The Impact of ABO Kodecytes as a Serological Teaching Tool

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Abstract

Background:

Skills in recognising a range of grades of haemagglutination are critical to safe practice in the preparation of blood for human transfusion. With the move from apprenticeship-style training of blood bank scientists to university-based laboratory science degrees, the skill of grading has probably diminished, due to a lack of availability of a range of grades to teach. The development of KODE technology, with the ability to insert synthetic blood group antigens into the red cell surface in controlled amounts, allowed the potential to develop a new grading teaching tool.

Methods:

KODE technology was utilised to produce panels of cells designed to have a range of agglutination grades (so called “kodecytes”). These were analysed by 102 students and 21 practitioners recruited over a two-year period. Participants performed a standardised grading exercise over either 1, 12, or 24 weeks. Statistical tools were applied to perform quantitative data analysis, and qualitative feedback was also sought.

Results:

Students did not have problems grading strongly agglutinating natural cells, or negative reactions. They did have problems grading weak and medium agglutinates (kodecytes). Students with initial grading problems improved their performance in the grading exercise over time ($p=0.02$). Both students and practitioners found the grading exercises to be valuable in improving grading ability and confidence in ability to grade, particularly with weak grades.

Conclusion:

KODE technology allowed the development of a robust and well-accepted teaching tool, to improve the teaching of grades of haemagglutination in the teaching environment.

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Attestation of authorship

“I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.”

Signature_____

Date_____

List of Abbreviations

AUT	Auckland University of Technology
BMLS	Bachelor of Medical Laboratory Science
CAT	Column Agglutination Technology
DAT	Direct Antiglobulin Test
FSL	Function-Spacer-Lipid
Gal	Galactose
GalNAc	N acetylgalactosamine
HTR	Haemolytic Transfusion Reaction
IANZ	International Accreditation New Zealand
IAT	Indirect Antiglobulin Test
MSCNZ	Medical Sciences Council New Zealand
MoAb	Monoclonal antibody
NZBS	New Zealand Blood Service
RCPA	Royal College of Pathologists of Australasia

Introduction

Red cell transfusion is a treatment for blood loss by trauma, and for anaemia where the underlying condition cannot be treated by pharmacological agents (Shander, 2008).

There are many risks associated with red cell transfusion, including the long-recognised incompatibility between patient and donor (Mollison, 1967), sepsis due to an infected unit of red cells (Theakston et al., 1997), and the more recently recognised complications of transfusion related acute lung injury (Chapman et al., 2009) and transfusion-linked inflammation (Mangalmurti et al., 2013). Incompatibility between patient and donor can cause haemolytic transfusion reaction (HTR), where the donor red cells are destroyed in the patient's system due to patient antibody specific for donor red cell antigen. Blood group antibodies of clinical significance that could cause a haemolytic transfusion reaction include, but are not limited to, those of the ABO, Rh, Kell, Duffy, Kidd and MNS systems. The most serious HTR occurs with antibodies of the ABO system (Issitt & Anstee, 1999).

Since the early 20th century, transfusion practitioners world-wide have relied on the in-vitro immunologic reaction between donor red cells (containing blood group antigens) and patient serum (containing blood group antibodies), to predict the *in vivo* response that a patient may have when those donor red cells are infused during a blood transfusion (American Red Cross, 2013).

The in-vitro compatibility test between donor red cells and patient serum is called a crossmatch and can be performed by a variety of techniques (Harmening, 2012). Other tests to predict donor red cell survival in patients are ABO and antibody screening. The latter checks patient plasma for the presence of clinically significant antibodies outside the ABO system (Harmening, 2012).

Patients and donors are routinely matched for the ABO and Rh(D) antigens in New Zealand, and for other system(s) when the patient has an identified antibody in that system which is potentially able to cause HTR. Electronic crossmatch (whereby an electronic blood management system selects ABO and Rh(D) compatible blood based on patient results), is used for patients who do not have clinically significant antibodies outside the ABO system. (New Zealand Blood Service 2008)

For many years, the gold standard test for measuring these in-vitro immunological (also known as serological) reactions has been the haemagglutination test (Walker, 1990), because this produces a visual and semi-quantitative reaction. Red cells which are positive for a specific blood group antigen can be held together in a red cell clump called an agglutinate, when the conditions are favourable. These conditions include presence of the specific antibody in the test serum, isotype of antibody, the concentration of antibody in the serum, accessibility of the antigen on the red cells, pH, temperature and ionic strength, and use of secondary bridging agents such as anti human globulin (Walker, 1990; Issitt and Anstee, 1999).

The reactions we observe *in vitro* are:

a. Agglutination, with a range of strengths. This may be direct (in saline and seen with IgM antibodies) or indirect (requiring the application of another technique and seen with IgG antibodies)

b. Mixed field agglutination, where some cells have been agglutinated and others have not

c. Haemolysis; complete or partial destruction of the red cells under test

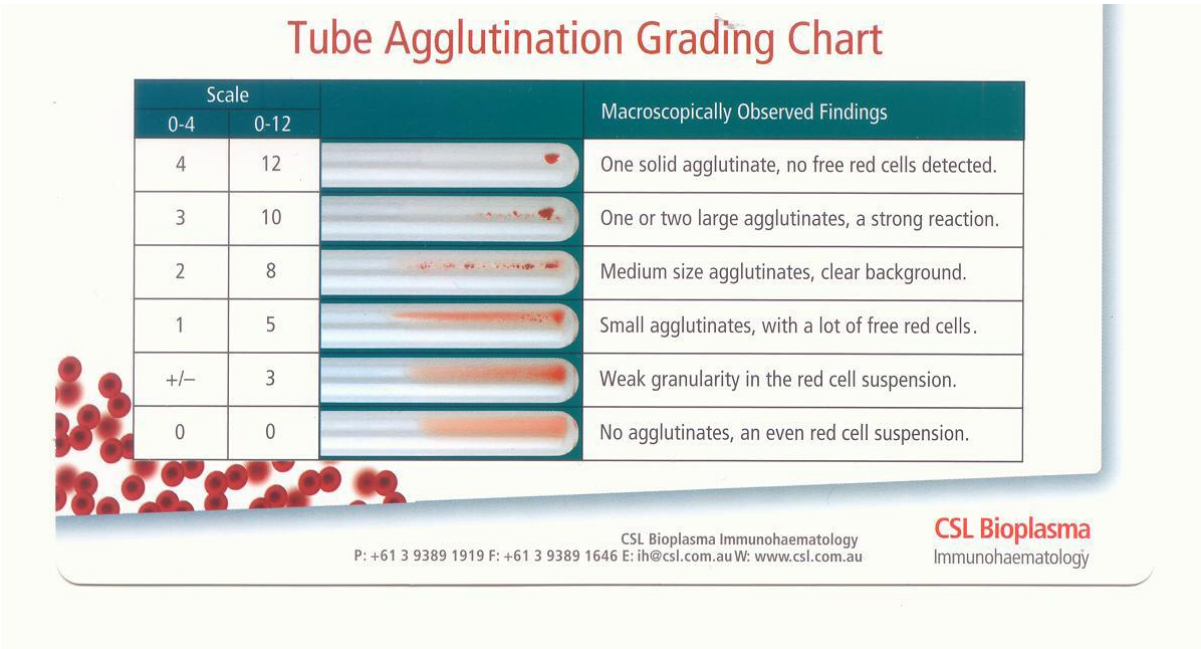
d. Non-agglutinated cells. This can indicate that no reaction has occurred, or that sensitisation with IgG antibodies has occurred, but has not been visualised by a secondary antibody

These serological reactions can be observed in different combinations in different platforms including the traditional tube technique, column agglutination technologies (Lapierre et al., 1990) and solid phase technologies (Rosenfield, Kochwa, & Kaczera, 1978) as cited in Harmening (2012).

Stronger reactions are generally associated with high titres of antibody, or when conditions are most favourable. Weaker reactions are usually associated with lower concentrations of antibody, lower numbers of antigen, poor antigen-antibody binding, or when conditions are not so favourable (Reverberi & Reverberi, 2007).

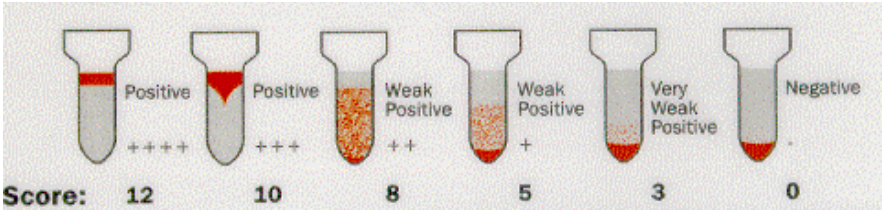
At least two grading/scoring systems are used internationally to assign a semi-quantitative result to the haemagglutination reaction (Roback, 2011). Both systems use the numeral 0 to denote a negative reaction (no agglutination observed). One system uses ±, 1, 2, 3 and 4 to assign grades to different strengths of positive. A second system uses 3, 5, 8, 10, and 12. The + symbol may or may not be added after the number in the positive grades; for example 3+, 5+, 8+ etc. The appearance of the agglutination, and the two grading/scoring systems are illustrated in Figure 1.

