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Development of an Automated Magnetic Silica Bead Based DNA/RNA Extractor

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Abstract

Most of these first few pages are generated by the file header/frontpage.tex

However, this file contains a lot of important (and messy) commands for setting up the document which should rarely need to be changed, so I've moved the abstract to a separate file (header/abstract.tex) using an \include{} command.

You could do the same with acknowledgements if you wanted to.

Certificate of Originality

I, Jarrod Herbert Stilp, hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at UNSW or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by others, with whom I have worked at UNSW or elsewhere, is explicitly acknowledged in the thesis.

I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the projects design and conception in style, presentation and linguistic expression is acknowledged.

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Acknowledgements

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Chapter 1

Introduction

1.1 Background

Needed: -Place in industry -Background of diagnosis -Requirement and fit in company -Existing equiptment

Within the healthcare industry, there is a continuous need for fast and reliable diagnostics of pathogens within patients. Identifying the presence of pathogens responsible for disease in a patient allows the appropriate preventive or corrective action to be taken and represents a crucial step in treating or preventing illness. This thesis was conducted for the benefit of and in collaboration with AusDiagnostics Pty Ltd.

Successful diagnostics, within the context of this thesis, can be summarised in three overall stages. Namely these are extraction, amplification and finally analysis. This thesis concerns itself only with extraction. It should be noted that not all pathogen analysis and/or commercial diagnostic processes follow these steps strictly, however the processes and technologies applied by AusDiagnostics follow this procedure. The stages of this procedure may be

summarised as follows:

1. Extraction To begin the diagnosis, a clinical sample is obtained from the patient. This sample may consist of cerebrospinal fluid, faecal matter, urine or others, depending on the disease to be diagnosed. These samples contain the target DNA or RNA which will later be analysed to determine the presence of the pathogen and hence disease. They also contain however a number of inhibitors to the process of amplification and analysis. Extraction is the process of removing said inhibitors and retaining only the target DNA or RNA. The result is referred to as a clean sample.

2. Amplifcation Amplification takes the clean sample and by one of many methods increases the overall count of the DNA. This may be with the intention of allowing multiple targets to be detected or to increase the sensitivity of the analysis.

3. Analysis Analysis uses one of many available methods to search for the presence of biomarkers within the amplified clean sample. The presence of the biomarker indicates the result of the diagnosis.

AusDiagnostics currently supplies customers with the instruments and chemical products required to complete stages two and three (amplification and analysis) of the diagnosis. This requires customers to purchase extraction equipment from alternative suppliers and represents a significant weakness and loss of profit. Research and development conducted by AusDiagnostics has determined that the optimal approach, when considering speed and efficiency, is super-paramagnetic bead based extraction. The beads utilised are of the shell-core variety. The core is composed of an iron oxide, which provides the super paramagnetic properties required for physical manipulation of the

beads via a magnetic field. The shell is comprised of silica, which via chemical modification has the propensity to bond DNA and RNA to the bead surface. The techniques developed by researchers at AusDiagnostics utilising the magnetic silica beads have been validated and verified via manual operation. This thesis concerns itself with the automation of the developed extraction process, to produce a commercially viable robotic instrument. This instrument will be referred to as the Gene-Plex Extractor.

The extraction process to be automated has a number of notable requirements. These include liquid handling via precise pipetting, including mixing and liquid transfer. Also required is manipulation of the magnetic silica beads to separate the bonded and hence captured target DNA or RNA, along with heating to a specified, constant temperature to act to increase the rate of the chemical processes. The automation of the extraction process will be achieved by integrating the required capabilities into the robot produced by AusDiagnostics to conduct the amplification stage of diagnosis. The instrument, based of the Gene-Plex platform and sold as the High-Plex Processor, is pictured in it's current application in Figure 1.1. The Gene-Plex platform is essentially a liquid handling robot. The robot carries out the amplification stage by precisely pipetting and transferring liquid mixtures between the tubes and instruments on the deck, using disposable tips. Each individual assay (an analysis conducted to determine the presence and amount of a substance within a volume) utilises an individual layout of components on the robots deck. This makes the platform highly configurable for differing setups.

