucket)
Total created surface area = $54.60m^2$

Pouring into the vats

Note: this is difficult to estimate, as clots break up significantly during pouring.
Average = 100% increase (after cutting into quarters)
Created surface area = $0.364m^2$ (per bucket)
Total created surface area = $54.6m^2$

Cutting in vats after pouring (3x3)

Basket dimensions
Length = 800mm
Width = 425mm
Depth = 300mm
Area created by Long cut = 2 × (Length) × (Depth) = 0.480m^2
Area created by Short cut = 2 × (Width) × (Depth) = 0.255m^2
Assuming each basket is cut 3x3:
Total created area per basket = 3 × (0.480m^2) + 3 × (0.255m^2) = 2.21m^2
Average number of baskets per day = 25
Total created surface area = 25 × 2.21m^2 = 55.25m^2

Combined total surface area created = 164.45m^2

Therefore, the average surface area per bucket is being increased by 73%.

Total surface area = 239.77 m^2

Appendix D – Feasibility of a Grid

Technical feasibility:

Blood coming down the tube takes approximately 2.5 minutes to fill a 20L bucket. Therefore, the flow rate is 8L per minute.

The volume of the baskets (in vat 1) is approximately 100L

Therefore it takes 12.5 minutes to fill each basket (assume they are all the same size).

The cycle of collecting-bleeding-emptying does not allow for the clots to be bled for long. The following cycle assumes:

- Collection time = 12.5min
- Clot/condemned wait = 40min
- Bleed time = 1hr 30min
- Time required to empty basket = 10min

Vat basket cycle times: There are 8 baskets in total

First cycle:

Basket	Fill time	Clot until	Bleed until	Time emptied
1	6:02	6:42	8:12	8:22
2	6:15	6:55	8:25	8:35
3	6:27	7:07	8:37	8:47
4	6:40	7:20	8:50	9:00
5	6:52	7:32	9:02	9:12
6	7:05	7:45	9:15	9:25
7	7:17	7:57	9:27	9:37
8	7:30	8:10	9:40	9:50

As shown, at 7:30am during the first cycle, basket 1 is still bleeding, and there is no room for further collection until 8:22am.

In order for the cycle to be sustainable with the current equipment, blood can only be drained for 35 minutes. This is not suitable, despite the increased surface area.

Therefore, in order to be able to bleed the clots for long enough with this method, we need another vat. The addition of 4 baskets of equal size would see the cycle as follows, with a bleed time of 1hr 30min:

Basket	Fill time	Clot until	Bleed until	Time emptied
1	6:02	7:32	8:07	8:17
2	6:15	7:45	8:20	8:30
3	6:27	7:57	8:32	8:42
4	6:40	8:10	8:45	8:55
5	6:52	8:22	8:57	9:07
6	7:05	8:35	9:10	9:20
7	7:17	8:47	9:22	9:32
8	7:30	9:00	9:35	9:45
9	7:42	9:12	9:47	9:47
10	7:55	9:25	10:00	10:00
11	8:07	9:37	10:12	10:12
12	8:20	9:50	10:25	10:25

This cycles shows that by the time the final basket has filled, basket 1 is ready for collection.

Appendix E – Further Testing

The following process actions & conditions are recommended to be tested.

Recommended actions to test:

Clotting:

- **The degree of coagulation that occurs before being cut.**
  - It is was concluded that breaking clots caused red blood cells to rupture. This was assumed be due to breaking the forces that hold the clots together, and in doing so rupturing the cell walls, releasing haemoglobin. If less coagulation has occurred (i.e. less force holding the clot together), then by this logic, less red blood cells would be ruptured when breaking a clot, releasing less haemoglobin.

Additional Variables:

- **The impact of pre-serum sitting in at the bottom the clotting vats amongst clots that are potentially dissolving:**
  - Dissolving clots may result in haemolysis (Arzoumanian, 2003).
  - Prolonged contact of serum or plasma with cells may result in hemolysis (Boyanton & Blick, 2002).
- **Any actions that were observed to potentially cause mechanical stress on red blood cells**. This includes:
  - The impact of whole blood impacting the bottom of the collection bucket when flowing down the tube.
  - Pre-serum dripping (from a height of approximately 50cm) onto the bottom surface of the clotting vats.
  - Draining pre-serum through a valve tap
  - Sieving pre-serum.
- **Processing temperature**
  - Exposure to excessive heat or cold can cause RBC rupture and hemolysis (Kroll & Elin, 1994)

Appendix F – Implementation Plan

This is an implementation plan for the recommended solution:

- The relationship between surface area ➔ serum recovery, and damaged surface area ➔ haemoglobin is well understood in an isolated, small scale scenario.
- It is therefore necessary to begin by validating this concept at a production scale.
- It is known that not damaging the clots will result in 20 mg/dL. This solution does not cause any damage, and will therefore be able to obtain this level of 20 mg/dL.

Proof of concept at full scale:

1. Manufacture a single steel grid that will divide a basket into 21x11 sections, and cover the entire depth of the basket (300mm).
2. Trial its use on a single vat basket:
    a. Place a plastic liner in one of the baskets that is easily accessible to the collection tube.
    b. Collect blood directly into the basket.
    c. Place the grid into the vat, and allow the blood to clot.
    d. Once fully clotted, remove the grid, and pull out the liner from underneath the clots, allowing the basket to drain.
    e. Note: this should be carried out in the first basket of a new vat cycle, so that a sample of the pre-serum drained can be taken for haemoglobin testing. Also, this will allow ABS to determine the volume recovery.

This test will determine:

1. Whether low haemoglobin serum can be obtained using this method.
2. The volume of pre-serum recovered using this method.
3. Whether it is feasible to pull out a plastic liner from the basket while underneath 100L of blood.
4. Whether or not the grid with ~4cm spacing will trap the clots in it when removed.

By completing one trail it will also determine how easily the grid can be cleaned, as post-operating procedures are a significant cost to ABS.

This trial should be repeated at least twice, and carried out over a whole day for a single basket.

Further Actions

If these trials are successful, it is recommended that 8 grids be manufactured (one for each basket), and a full production run is trialled with this system. Note that this will involve less blood being collected, as with only two vats there is not enough capacity to clot and drain the blood at the same rate it comes down the collection tube.

If this full production trial is successful with both haemoglobin levels and volume recovery, it is recommended that an additional vat (same size as the current two) is purchased (as well as four more grids), allowing ABS to use this method and not having to sacrifice on volume.