Figure 1. bioCSL Bioplasma tube agglutination grading chart (reproduced with permission)



As can be seen in Figure 1, the assignment of grades (regardless of which system is used) is somewhat subjective. For instance, the size of the agglutinates might be somewhere between that of those depicted in the 1+ and the 2+ reactions. The scientist does not have the option to record 1½+, and must make a decision to record the reaction as either 1+ or 2+, because 1½ is not a recognised score. Repeated centrifugation of the tube may change the strength of the agglutination, and should not be undertaken for this reason. However, when the scientist is unsure, it is difficult for them to seek a second opinion without re-centrifuging the tube, as the cell button has dispersed. This subjectivity, and the instability of the agglutinate, are limitations of the tube technique. In column agglutination technology (CAT), because the agglutinates are immobilised in sephadex gel or glass beads (Lapierre et al., 1990), the reaction is somewhat less subjective to grade. Typical appearance of grades of CAT agglutination are shown in Figure 2 (Nicol, 1998). However, once again the appearance and size of the agglutinates may be somewhere between those shown in the published pictures, and a judgement still has to be made by a manual operator. With automated readings of CAT cards and cassettes, the subjectivity is further reduced. However, not all tests performed in a blood bank can be performed in CAT (manual or automated) and the ability to grade a tube reaction, subjective though it may be, is still an important skill. Additionally, during natural disasters, a laboratory may lose access to automated analysers and need to revert to manual techniques such as tube blood grouping. Correct tube technique is an important skill that needs to be taught to trainees, and maintained in practising blood banks.

Figure 2. Grading of serological agglutination reactions in column agglutination technology (Nicol, 1998)



Various strengths of reaction are routinely seen with patients, and it is imperative that scientists can recognise a range of reactions, ranging from strong to weak. Failure to recognise a positive reaction (be it strong or weak) can result in issue of incompatible blood, causing potentially serious consequences for a transfused patient. It is important to appreciate that a weak reaction can be as clinically significant as a strong one.

A natural occurrence on human red cells is variation in antigen copy number. For example, subgroups of A exist in the ABO system, whereby different alleles at the ABO locus produce transferases which add the terminal sugar N acetyl galactosamine (GalNAc) to the substrate molecule with different degrees of efficiency (Olsson et al., 2001). This leads to different numbers of molecules expressing GalNAc on the red cell surface. Group A individuals are split into subgroups of A based on these different alleles at the ABO locus, with subgroups A_1 and A_2 accounting for the great majority of group A individuals (Daniels, 2002). Rarer subgroups of A also exist. Frequency, antigen copy numbers and typical reactions with anti-A are shown in Table 1.

Table 1. Characteristics of subgroups of A

Subgroup of A	Phenotype frequency*	Typical reaction with Anti-A**	Antigen copy number per red cell ($x10^3$)**
A_1	0.34	4+	810 - 1170
A_2	0.10	4+	240 - 290
A_3	<0.01	2+ mixed field	35
A_x	<0.01	±/0	5
A_{end}	<0.01	± mixed field	3.5
A_m	<0.01	±/0	1
A_y	<0.01	0	1
A_{el}	<0.01	0	0.7

*Caucasians (Daniels, 2002; Reid & Lomas-Francis, 2007)

** (Harmening, 2012)

It is rightly argued that incorrectly identifiying red cells with weak reactions, such as Ax, is of no clinical consequence (Klein & Anstee, 2013) , however weak reactions are typically encountered when cross-matching a patient serum, where low levels of ABO antibodies are common (Knowles, Milkins, Chapman, & Scott, 2002).

Trainers and practitioners of transfusion laboratory science have no control over the natural biological variation in antigen copy number. This can affect performance of a test. Unlike biochemical tests, where molecules can be added in measured and known amounts, the quantitative contribution of a natural red cell antigen to a reaction is relatively uncontrolled (Henry, 2009). This can lead to problems, ranging from prozone to variable detection or non-detection of a low copy number antigen. Knowles et al., (2002) report a “decline in proficiency in detecting weak ABO incompatibilities”, in analysis of data from national external quality assessment scheme over the period 1985 – 2000.

The *in vitro* testing in medical laboratories in New Zealand is performed by either medical laboratory scientists, or medical laboratory technicians, both of whom must be registered and deemed competent to hold annual practising certificates (Medical Sciences Council New Zealand, 2013).

Until the 1990’s, trainees of medical laboratory technology in New Zealand served an apprenticeship in hospital or community laboratories, and completed qualifications whilst working under supervision on patient samples. In this system, trainees usually received a lot of practical training, and exposure to a large range of clinical variations (but not always).

In the 1990’s, the qualification leading to registration as a medical laboratory technologist changed to a degree named Bachelor of Medical Laboratory Science (BMLS). The degree is offered by three New Zealand Universities and, upon completion, graduates apply to register as medical laboratory scientists with the Medical Sciences Council New Zealand (Medical Sciences Council New Zealand, 2013).

Within the degree, students specialise in two subjects and Transfusion Science is one of the available specialties.

The Auckland University of Technology (AUT) degree consists of :

- first year of general sciences
- second year, in which the disciplines of Clinical Chemistry, Cytology, Haematology, Histology, Immunology, Medical Microbiology and Transfusion Science are introduced
- third year, in which two of these disciplines are taught to an in-depth level
- fourth year, in which students learn the practice of their two specialising disciplines as interns in an accredited medical laboratory. These internships are known as clinical placement. 450 hours must be completed in each clinical placement

Each year of the degree is made up of two 12-week semesters. There are four Transfusion Science papers in the degree; one in the second year, two in the third year, and one in the fourth year. The clinical placement in Transfusion is conducted in the New Zealand Blood Service (NZBS), or a combination of NZBS and district health board blood banks.

A further qualification recognised by Medical Sciences Council New Zealand (MSCNZ) for registration as a medical laboratory scientist is the Graduate Diploma in Science, offered by AUT and Massey Universities. This qualification consists of selected papers from year two, three and four of the BMLS and includes the Transfusion Science papers for students working in an accredited transfusion laboratory.

In both qualifications, AUT teaches the students with lectures and wet laboratories in the university. For each paper students attend one 2-hour lecture and one 4-hour laboratory per week, over a 12 week semester. In class laboratory setting, exercises are produced to mimic tests and reactions seen in a blood bank.

There are advantages and limitations to training in both apprenticeship and university settings.

Apprenticeship models of training are well established and still in place for many practitioners such as builders, plumbers and electricians. The apprenticeship model is one of learning a skill through practise in an environment where that skill is normally practiced, alongside someone who is already skilled in a particular range of tasks. There are many advantages to this way of learning. Firstly the student is usually taught one-on-one. Secondly, there is the opportunity for two-way communication in the apprenticeship environment, where questions arise very naturally from handling something and repeating a task many times. Learning occurs through observation, interaction and variety of practice. For example, in the blood transfusion setting, the student might ask the "teacher"; *Why is the method I use for this antisera different to the method we used yesterday with a different antisera?* In the university classroom setting, learning occurs differently, with one lecturer delivering information to a class of students. Although there is always the opportunity to ask questions and perform practical work, the environment may not stimulate questions so naturally, and the students may feel more embarrassed to ask something in front of their peers. Without access to patient samples, the university is forced to "mock-up" samples to mimic patient conditions, where agglutination reactions are usually limited to strong positive, or negative. The variety of practice may not be as great as in the working laboratory.

Apprenticeship models of training also lend themselves very well to measurement of an individual's competency. This is so because it is very easy to count the number of hours spent on an individual task, and track improvement and mastering of that task. Practical contact hours in university teaching laboratories form a large part of the BMLS. The number of hours is set and student attendance is required and recorded. However, when one lecturer has up to 40 students in class at a time, and students work in pairs or small groups, it is not so easy to know how much time each individual has spent on a task, or whether they have truly mastered it. The usual way to assess practical mastery is by practical examination. This usually highlights the student who

fails to perform the task well enough to get the correct result, but does not give the examiner the opportunity to watch every aspect of the student's laboratory practice.

On the other hand, the apprenticeship model may not be as robust as the university classroom in producing a practitioner with an in-depth theoretical understanding of the task. In the workplace, the range of reading materials may be less, time may be a constraint and the teacher may be skilled in the practice but not the theory of the task. Emson (2013) states that the aim of study in the AUT School of Applied Sciences, is that graduates "will be adequately prepared to utilise and apply their knowledge and skills on completion of a programme, in addition to possessing the knowledge and skills to contribute to the pool of scientific knowledge with further postgraduate study" (p. 24).

The fourth year of the BMLS is an apprenticeship model within the degree, and is seen as a strength of the degree by many within the profession. Employers of graduates also comment that graduates need more "work-ready" experience in the laboratory once they are employed than did the newly qualified apprentice, but that they are better at problem solving and critical thinking than previous apprenticeship trained graduates (unpublished observations).

Approaches to teaching Transfusion Science to scientists and/or physicians at learning institutes other than AUT include:

- Lectures, computer labs and laboratory classes, with clinical placements/internships (Massey University, 2014) (University of Otago, 2014)
- Apprenticeship style learning in a laboratory supported by theory delivered by distance education (Massey University, 2014)
- Virtual learning sites (Dunstan, Devenish & Kevill, 1997)
- A mix of apprenticeship , formal lectures and patient conferences (Stanford School of Medicine 2014)

It was noted after conducting a web-based search to look at different educational forums practised in teaching Transfusion Science, that the number of web pages concerned with the training of doctors far outweighed those for scientists (unpublished observations). Training for doctors usually included practical applications of transfusion for the patient, as the doctor sees the patient daily. The medical laboratory scientist does not have the opportunity to see the patient. This may be a disadvantage, as remembering a patient and their clinical outcome may enforce learning through empathy with a person, rather than just their sample. The medical laboratory scientist remains the "hidden health professional", as there is not the opportunity to interact with the patient in the same way that other health professionals do.