Fig. 1.1: The Gene-Plex liquid handling robot platform, as implemented as the High-Plex Processor.

1.1.1 The Extraction Process

In order to allow the aim and scope of the work to be clearly defined, a condensed overview of the extraction process developed by AusDiagnostics is presented. It should be noted that unless explicitly stated, all operations are to be automated.

The extraction process requires that the clinical sample undergoes a number of chemical steps across different locations on the robot. To aid in understanding the liquid handling involved, Figure 1.2 displays the important sites on the deck. The process will begin with the operator manually loading 24 individual clinical samples into the location labelled "Clinical Samples". In order to conform to existing products used by customers, it is then required that the chemical processing takes place in the locations labelled "Samples". In order to transfer the liquid between locations, the robot will pick up 1000μL tips from the locations marked "Tips".

The sample processing locations must accept modules that fit within the standard block size (SBS) format. These blocks are required to each accept 8 cassettes, one for each sample (pictured in Figure 1.3). Each cassette includes 6 tubes, which will be the site of a particular chemical reaction in order to extract the target DNA and RNA:

1. 500μL of clinical sample is pipetted into tube location 2, as marked in Figure 1.3. Within this tube as supplied by AusDiagnostics, there will already be 10μL of magnetic bead mixture along with 440μL of lysis buffer. The lysis buffer will destroy the cell walls, releasing the target DNA or RNA to be bound to the magnetic silica beads. This process is

required to take place at 60°C in order to increase the rate of reaction Due to the low volume of liquid in this step, it is to be completed in one of the 1mL low profile tubes. This aids with liquid handling precision.

2. The target DNA or RNA is now bound to the beads, which are suspended in the waste liquid (supernatant). In order to capture only the bead suspension, the entire liquid mixture is aspirated via pipette tip and subsequently move to the location marked "Waste Separation". In this location, a magnetic field is to be applied to capture the magnetic silica beads within the pipette tip. While captured, the supernatant must then be expelled into a waste container in this location. The tip now contains only the magnetic beads with bound targets.

3. Despite the supernatant having been expelled, there will still be a significant amount of waste retained on the bead surface. To remove this and clean the beads, a sequence of 3 wash steps must then occur in tube locations 1, 5 and 6. To achieve this, the beads are first re-suspended in the tube, which contains 800μL of a particular wash buffer. The suspension must then be mixed via pipetting in order to to ensure the bead surface is properly exposed. The entire liquid mixture is then aspirated once again and transferred to the waste location, where via the same means as step 2, the waste liquid is disposed of while the beads are retained. This process is repeated 3 times in the tubes noted above to ensure no waste matter clings to the bead surface.

4. With the beads now clean and still bound to the target, the mixture is transferred to location 3 of the cassette. The elution buffer is used to break the bond between the target DNA and RNA and the magnetic silica beads. This process is also required to be completed at 60°C to

reduce the time required. Following this step, the target DNA or RNA of interest is now contained within the elution buffer, along with the magnetic silica beads which are now waste.

5. The final step is to capture only the target DNA or RNA within the elution buffer, leaving behind the magnetic beads. This is required to take place in tube location 4. This is a low volume reaction, containing only 100μL of elution buffer and is therefore completed in the final small profile tube. The pipette must then aspirate this mixture, following bead removal, and contain only the elution buffer and target mixture required for amplification in stage 2 of the assay.

1.2 Aim

This work aims to develop and integrate into the Gene-Plex Robot Platform the hardware necessary for carrying out the described chemical processes, the required magnetic manipulations and the controller necessary to maintain the stipulated constant 60°C temperature.

1.3 Scope

The features required by the Gene-Plex Extractor, over those already part of the Gene-Plex Processor, can be grouped into 3 overall categories. These are detailed below, including clear definition of their inclusion or exclusion from

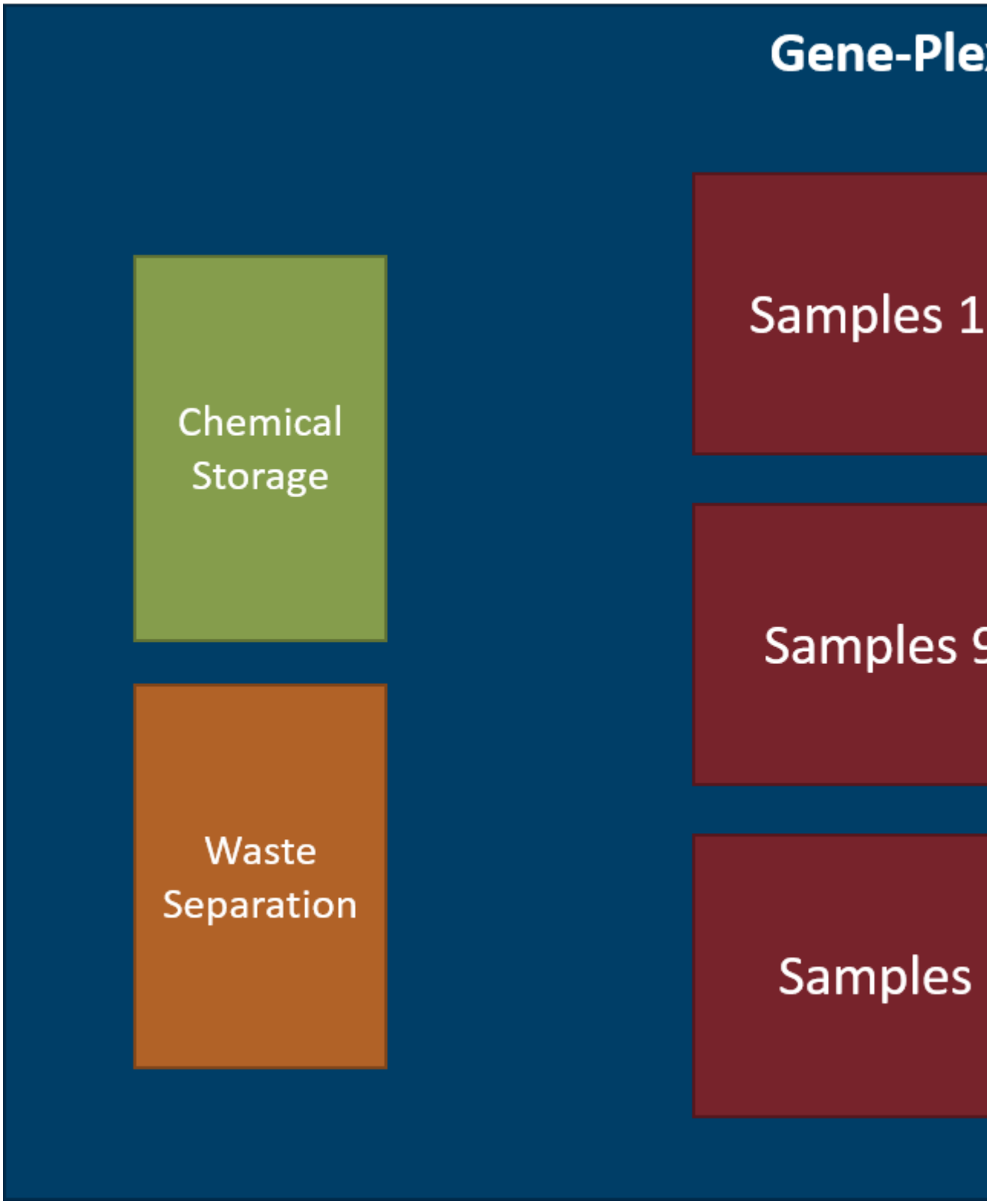

Fig. 1.2: The layout to be used for the Gene-Plex Extractor.