In the AUT setting, the teaching and learning model of lectures, laboratory classes, and a small number of computer labs is used. In the laboratory classes, students are taught using blood donor samples with the support of NZBS, who supply red cells that are expired or otherwise unsuitable for transfusion. These donor samples are used to mimic patient and donor samples.

Red cells and antibody (sourced from either human or commercial antisera) are selected by specificity to enable positive and negative reactions in a haemagglutination test (hereafter referred to as agglutination).

A range of assays and clinical scenarios is taught, using:

- Red cells which are positive for a selected antigen
- Red cells which are negative for a selected antigen
- Commercial antisera
- Human anti-D

In ABO and Rh(D) phenotyping, donor red cells of various blood groups are tested with commercial anti-A, anti-B and anti-D.

In the antibody screen and antibody panel, red cells are selected for their D antigen status and patient serum containing anti-D is mimicked to contain a range of red cell IgG antibodies reactive by the indirect antiglobulin test (IAT). For example, if the lecturer wants to teach an antibody identification panel containing anti-Fy^a, the D positive cells would be labelled Fy^a positive, and the D negative cells would be labelled Fy^a negative in the antibody identification panel. The student tests the cells against a serum containing anti-D (labelled to masquerade as a patient specimen), using the IAT to detect the anti-D reacting with the D positive cells, and not with the D negative cells. On reading the panel profile, the student believes the specificity of the antibody to be anti-Fy^a, due to the mimic labelling. In this way, anti-D, and D positive and D negative cells have been used for many years to teach identification of a range of IgG antibodies. Anti-D was chosen because it was originally readily available, and D positive and D negative cells were also readily available, and easy to identify. Serums with a range of specificities of blood group allo-antibodies are not readily available to the university. The strategy also minimises cost, as the readily available D positive and D negative cells are much cheaper to source than commercial antibody identification panels.

This strategy has produced graduates who are very acceptable in the workplace (unpublished observations) but has significant limitations:

1. Due to the lack of variety of clinical samples, there is no ability to teach less commonly encountered phenotypes when working with IgM monoclonal antibodies. For example, there is no ability to teach weakened antigen expression, as may be seen in ABO subgroups (Salmon, Lopez, Catron & Bougerra, 1976), disease states (Reid & Lomas-Francis, 2007; Olsson et al, 2001) or inherited D variants (Reid & Lomas-Francis, 2007). All samples obtained from NZBS are from healthy individuals. The frequency of ABO subgroups and D variants is not high (Reid & Lomas-Francis, 2007) so the likelihood of these being available to the university is low.

2. Due to the lack of variety of clinical samples there is limited ability to teach a range of agglutination reactions of different strengths. To practice detection of IgG antibodies, one anti-D serum is used over several months, as it is supplied in 200ml volumes. As the titre of one donation is constant, the same agglutination strength will be seen with this serum with the D positive cell over its lifespan. Therefore there is no ability to teach students to recognise different agglutination strengths with an antibody panel, or to reflect the reality of different antibodies reacting at different strengths.

3. There is no ability to teach dosage in practise. Dosage is a phenomenon seen with antibodies of Rh, Duffy, Kidd and MNS systems, whereby the antibody reacts more strongly with antigen positive cells which are the result of homozygous gene expression (Harmening, 2012). Because the antibody used is always anti-D, this cannot be demonstrated in practise.

These three limitations, which all amount to an inability for students to practise grading a range of strengths of agglutination, meant that in 15 years of teaching, no students were identified who lacked that skill. Undoubtedly those students existed, but the lecturers did not have the tools to identify them. The ability to teach this skill had been lost in the move from the apprenticeship style of training to the university environment. Therefore the university was probably failing to detect students who were not fully competent in grading. The researcher's own experiences in teaching students support this statement. For example, in a second-year class practical test in August 2013, 40 students were presented with samples to perform ABO, Rh(D) and direct antiglobulin test (DAT) on. One sample was DAT positive. This DAT positive sample was prepared in the laboratory by incubating an O Rh(D) Positive cell with human IgG anti-D, to mimic a patient with a positive DAT. Because the human IgG anti-D had blocked the Rh(D) antigen sites, there were fewer sites left to react with the reagent anti-D which students used to perform the Rh(D) phenotype. This cell was giving a 2+ reaction with the anti-D. Nineteen of the forty students (48%) missed this weak reaction and called the sample Rh(D) negative (unpublished observations). Up to this point in their training, this cohort of students had not experienced a reaction as weak as this. Therefore we know that there is a problem with recognising weak reactions in untrained students.

There are also issues which impact on the teaching strategy at AUT:

a. The specimens sourced from the donor are screened for hepatitis and human immunodeficiency viruses to minimise risk to students. However, human samples can never be guaranteed to be risk free.

b. Donor red cells which are unsuitable for clinical use (including those which are expired) and anti-D are becoming more difficult to source. These are precious donor resources and are less readily available than in the past (Dean, 2000).

Some of these limitations and issues are overcome when the student moves from the university laboratory to the clinical placement. However, as the clinical placement is only 450 hours in

duration, students may not be exposed to some clinical scenarios, particularly in smaller laboratories. For example the laboratory may not see a haemolytic transfusion reaction in a patient in the training period. Practical training in transfusion science can be conducted at 3 levels:

a. Basic level for undergraduate students
b. Advanced level for practitioners
c. Ongoing training and testing to assess competency in both students and practitioners

Some of the identified issues may also be problematic for practitioners trying to maintain competency, with demonstration of competency being one requirement to hold an annual practising certificate. A practitioner working in a smaller laboratory, with low sample numbers, or under a policy of referring samples with unresolved serological anomalies to a national reference centre, is confined by the same limitations.

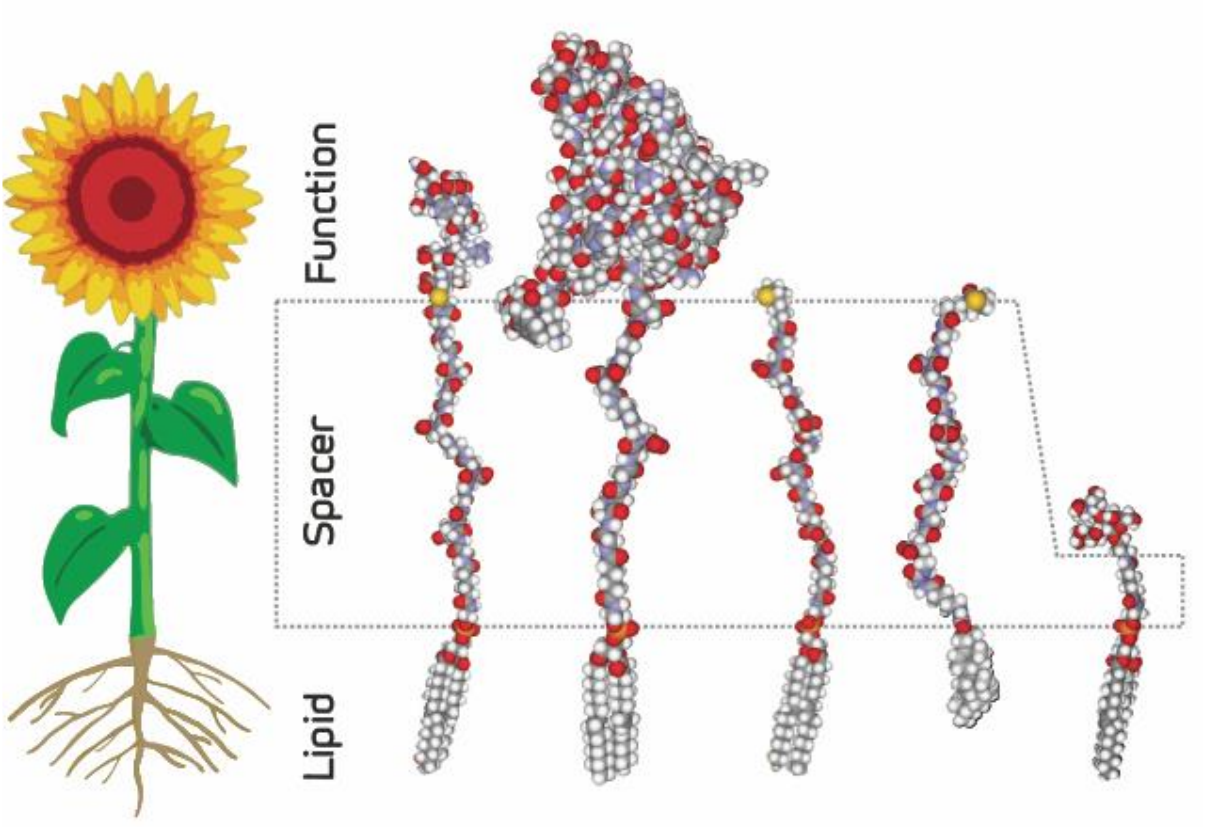
A solution to some of these limitations may be to use red cells modified with synthetic blood group molecules, able to be added to red blood cells in known and controlled amounts. KODE technology allows the modification of surfaces with synthetic molecules, including blood group molecules (Frame et al., 2007; Hult et al., 2011). Glycolipids have a natural ability to insert into red cell membranes due to their hydrophobic tail (Sneath & Sneath, 1955). This phenomenon is exploited in KODE technology, which allows synthetic glycolipids to insert naturally and stably into red cell membranes, in controlled and variable concentrations (Frame et al., 2007; Henry, 2009; Blake et al., 2011).

"Such synthetic constructs can be made to express a potentially unlimited range of carbohydrate blood group determinants" (Frame et al., 2007)

The synthetic glycolipids are called "FSL" and a cell modified with FSL construct is called a "kodecyte". Kodecytes retain the normal properties of unmodified red cells; that is their shape, size and osmotic fragility are all unchanged by the addition of construct. They behave as an unmodified cell would in in-vitro immunological assays, and retain normal storage life in red cell preservatives (Henry, 2009).

There are three components to the construct; the lipid tail, the functional head and a spacer separating the two. An easy to visualise model is to liken the construct to a flower, with the tail represented by the flower root system, the spacer by the stalk, and the functional group by the head of the flower. The functional group can be either protein or carbohydrate, and simple or complex.

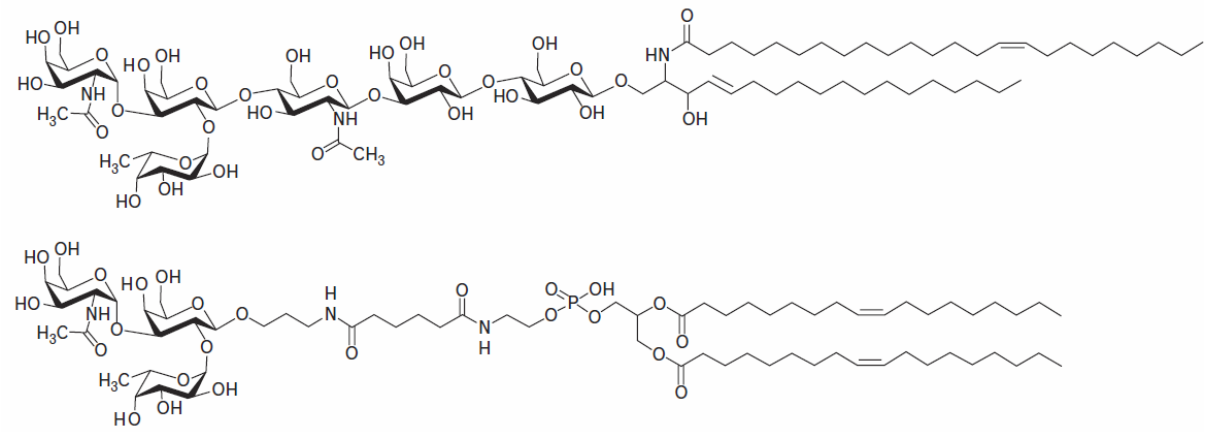
Figure 3. Examples of function-spacer lipid (FSL) constructs with analogy to flowers, where the functional group of the molecule is represented by the flower head, the spacer by the flower stalk, and the lipid by the flower root system . From left to right FSLs shown have functional groups of a simple peptide, a more complex peptide, biotin, biotin with a different lipid, and a tri-saccharide. (reproduced with permission)



The ability to have natural cells modified with FSL construct, gives a hitherto unseen ability to control number of antigens on the red cell surface and therefore to control the strength of a reaction. This is a critical improvement in blood group serology. KODE technology allows a selected antigen to be added to the red cell surface in controlled and reproducible amounts (Frame et al, 2007), thereby overcoming the problems inherent to variation in antigen copy number.

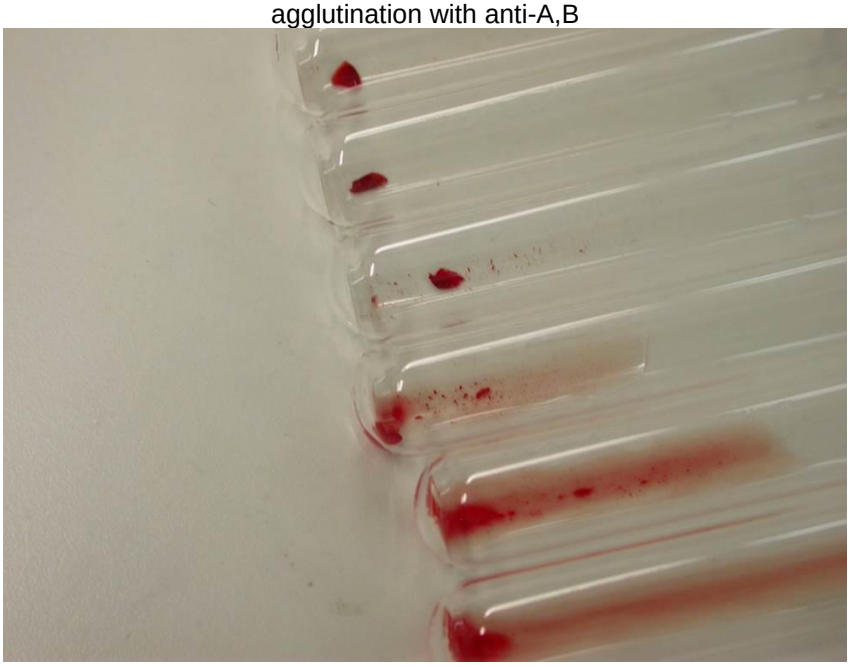
FSL-A antigen is one of many KODE constructs. Figure 4 (Henry, 2009) illustrates the difference between a natural A antigen (at the top of the figure) and an FSL-A (in the lower portion of the figure). The chemical nature of the epitope head which binds to the antigen binding portion of anti-A is the same, but the insertion of the spacer, and the lipid tail of dioleoyl-phosphatidyethanolamine, differentiate it from natural A antigen.

Figure 4. Natural glycosphingolipid A type 2 vs FSL-A(tri) (reproduced with permission)



Another KODE construct is one which consists of a mixture of synthetic blood group antigen A-like molecule FSL-A (FSL-A(GALNa3[Fa2]GALb)-SA1-L1) and B-like molecule FSL-B (FSL-B(GALa3[Fa2]GALb)-SA1-L1). When this is added to human group O red cells, it transforms the blood group of those O cells to AB. This is interesting in itself, but it is the aspect of control here that is important. By adding the A+B construct in different concentrations, a range of cells is created which express the A and B antigens in different amounts. Therefore, from one cell which is homogeneous in terms of serological reactions, many can be created, with a spectrum of grades. Figure 5 illustrates group O natural red cells modified with decreasing concentrations of FSL-A+B construct to produce a range of agglutination grades from strongly positive, through medium and weak positive, to negative. This allows students to practise grading a range of agglutination strengths.

Figure 5. Kodecytes of different A+B concentrations produce a range of different grades of agglutination with anti-A,B



KODE technology also reduces the infectivity risk of multiple donor samples. Although all donors are widely screened for blood borne infections, they could still be in the period between infection and sero-conversion known as the “window period”. Using a small pool of current group O blood donors who have donated for many years and been screened many times to create kodecytes constitutes a lower risk for students. Small volumes of these red cells can be used to create kodecytes for classroom teaching, thus utilising a precious resource effectively. KODE also addresses the problems of low availability of samples which would yield weak reactions. Instead of having to wait for these to become available at an unpredictable time, KODE technology is available on demand at any time.

The ability to add the FSL-A+B construct to O cells means researchers have been able to create serological mimics of the naturally occurring weaker subgroups of A and B (Frame et al., 2007; Hult et al., 2011). Laboratories can use such modified cells to teach recognition of subgroups and blood grouping anomalies and their resolution.

In 2004, and again in 2008, Royal College of Pathologists of Australasia (RCPA) included A+B kodecytes in a survey in which over 300 laboratories participated. Laboratories blind-typed samples including FSL-A-kodecyte (weak), (with serologic equivalence to a natural $A_{weak,}$ and expected to yield 1+ agglutination reaction) and FSL- A-kodecyte (medium), (expected to yield 1+/ 2+) agglutination reactions. Not surprisingly, techniques and testing platforms varied widely amongst the 300 laboratories, and included manual tube and tile, and manual and automated CAT and microplate technologies. Antisera used also varied widely.

Henry (2009) analysed the results of this longitudinal survey and results supported the need for serologist training in the recognition of weak and medium strength agglutination reactions. Less than 50% of participating laboratories correctly graded the A-kodecyte (medium) in both years, with errors showing a mixture of over and under-grading, and missing it completely and reporting as negative. Laboratories showed only marginal improvement between 2004 and 2008. In both years, more than 70% of participating laboratories missed the A-kodecyte (weak) (1+), reporting it as negative.

Quality surveys are a valuable quality assurance tool and required by most accreditation bodies accrediting medical laboratories, for example International Accreditation New Zealand (IANZ). The value of surveys where participants all test the same sample is that it can highlight inadequacies in technology, or individual scientist performance, or systemic failures. This can otherwise be difficult to detect, since a negative reaction in the absence of anomaly does not alert the operator to test failure. The missing of weak reactions in the RCPA survey is a good illustration of this principle.

The scarcity of medium and weak reactions to grade is a problem not only in universities, but also in practising laboratories. The results of the RCPA surveys (as cited by Henry, 2009) showed that there is a need for a tool for in-service training to allow practitioners to maintain competency in grading.