Fig. 1.3: The tube format to be used as the site of the sample processing.

the scope of this work.

1.3.1 Mechanical

The summary of the extraction process to be implemented, given in Section 1.1.1, revealed a number of missing mechanical components when compared to the description of the Gene-Plex Processor given in Section 1.1.

SBS Reaction Module As was noted in Section 1.1.1, the extraction process is to take place within cassettes consisting of 6 tubes. These must be located in the 3 SBS deck locations as marked in Figure 1.2, with each SBS location accepting 8 cassettes. The design of the module which will accept these cassettes is within the scope of this thesis and will form a large component of the design work undertaken.

Heating Element In order to create a commercially viable product, it has been stipulated that the tubes within each cassette where the the lysis buffers and elution buffers are held (tubes 2 and 4), a temperature of 60°C must be maintained for the duration of processing. This is a core requirement of the extraction process and is included in the scope of this project.

Magnetic Separation The Gene-Plex Extractor can be seen to require magnetic manipulation of the silica beads in two different locations. In order to allow the extracted DNA or RNA to be captured after separation from the beads in tube 4, the beads must be retained in the tube while the liquid is aspirated by the pipette. Secondly, the magnetic beads must be captured within the pipette tip during the expulsion of the waste supernatant after all wash steps are completed. The magnetic separation required in both of these processes is defined to be within the scope of this work.

Waste Disposal Following the wash steps and the subsequent separation of the magnetic beads and the bound DNA or RNA from the wash buffer, the supernatant must be hygienically disposed of. This waste disposal method is required in order to ensure that no contamination occurs between the clinical samples being processed. It is also crucial to ensure that no contact can occur between the operator and the biological matter which is a by product of the extraction process. The development of this waste disposal hardware is included in the scope of this work.

1.3.2 Electronic

In order to account for the newly integrated capabilities in the Gene-Plex Extractor, some modifications to the Gene-Plex Processor electronics are required. Due to the temperature control requirements of the extraction process,

an electronics board capable of communicating with the robot software and driving the heating elements appropriately is required. This however is not included in the scope of this work for a number of reasons. One of the components of the Gene-Plex processor is a device called the MTX Cycler. This device controls the temperature of the already extracted sample (during stage 2, amplification) precisely between set temperatures to enable PCR (Polymerase Chain Reaction) to occur and hence amplify the sample. The MTX Cycler controls the temperature of liquid volumes significantly smaller than those involved in extraction and in a different form factor, making it unsuitable for this application. It does however provide the necessary electronics and interfaces required to drive any standard form of heating element used here. Further to this, the electronic board used to control the MTX Cycler is currently being integrated into a newly developed control board for the Gene-Plex Platform by AusDiagnostics engineers. This new board will also be capable of driving the heating elements selected for this application. Therefore, due to the redundancy of work on this component of the Gene-Plex Extractor and due to a suitable requirement existing, the development of the required electronic is not within the scope of this work.

1.3.3 Software

In order to enable the Gene-Plex Platforms controlling software to utilize the newly integrated capabilities, two main components of software must be created:

Temperature Controller In order to control the heating elements to maintain a stable and accurate 60°C for the lysis and elution stages, a temperature controller must be implemented. This temperature controller must be designed according

Fig. 1.4: Full caption for the Mechatronics Logo. Designed by Wei Hua Chen.

to the temperature response of the heated hardware using appropriate methods and implemented as a controller in software. This element of the Gene-Plex Extractor's requirements will also form a core part of this work and is included within the project scope.