Thus KODE technology can be used in both the university and the practising laboratory setting, and students trained in the tool at university would already know how to apply it when they graduated. By exposing students in their university years, and then again in the laboratory to the KODE teaching tool, there may be a very seamless transition in learning in years to come. Henry (unpublished observations), extrapolated the learning from the Henry (2009) study to the university environment, believing that students might also benefit from practising such grading tasks.

The features of control and reproducibility of KODE technology have also allowed the development of quality control systems which have been adopted by some blood services throughout Australasia in a commercial product known as Securacell ®. Group O human red cells have been modified with known concentrations of synthetic A and B KODE FSL constructs, to produce cells expressing A and B antigens in controlled and varying amounts. Low copy numbers of A antigen can be used to ascertain the ability of an anti-A reagent to detect a subgroup of A (Frame et al., 2007). This is far superior to the traditional control of A_2. Although A_2 cells do contain a lower number of A antigens than A_1, they still contain a vastly higher number of A antigens compared to the number controllably added in the A_{weak} Securacell cell. Modern monoclonal antisera are both sensitive and specific, compared to traditional polyclonal blood grouping reagents, and A_2 cells no longer provide a valid control of an anti-A reagent's ability to detect a small copy number of A antigens. According to international guidelines (Franceso, 2003; James, 2005, as cited by Hult et al., 2011) monoclonal ABO typing reagents are required to be capable of detecting A_x and B_{weak} subgroup red cells. However, as A_x and B_{weak} are rare, most laboratories do not have access to them to check compliance of their antisera. Secondly, the weak subgroups A_x and B_x are poorly defined, and represent a range of cells from different individuals, so that the antigen copy number can vary by more than 200% (Daniels, 2002).

Using the serological KODE mimics instead overcomes this problem, as it is possible to use the FSL-A+B mixture, (or FSL-A or FSL-B singly) at a concentration to produce a cell which will react with the same weak serological grade as an A_x or B_{weak} cell. This is a far superior tool to check on the sensitivity and avidity of a typing reagent than an A_2 cell, which is sometimes used by a lab when A_x is not available.

Henry (personal communication 2014) found that an anti-A may be degraded to only 10% of original functional activity, and still yield a 4+ agglutination reaction with an A_2 cell, whereas the A_{weak} serological mimic had ceased to agglutinate with this reagent.

The FSL-A+B(tri) serologic teaching kit (KODE 109730-2-TUO) consists of a mixture of FSL-A and FSL-B antigen constructs, which allows anyone using the kit to prepare A+B kodecytes at different concentrations. Contributing to the research for this thesis, these kits have recently been used as survey samples in New Zealand to test the ability of practitioners to detect a range of serological grades with antisera (T. Shaw, personal communication, June 5, 2013). In this way, practitioner competence has been assessed in an internal quality survey. The kit had never been

assessed in a university teaching situation, so this study allowed the evaluation of this novel tool with students studying Transfusion Science at AUT.

The aims of this study were:

1. To develop a robust and reproducible serological-grading teaching tool, suitable for use in a university teaching environment
2. To ascertain if there was a measurable impact of kodecytes on grading outcomes in a university teaching environment
3. To determine ability of kodecytes to highlight grading problems in students
4. To evaluate participants' perceptions of the impact of kodecytes

Methods

Section A: Tool development

Preparation of Kodecytes

FSL - A+B (tri) serological teaching kit is an existing technology (KODE 109730-2-TUO). The kit contains a mixture of FSL blood group A and B trisaccharide construct designed for teaching/training purposes, mimicking natural subgroups of A and B with varying but controlled amounts of A and B antigens. The product contains a mixture of 0.5 mg of FSL-A (FSL-A(GALNa3[Fa2]GALb)-SA1-L1) and 1.5 mg of FSL-B (FSL-B(GALa3[Fa2]GALb)-SA1-L1). The kit was used to conceptualise and develop exercises to assess ability to grade tube agglutination reactions, based on the principle that different concentrations of FSL-A+B construct inserted into red cells, will give a controlled range of grades of agglutination.

Graphically illustrated in Figure 6, preparation of A+B kodecytes was as follows:

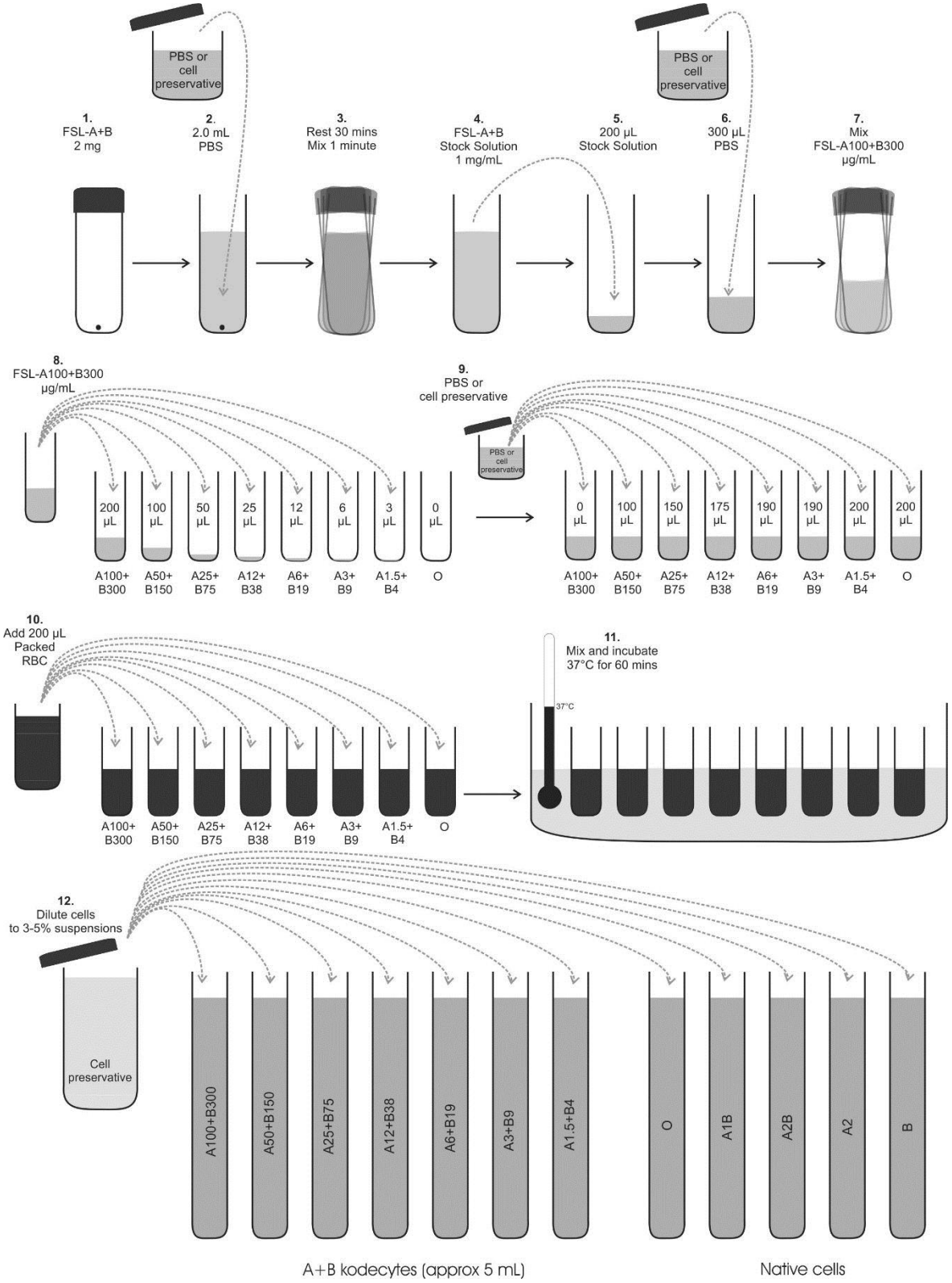
1. The vial containing the 2mg of FSL-A+B(tri) construct (KODE 109730-2-TUO) was thawed and opened
2. The construct was reconstituted to a 1 mg/mL stock solution by the addition of 2.0 mL of Celpresol (CSL 06332301)
3. The vial was allowed to rest for 30 minutes then vortexed for 60 seconds
4. 200 µL of 1 mg/mL construct was dispensed into a 3ml glass tube
5. The construct thus dispensed was labelled “stock solution”
6. 300 µL of Celpresol was added to create 500 µL of a working solution containing 100 µg/mL of FSL-A and 300 µg/mL of FSL-B (FSL-A100+B300)
7. The FSL-A100+B300 working solution was mixed by vortex for a few seconds
8. Volumes of the FSL-A100+B300 working solution were dispensed into eight glass tubes according to Table 2
9. Volumes of Celpresol were dispensed into the eight glass tubes according to Table 2. A tube into which no construct was added was also prepared as a process control (A0+B0). The final volume is approximately 200 µL in all tubes . The kit package insert (Henry & Perry, 2011) states “as the correction volume is non-reactive the delivery volume can be rounded to the nearest 10 µL volume”
10. 200 µL of washed hard-packed fresh group O red cells from a research-group volunteer was added to all tubes to create 50% suspensions
11. All tubes were mixed and incubated at 37°C for 60 minutes
12. Tube contents were transferred to 15ml plastic capped tubes, with contents rinsed with Celpresol to ensure maximum cell recovery. Celpresol was added to each tube to a final volume of 6ml mark to create 3% suspensions of A+B kodecytes. This series of A+B kodecytes were stored at 4°C for up to six weeks

Table 2. Preparation of working dilutions of FSL-A+B

Tube identifier	Volume µL FSL-A+B	Volume µL Celpresol	Total volume µL	[FSL-A] µg/ml	[FSL-B] µg/ml
A100+B300	200	0	200	100	300
A50+B150	100	100	200	50	150
A25+B75	50	150	200	25	75
A12+B38	25	175	200	12	38
A6+B19	12	190	202	5.7	18.1
A3+B9	6	190	196	3	9.4
A1.5+B4	3	200	203	1.5	4.5
A0+B0	0	200	200	0	0

The numbers used as tube identifiers in Table 2, and to name the kodecytes used in panels throughout the study, refer to the concentration in µg/ml of FSL-A and FSL-B respectively. For example “A100+B300” indicates a kodecyte that has been prepared with natural red cells modified with FSL-A+B containing FSL-A at 100 µg/ml and FSL-B at 300 µg/ml. It does not give any information about the copy number of A and B antigens; this is unknown. (S.M Henry, personal communication, February 2014)

Figure 6. Preparation of kodecytes.
The diagram represents the numbered steps of preparation



Reproducibility trials

To assess consistency of A+B kodecytes manufactured over the study, each batch was tested one week after manufacture. One week was chosen for convenience and standardisation. The monoclonal antibody reagents used to assess the kodecytes were the same throughout the study, and are listed in Table 3. Batches of kodecytes were tested with anti-A (MoAb-A3) and anti-B (MoAb-B3) by saline techniques in both tube and CAT. Results were compared to reference results. The reference range was as established by KODE Biotech for the CAT method, and by consistent results gained in this study for the tube technique (as no reference range existed before this study).

Table 3. Monoclonal antibody reagents

Abbreviation	Full Name	Manufacturer	Catalogue #	Lot #	Expiry Date*
MoAb-A1	Epiclone anti-A	CSL	02611305	026134801A	25/02/14
MoAb-A3	Epiclone anti-A	CSL	02611305	026134201A	18/08/2013
MoAb-B3	Epiclone anti-B	CSL	02661305	026634601B	17/12/2013
MoAb-AB1	Epiclone anti-AB	CSL	02671305	026728302B	08/10/2013
MoAb-D1	DiaMed anti-D	Diamed		20093.34.1	01/08/08

*No reagents were used past expiry date

The method for testing reproducibility in tube was as follows:

1. 5% cell suspensions of each kodecyte A100+B300 – A0+B0 were prepared by adding 50µl packed kodecyte to 950 µl Celpresol
2. Glass tube series were labelled with kodecyte and antisera identifiers
3. To each of the anti-A series, one drop (≈50µl) of MoAb-A3 was added
4. To each of the anti-B series, one drop (≈50µl) of MoAb-B3 was added
5. To both sets of tubes, one drop of appropriate kodecyte was added
6. Tubes were incubated at room temperature (≈20°C) for five minutes
7. Tubes were centrifuged at 450g for 15 seconds
8. Tubes were examined and grades were recorded for each tube
9. A pass/fail was awarded to each tube. A pass was defined as a grade that was no greater than 1 interval difference between the reference and the test sample

The method for testing reproducibility by CAT was:

1. 1.6% cell suspensions of each kodecyte were prepared by adding 10µl packed kodecyte to 615 µl Celpresol
2. CAT cards (NaCl, enzyme test and cold agglutinins cards (Biorad 005014) sufficient for all kodecytes to be tested with anti-A and anti-B, were labelled, where 1 kodecyte was tested against 1 antisera in each well
3. 50 µl anti-A was added to all wells where kodecytes were to be tested with anti-A
4. 50 µl anti-B was added to all wells where kodecytes were to be tested with anti-B
5. 50 µl of the appropriate kodecyte was added to all wells
6. Cards were centrifuges in Diamed ID 12SII on automatic (10 mins at 85g)
7. Reactions were graded according to published criteria (Nicol, 1998) and recorded
8. Results were compared to reference range. A pass was defined as a grade that was no greater than 1 interval difference between the reference and the test sample.

Section B: Study Design

Participants

Participant characteristics

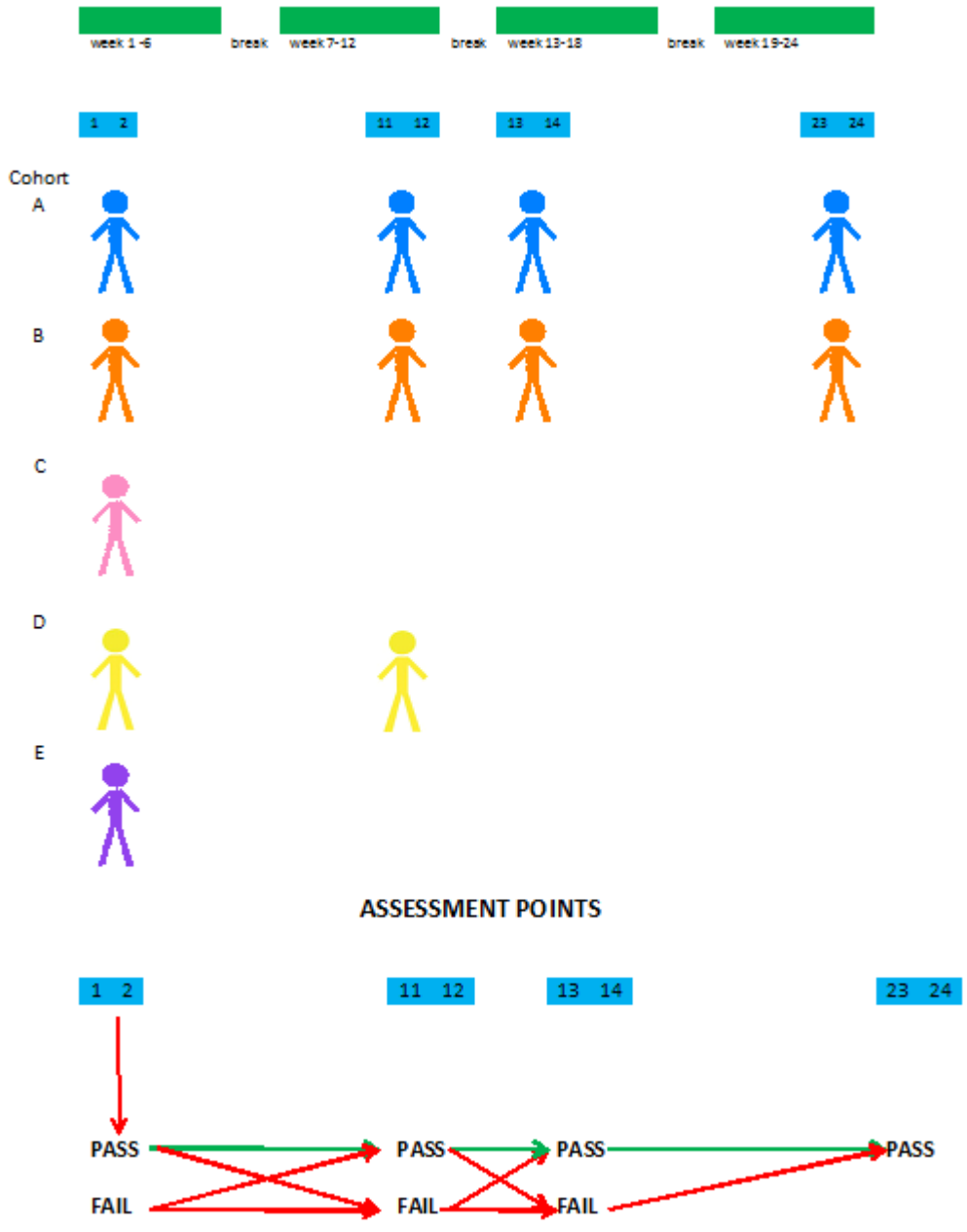
A total of 102 students and 21 practitioners were recruited to the study over a two-year period. The students were studying in Transfusion Science papers in either the second or third year of the AUT BMLS, or the Graduate Diploma in Science. The practitioners were recruited by two methods. Thirteen attended a one-day workshop at AUT, and used kodecytes in a variety of exercises. Eight practitioners from two of ten laboratories invited to participate in an electronic notice of the study consented, and consumables and instructions were sent to the participants in their own working laboratories.

Participants all started the study in the same way. They were then split into four cohorts, for the purpose of data analysis, based on two criteria:

- length of continuous enrolment in the study (1 week, 12 weeks or 24 weeks in two 12 week blocks). Length of study enrollment depended on the students' enrolment in varying university papers
- prior grading experience relative to kodecytes and natural (untreated) red cells (table 4)

Cohort criteria are shown in Table 4 and Figure 7. Figure 7 graphically depicts the participants and the nature of their participation. Cohorts of participants are represented in Figure 7 in different colours, to represent their different characteristics.