Routine Addition A was briefly noted in Section 1.1, the Gene-Plex Platform is highly adaptable in its ability to perform assays requiring differing liquid handling operations. The platform achieves this by utilizing a number of library routines which may be called upon the assay requiring a particular movement, such as expelling liquid, moving to a certain robot position etc. While the vast majority of these library routines will be directly effective within the Gene-Plex Extractor, due to the newly integrated capabilities further routines will be required. These will include routines for the mixing of liquids in the cassette tubes, magnetic separation and a table of definitions which specifies the locations of each of the tubes and pieces of deck hardware. These software additions are not included within the scope of this work and will be implemented by software engineers at AusDiagnostics.

1.4 Methodology

Figure 1.5 and subfigure 1.5b or b contains ... Table 1.1.

Citations: Luce [?] wrote something important.

(a) Subfigure caption. (b) Subfigure caption.

Fig. 1.5: Overall figure caption.

Classification	Cost	Description
CLEAR	1	Good for traversing
OBSTACLES	∞	Definitely not traversable
UNKNOWN	4 if distant, ∞ if close	Not classified
EXPENSIVE	In range $[2, 50]$	Traversable but should be avoided

Table 1.1: Example table.

Chapter 2

Literature Review

The thermal requirements for the extractor to be developed, as outlined in Section **??**, are similar in nature to those of a Polymerase Chain reaction (PCR) Thermal Cycling Device, albeit with simplified operations. The requirements of a PCR thermal cycler are driven by the need to cycle the temperature of an array of tubes containing sample liquid through temperatures of 55°C, 72°C, followed by 92°C [1]. Due to the process involving upwards of 20 cycles between these temperature set points, ramp times for this change in steady steady state must also be minimised to ensure the total processing time is commercially viable. The thermal requirements of the Gene-Plex Extractor are highly matched, with two exceptions. In this application the temperature of the sample liquid shall be held at a constant 60°C, as determined by research conducted by AusDiagnostics, as opposed to the multiple temperatures of a PCR cycler. Secondly, the cool down ramp rates for a PCR cycler are of equal importance to it's ramp rates during heating, in order to achieve temperatures lower than the current steady state in adequate times [2] [3]. In the application of the Gene-Plex Extractor, there is no need to pay attention to cooling ramp rates. Due to the similarities evident between these two applications, a great

deal of information may be gathered from PCR cycler design.

In order to generate the heat necessary to reach the required sample temperature, some form of thermal pump is required. A Thermoelectric Cooler (TEC), also know as a Peltier, is often used for liquid sample thermal cycling [4]. A TEC is a solid state heat pump, controlled by the directional application of electric current across it's two terminals [5]. The direction of the applied current determines the direction of heat pumping across the module [5]. TEC's are constructed of pellets of n-type and p-type bismuth telluride semiconductors, connected in an alternating series [6]. The connections, made of copper, are bound to a substrate of ceramic alumina. This substrate forms the surface by which the heat generated is transferred. The TEC has shown through the reviewed literature to be common in PCR thermal cycler applications due to the above mentioned ability to pump heat in either direction, an important implication for thermal control.

Despite the TEC's ability to actively remove heat from a component, other active temperature removal methods exist. A passive approach to heat removal is that employed by a heat sink, or any simple surface area [7] [8]. This differs from an active approach, where airflow over the heat sink surface area is controlled via a fan, for example [7]. This leads to simpler possible options for heat pump selection, namely electronic resistive heaters. Despite no reviewed literature describing these devices as the main heat source in thermal control for PCR, they have been demonstrated to provide a crucial supporting role by Williams et al. The thermal cycler described in this work utilizes resistive heat strips to control the temperature of a sealing lid, to prevent sample evaporation at the upper temperature of a PCR thermal cycler [9]. While the application

noted here differs from the intended application within the extractor, where the device will be the primary source of heat, it demonstrates the feasibility of driving component temperature to a steady state value and hence represents a design possibility.

In order to be able to control the temperature of the liquid sample as required, a method of evacuating heat must be present. A passive method alone, such as a heat sink in isolation, will not allow the process controller to drive a reduction in heat beyond the limits of conduction to the surrounding air [8] and as such performance will not be satisfactory. Along with heat dissipation being essential for control, the TEC elements have a maximum temperature differential of which they are capable of generating across their surfaces. This imposes the following constraint on the thermal system:

$$T_{hot} - T_{cold} \leq dT_{max} \tag{2.1}$$

This has been noted as highly significant in PCR cycler design [5], where one end of the TEC must be kept at ambient temperature to enable the maximum steady state value of 92°C to be reached safely. It should be noted that due to the maximum steady state temperature in this application being limited to 60°C and assuming a reasonable TEC differential limit, dT_{max}, this constraint is not critical. However, a TEC becomes less efficient as it's cold side temperature is increased [10]. As such, a method of heat evacuation must be present.

In order to create a thermal system which is controllable within the context of stability and speed of response, attention must be paid to the thermodynamics of the device. Due to manufacturing constraints and standards, the

tube which contains the sample itself is not optimal. A wall thickness of 1mm results in poor heat transfer characteristics from the heated block to the sample [11]. Another source of poor dynamics is any regions of mechanical connection between the heated block and external components [9]. Recommendations from the reviewed literature included the use of gasket material to limit thermal material between any surface contacts (Williams et al specifically recommends the use of ethylene propylene), along with the use of a groove extending through the majority of the thickness and hence limiting heat transfer to the mechanical connections [12] [10]. Mechanical connections are however only one cause of significant thermal gradient within the device. Care must be taken to reduce the severity and frequency of the occurrence of these gradients to maintain stability and performance in temperature response. After heat pumping has ceased, a temperature gradient will persist for a period of time that is proportional to the square of the distance between the hot and cold locations [9]. This has important implications for the number of sample tubes that may be heated within a single block. If the size of the sample array to be heated is too large, difficulty will be encountered achieving a uniform and stable steady state temperature [9]. Widomski et al note that despite efforts to reduce heat loss and hence gradients around the system boundaries via methods such as insulation, the region of location of the heat source will continue to maintain a higher temperature. To counter this, it is suggested to use a rod of material with lower thermal conductivity (such as stainless steel within an aluminium block) to reduce the heat retained in this volume [10].

A number of measures may also be taken directly related to the heated block itself to ensure optimal thermal response. The concepts of static local balance and static local symmetry may be applied to obtain desirable heat

transfer within the medium [9]. A state of static local balance refers to a state of constant temperature where all heat sinks are equalled by heat sources in a local area [9]. This essentially describes a state of thermal equilibrium, where the following condition is satisfied:

$$Q_{in} = Q_{out} \tag{2.2}$$

To ensure this balance is obtained, a device design should not incorporate overly effective heat sinks, without a proportional increase in the heat source capacity, and visa versa. The condition of static local symmetry requires that for a constant temperature and within a local region, the center of mass of the heat source is coincident to the center of mass of the heat sink [9]. Satisfying this condition will ensure that a constant, steady state temperature is achievable, without the presence of a permanent temperature gradient between the two centers of mass. The selection of heated block material along with manufacturing methods may also act to improve transfer properties. Aluminium 6061 is recommended due to its purity [9], which aids in even heat transfer [13]. It is further recommended that the heated block be manufactured via machining, due to the homogeneous structure that results. This is beneficial in reducing any temperature gradients that may occur within the heated element due to mechanically or otherwise joined components or impurities or inconstancies that exist as a result of other manufacturing methods such as casting.

To utilize the thermal system to control the liquid sample temperature accurately, a method of obtaining a feedback signal is required. Complications exist however due to the constraint that the sample temperature itself

cannot be directly measured. Due to the thermal transfer characteristics discussed above, the placement of the sensing device is critical to the stability of the controlled substance [12]. Vilchiz et al studied the issues of sensor hardware placement and provide numerous recommendations to increase baseline stability. Using experimental methods, it was verified that the following one-dimensional heat equation holds:

$$s = \frac{L}{a} \tag{2.3}$$

Where s is the time taken for energy to flow from location A to location B, L is the distance between the two locations, and a is the thermal diffusivity of the material. This is relevant as it allows the time lag between a controlled heating action at the heat source to the arrival of the generated heat at the location of interest to be determined. The experiment conducted tested two temperature sensing cases. One sensor was placed within the center of mass of the heated block, while the other was located as close as was possible to the heat source. The results obtained found that "there is a general advantage of control via sensing close to the heater, whether by temperature measurement, or by heat-flow control sensing." [12]. Equation 2.3 is accountable for this result. When the sensor is placed at a greater distance to the heat source, the control effort is determined using a measurement that is not yet equalised due to the more severe temperature gradient. This error in feedback signal is reduced by the recommended placement strategy, such that "the reduction in the magnitude of the heater-induced oscillations achieved by this control strategy is at least one order of magnitude better than that of the temperature control at the core" [12].

A number of aspects of the experiments and findings the work of Wilchiz et al should however be considered. The sensing device used to make the presented conclusions were thermocouples, one of many sensing devices available. Despite the variances in accuracy and stability of measurements that are expected between differing sensing technologies, the results can be assumed independent of any associated error. Any error as a result of sensor output stability is of an insignificant order to magnitude of time when compared to the 1.07 min thermal lag situation generated by the experiment. Furthermore, one would expect a sensor technology of differing accuracy to produce a controlled output different to the desired reference input. This error is however not influential to the results given, which are concerned exclusively with the stability of the controlled response. The focus of the experiment was the application of thermocouple technology in Tian-Calvet microcalorimeter devices. This does not detract from the relevance of the reviewed work for a number of reasons. The motivation for the study was to improve baseline stability in temperature control, via the application of improved temperature measurement strategies; a general outcome that is relevant to the work of this thesis. Further to this, the scale of the experiment conducted closely resembles the device to be developed, with the distance between sensor locations set equal to 3.5cm. Therefore, despite the differing applications of the work conducted, the recommendations and strategies found may be concluded as directly applicable.

The literature reviewed has presented various differing selections of temperature sensing technologies, the most prominent of which have been thermocouples and thermistors. Despite the overlapping application, the specifications of the two technologies are significantly different. The thermocouple has a large temperature range, with measurements capable of ranging from

-270°C to 1800°C compared to the limited range of thermistors of usually around -90°C to 130°C [14] [3] [15]. The thermocouple and thermistor possess similar response rates along with similar accuracy, however due to the wider resistance range available, thermistors are able to achieve a high measurement resolution [14] [3] [15]. The two significant differences between the technologies are power supply requirements and linearity. Due to the welding of differing metal conductors at the measuring point, the thermoelectric effect results in a temperature dependant voltage being generated across the thermocouple terminals [14] [15] [3]. While this means the sensor does not require a power supply, it can introduce complications requiring a reference/compensating wire. Furthermore, changes to the conductor materials such as corrosion require that the sensor be calibrated on a regular basis, often as frequently as 3 months [14] [3] [15]. Thermistors use semi-conductive material that changes resistance with temperature. As such, measuring the temperature requires a current be passed through the device [14] [3]. While the output voltage of thermocouples changes linearly with temperature, this is not true of thermistors. Hence, the output signal requires linearisation before it may be of use [3]. In commercial applications, thermistor technology is often also preferred due to its cost effectiveness [15]. While Resistive Thermal Detectors (RTD's) and Integrated Temperature Sensors appeared in a number of reviewed sources, they are not considered in depth due to the high level of signal conditioning required [15] [14] and the lack of observed uses in relevant thermal control applications.

With the necessary hardware in place and optimally set up, as has been discussed above, controller design and theory may be considered. The large majority of the reviewed literature, along with the instruments currently em-

ployed by AusDiagnostics, recommend or employ the Proportional Integral Derivative (PID) controller. As summarised by Vilchiz et al, "Although other types of controllers are also feasible, e.g., those based on fuzzy logic or artificial neural networks, the simplicity, good response, and low cost of PID-based control loops have motivated their popularity in industrial applications" [12] [5]. Despite the common application of the PID controller in thermal control application, if a TEC is the selected source of heat, complications are presented. Due to the non-linearity of the device, PID coefficient selection involves the process of trial and error [5]. The strategies for PID design given were however applied to the thermal cycling requirements of PCR. This requires that 3 separate temperature controllers be implemented for each of the temperature set points, to ensure the required performance is met equally at each [5]. While the characteristics of the PID controller are not effected by this difference, it does significantly reduce the complications of coefficient selection. Further to the controller to be implemented itself, a number of sources give recommendations regarding relevant hardware. Cellatoglu et al investigated the dynamic performance of a process control system, with a focus on temperature controllers. The findings reveal that the word length of the ADC utilised is an important factor in reducing output error. A case study was conducted to assess the impact of increased word length on error, with results suggesting that 8 bits is the optimal value [16]. It was further found that an increase in word length beyond this point yields no reduction in error. The analysis conducted was however completed as a simulation, with no experimental verification evident. The electronic hardware implemented in other sources, such as the work of Shirafkan et al, differs from this recommendation. While not providing evidence to suggest the 8-bit recommendation is problematic, the successfully implemented controller utilized a 24-bit ADC.

To ensure the target DNA is extracted as required, the device must include a robust means of capturing the magnetic beads. Despite offering an alternative means of magnetic bead manipulation, electromagnets are not investigated for application in the thesis due to a high level of complexity that is not required for this static, constant force implementation. Therefore, the review of literature focuses on permanent magnets. While there are numerous compositions of magnets available, the rare earth variety and in particular the Neodymium composition (ND-Fe-B) are most suitable. Neodymium magnets offer the strongest solution, with an energy product of up to 56 MGOe [17]. Good mechanical characteristics and energy to size ratio make them suitable for fastening within a manufactured product, without undue difficulty. Importantly, given the requirement to capture beads within the heated block element, Neodymium magnets are stable in temperatures near ambient. Progressive loss of magnetism occurs at temperatures greater than 80°C [17], providing a 20°C margin of safety between the operating temperature of 60°C. This composition also holds a very high coercive force [17], allowing it to resist demagnetisation in the presence of external magnetic fields that will be present within the GenePlex Extractor, as a result of other hardware. Despite the specification of the superparamagnetic silica beads being a constraint determined by completed research at AusDiagnostics, a number of characteristics should be noted in order to obtain reliable bead capture capabilities. Shevkoplyas et al describes the force acting an a superparamagnetic bead due to an applied force. The context of this paper is the motion prescribed by the beads within a microfuidic chamber, however the general characteristics of the interaction between the beads and magnetic field are applicable to the capture required here. In order to saturate a suspension of superparamagnetic beads, an external mag-

netic field greater than 0.5T must be applied [18]. Saturation is the state where all beads magnetic poles are aligned. In a state of saturation, the magnetic beads behave simply as permanent magnets [18] [19]. In order to ensure the complete separation of the magnetic bead dispersion within the sample liquid, this condition must be achieved.

The literature reviewed has allowed a number of critical elements of thermal control and magnetic separation, being the main requirements of the extraction system, to be recognised and evaluated within the context of this thesis. The similarity between the processes demanded by the extraction technique and the works discussed allow the information gathered to be applied directly to the benefit of the developed instrument. Those works which were an exception to this allowed general strategies and design characteristics to be collected, which may be applied with consideration in future development.

Chapter 3

Methodology

Chapter 4

Results

Chapter 5

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Chapter 6

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Chapter 7

Future Work

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Appendix A

Raw Results