Figure 7. Characteristics, nature of participation and assessment of study participants



Participants in blue (cohort A) represent third-year BMLS students and Graduate Diploma students, who started the study with no experience of kodecytes. They stayed on the study for 24 weeks over two semesters. Cohort B (orange) are third year BMLS, with prior kodecyte experience and enrolled for 24 weeks. Pink (cohort C) are second year BMLS students, with no kodecyte experience, who used kodecytes only at the first assessment. Yellow (cohort D) are third year BMLS, with prior experience, who remained in the study for 12 weeks. Practitioners are represented in purple (cohort E). They had not experienced kodecytes, and were assessed at only one time point.

Table 4. Participant cohort criteria

Cohort	(n)	Prior grading with kodecytes	Prior grading with natural cells	# of weeks in contact with kodecytes	Student/ Practitioner	Date of enrolment (Y)
A	20		√	24	Student	2012 and 2013
B	7	√	√	24	Student	2013
C	75		√	1	Student	2012 and 2013
D	7	√	√	12	Student	2012 and 2013
E	21		√	1	Practitioner	2013

Ethics

Ethics approval from AUT Ethics Committee AUTEC (#11/323) is appended (Appendix 1) together with information sheets (Appendix 2), consent forms (Appendix 3) and questionnaire (Appendix 4). All participants were anonymised.

For students, participation in the teaching session was not optional. Participation in the research was voluntary, requiring informed consent. The distinction between the teaching and the research was the requirement for informed consent for inclusion of data in thesis and publications.

The researcher was also the teacher and head of programme for the students, and therefore held academic power. An independent academic from another Faculty performed all student recruitment, in the absence of the researcher. This person also held the student consent forms, and anonymised the data for the researcher, so that the researcher never knew which students had consented to participate.

Students were recruited during a laboratory class by the independent academic. All students in Transfusion Science papers experienced teaching with kodecytes, but participation in the research was voluntary. Giving of informed consent to have results included in this thesis and subesequent publications formed the distinction between teaching and research. Ninety percent of students gave consent.

Section C: Design of data collection exercises

Design

As stated in the introduction, the aims of this study were:

1. To develop a robust and reproducible serological-grading teaching tool, suitable for use in a university teaching environment
2. To ascertain if there was a measurable impact of kodecytes on grading outcomes in a university teaching environment
3. To determine ability of kodecytes to highlight grading problems in students
4. To evaluate participants' perceptions of the impact of kodecytes

This study was designed to provide both cross-sectional data (all cohorts) and longitudinal data (cohorts A, B, D). One standardised exercise, hereafter known as "the grading exercise", was performed by all cohorts, a variable number of times.

When testing the impact of an experimental invervention, it is normal to have an intervention group who receive the intervention (in this case teaching with kodecytes) and a control group who do not receive the intervention (Clark-Carter, 2005). This raised ethical issues in this study; knowing that teaching is likely to improve performance, and performance affects employment opportunity, it was not deemed ethical to form a separate control group amongst the students. Cohort C was broken into two subgroups by random split into intervention group (n = 38) and control group (n = 37). The split in each of years 2012 and 2013 occurred by the laboratory stream in which the students self selected upon enrolling into their degree papers (with one stream in each year forming the control group and the other the intervention group). Intervention and control group performed the grading exercise twice in one teaching session. The control group was presented with 12 kodecytes and natural cells to grade (test 1). They were then presented with a further 12 kodecytes and natural cells to grade (test 2). After both exercises had been completed the students received their feedback.

In the intervention group, students also received 2 sets of 12 kodecytes and natural cells to grade. However, they received their feedback after performing test 1, and before performing test 2, and so were informed of the results, and had a chance to evaluate their skills before attempting the second set. In this way, the control group still had the potential to benefit from the teaching experience, but formed a non-intervention group.

There was not the same ethical issue with practitioners and the group who attended the one-day workshop were randomly split into two groups by the laboratory bench where they independently chose to sit at the beginning of the day.

Participants in each of longitudinal cohorts A, B and D performed the grading exercise weekly over 1, 12 or 24 weeks. For students completing Transfusion Science papers in semester one, weeks 11-12 are the end of the learning as far as weekly grading is concerned. For students

advancing to higher-level Transfusion Science papers, weeks 11-12 and 13-14 are the mid-point of the learning, and weeks 23-24 the end. It was important to collect data at weeks 13-14 as well as 11–12, because there is a five week break in learning between these two time points, whilst students have their examination and mid-semester break.

Participants in cross-sectional cohorts C and E performed the grading exercise twice in one day.

The Grading Exercise

The grading exercise was performed by tube method. The researcher selected a panel of 12 kodecytes and/or natural cells in categories of negative, weak, medium and strong agglutination, to form a panel to present to participants in random orders. The cell pool from which these cells were selected is shown in Table 5. Selection of cells in each of the categories of negative, weak, medium and strong agglutination was based on an attempt to have an approximately equal number in each category of aggluination strength by the end of the study.

Known control cells (A_1, A_2, B and O) were also supplied, as it is normal practice to include samples with known expected results, to ensure a technique is working on a particular day in a particular set of conditions.

Participants were blinded to the identity of the 12 panel cells.

It was decided in the interests of time, resources and standardisation to test the cells only against anti-A, rather than both anti-A and anti-B. Over the 2 years of the study, the monoclonal anti-A (MoAb-A1) came from 3 different batches, but was the same clone formulated to the same specification.

Table 5. Cell and agglutination categories

Tube identifier	Expected grade (tube) with anti-A	Agglutination strength	
		kodecyte	natural cell
A100+B300	4+	Strong	
A50+B150	4+	Strong	
A25+B75	3+	Strong	
A12+B38	2+	Medium	
A6+B19	1+	Weak	
A3+B9	0	Negative	
A1.5+B4	0	Negative	
A0+B0	0	Negative	
A1	4+		Strong
A1B	4+		Strong
A2	4+		Strong
B	0		Negative
O	0		Negative

The researcher (an experienced serologist) followed the same standard protocol of the grading exercise each time and revealed her results to the participants for them to mark against. In order to reduce bias by the researcher in reading the kodecyte reactions, the researcher covered the identity of the kodecytes or natural cells on the worksheet, thus blinding herself to the results before grading her set of tubes.

Two sample panels with typical grades are shown in Table 6.

Table 6. Two examples of typical teaching panels with natural cells & kodecytes interspliced, tested with anti-A

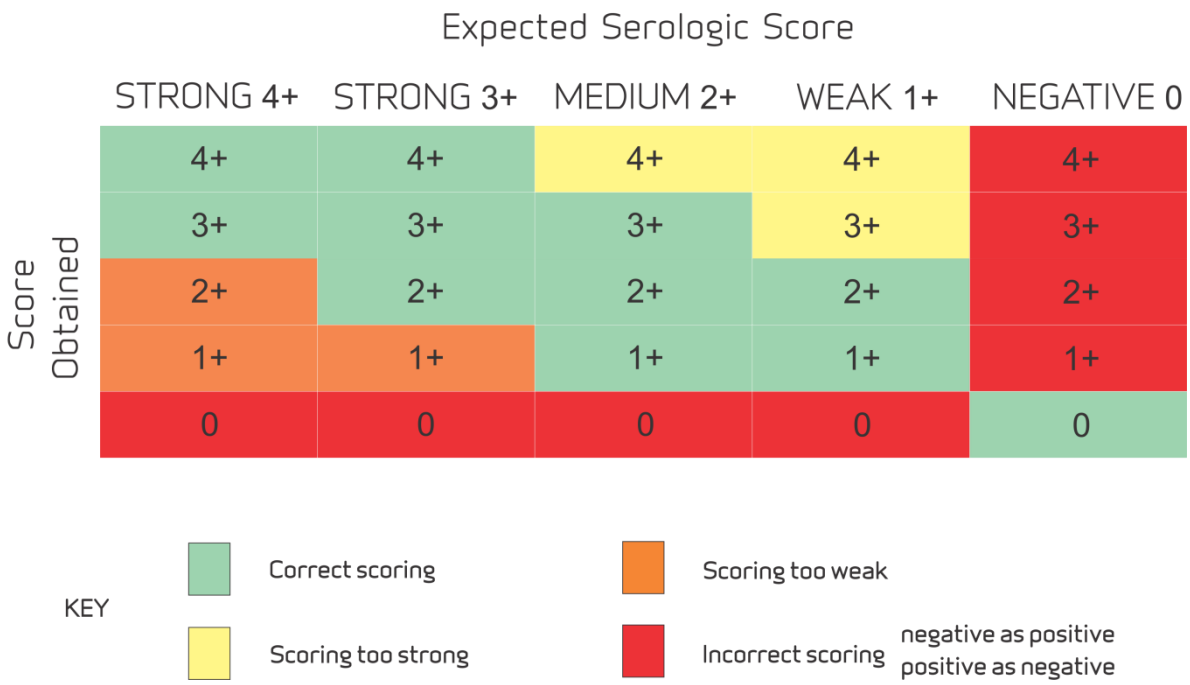
Panel A	negative	strong	strong	weak	negative	medium	strong	negative	strong
Panel cell	O	A_2	A100 B300	A6 B19	B	A12 B38	A25 B75	O	A100 B300
Typical grade	0	4+	4+	1+	0	2+	3+	0	4+

Panel B	strong	strong	strong	weak	weak	weak	weak	negative	negative
Panel cell	A100 B300	A100 B300	A50 B150	A6 B19	A6 B19	A6 B19	A6 B19	A0 B0	A0 B0
Typical grade	4+	4+	4+	1+	1+	1+	1+	0	0

The grading exercise method followed by all cohorts was as follows:

1. Participants received 12 unknown kodecytes and/or natural cells in tubes labelled 1 to 12 , and four known control cells labelled A_1, A_2, B and O
2. Participants also received a standard testing protocol (Appendix 5) and a bottle of monoclonal anti-A (MoAb-A1)
3. Participants added 1 drop of MoAb-A1 to each tube
4. Participants incubated all tubes for a timed five-minute period at room temperature
5. Participants centrifuged the tubes for 15 seconds at 447g in a Diacent 12 centrifuge
6. Using a 10x magnification eyepiece, participants graded and recorded all results on their worksheet, and handed a copy of their results to the researcher. Grading scale 0-4+ (Figure 1) was used.
7. Participants self-marked their results against the criteria shown in Figure 8

Figure 8. Criteria for students self-marking against results of an experienced serologist



An attempt was made to minimise variables other than grading ability, such as lighting, magnification and centrifugation speed. Overhead lighting was fluorescent, with the same number of lights turned on each week. A standard set of 10x magnification eyepieces was used. The same make of centrifuge (Diacent 12, Diamed, Cressier, Switzerland) was used by each student each week. Reading technique with an eyepiece is in itself a variable (for example the degree to which the tube is shaken to resuspend the cell button will influence the result). In this study, all students were taught a standardised technique by the experienced serologist. Acquisition of this skill is taught in the student classes as part of the normal teaching programme, and students enrolled in this study had already mastered that skill prior to enrollment. Whilst no formal attempt was made to assess reading technique, observation suggested a high degree of standardisation of students' technique.

Kodecyte grades were clustered as; strong (grade 3+ and 4+), medium (grade 2+), weak (grade 1+). An acceptable result for the expected medium or strong grades was "no greater than one grade from the grade reported by the experienced serologist". An acceptable grade for an expected weak positive reaction was either a 1+ or 2+. Only a negative grade was acceptable for true negative reactions. An error was defined as falling outside these criteria.

Data Analysis

Simple numerical data were counted and converted to percentages to show the ratios of students correctly or incorrectly grading each category of natural cell or kodecyte. This was done collectively for combined student cohorts A, B C and D to give a single "shapshot" view with large data numbers.

Statistical methods

To ascertain if there was a measurable impact of kodecytes on grading outcomes (research aim 2 as stated on page 30), two statistical questions were posed:

1. Is the difference between test one and test two in cohorts statistically significant? Results from cohorts C and E were suitable to study for this research question, as they had no prior exposure to kodecytes, and were split into intervention and control groups for the exercise.
2. Is there a statistically significant difference between students' ability to grade in individual students over time?

Data used to answer each of these questions are summarised in Table 7:

Table 7. Summary of statistical questions linked to cohort and statistical tool

Statistical question	Cohort	Contact weeks	Statistical Test	Significance level	Type of analysis
1.	C	1	Fisher's exact	$p \leq 0.05$	Cross sectional
1.	E	1	Fisher's exact	$p \leq 0.05$	Cross sectional
2.	A	1-2 11-12 13-14 23-24	Fisher's exact	$p \leq 0.05$	Longitudinal

In each data week analysed, results for positive grades (kodecytes A100/B300, A50/B150, A25/B75, A12/B38, A6/B19 and natural cells A_1, A_1B and A_2) and negatives (kodecyte A0/B0 and natural cells O and B) were analysed separately

Fisher's exact test was applied to data from cohort C's test one, compared to their test two results. Results for combined grades of positive agglutination were considered separately from negatives. Fisher's exact test was also applied to data from cohort A's results at different time points to test if there was significant difference between percentage of grades correct at weeks 1- 2 compared to weeks 11-12, weeks 11-12 compared to weeks 13-14, and weeks 13-14 compared to weeks 23-24. Weeks 1-2 represent the beginning of the semester and the learning experience.

To determine ability of the use of kodecytes to highlight grading problems in students, subsets of data were examined. A subset of students from cohort A was identified as initially having had problems with grading. This was defined as having ≥3 errors in the first 2 weeks. As students graded 12 tubes per week, in two weeks most had graded 24 reactions, and ≥3 errors represented just over a 10% error rate.

The data was separated into weeks 1-2, 11-12, 13-14 and 23-24 and Fishers exact test was applied to see if there was a statistical difference between time points in completing the study.

Survey design

To evaluate participants' perceptions of kodecytes, a questionnaire (Appendix 4) was given to cohorts A, B, D, E and the 2012 occurrence of cohort C. Participants rated their own technical performance and confidence level, before and after the use of kodecytes, on Likert scales, where 1 equalled poor and 5 equalled excellent. Likert scales are involved in research that employ questionnaires (Likert, 1932).

Data from Likert scales was counted for cohorts of students and practitioners, to provide quantitative analysis of participant perception of kodecytes.

Participants were also asked to reflect on the question "Do you consider kodecytes to be a valuable teaching tool? Please explain why or why not". To analyse this feedback, numbers of participants answering "Yes" or "No" were counted, and open comments analysed for qualitative themes.

Results

Section A: Tool development

Reproducibility trials

Ten batches of A+B kodecytes prepared over a two-year period by the method described produced highly reproducible results when tested with anti-A and anti-B in both tube and CAT. Photographs of typical CAT results are shown in Figures 9 and 10. Of a total of 10 times the kodecytes were prepared, reproduciblity trials were performed eight times and passed seven times. When assessed in tube, kodecyte panels met the criteria for consistency of grade 98% of the time (125 of 128 reactions). There were three grading discrepancies, or reproduciblity failures, as shown in Table 8. In CAT (Table 9), kodecyte panels met the criteria for consistency of grade 98% of the time (118 of 120 reactions) and showed two reproduciblity failures.

Reproducibility trial failures did not affect the study, as in each exercise participant gradings were compared to the experienced serologist's gradings, not to the expected grade of each strength of kodecyte. The failures would probably be due to errors in preparing dilutions rather than failure of the FSL construct. As seen, all five failures were the endpoint of the titre (although batch 8, tube 9 did have lower overall values).

Figure 9. Result of reproducibility trial of A+B kodecytes with anti-A in CAT

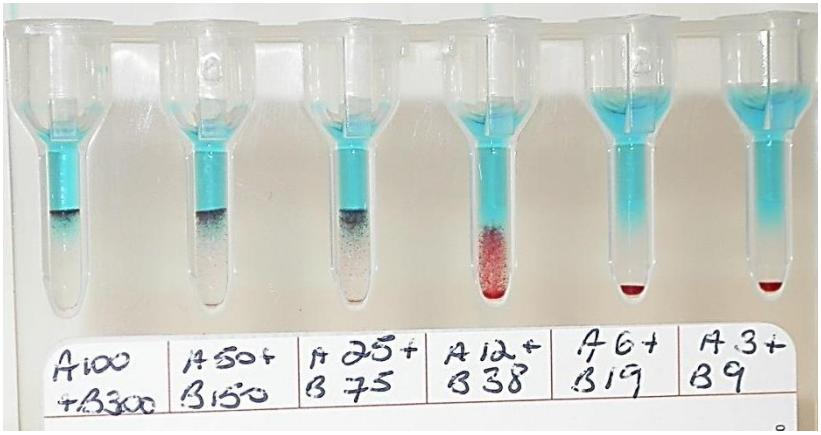


Figure 10. Result of reproducibility trial of A+B kodecytes with anti-B in CAT

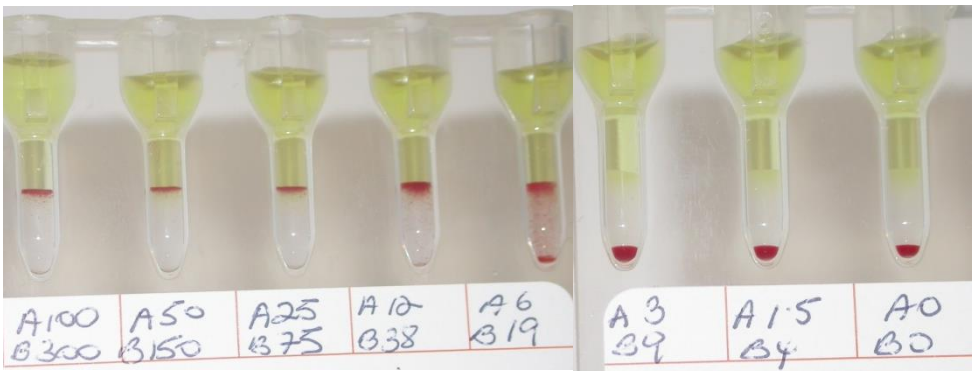


Table 8. Reproducibility trial results of A+B kodecytes in tube

Antisera	kodecyte	Reference grade	Batch grades for each of 8 batches								PASS/FAIL
			Batch #								
			1	2	3	4	5	6	7	8	
anti-A	A100+B300	++++	++++	++++	++++	++++	++++	++++	++++	++++	PASS
MoAb-A3	A50+B150	++++	++++	++++	++++	++++	++++	+++	++++	++++	PASS
	A25+B75	+++	+++	+++	++++	+++	++++	++++	+++	++++	PASS
	A12+B38	++	+++	+++	++	++	+++	++	++	+++	PASS
	A6+B19	+	++	+	±	+	++	+	0	++	PASSS
	A3+B9	0	±	0	0	0	0	0	0	0	PASS
	A1.5+B4	0	0	0	0	0	0	0	0	0	PASS
	A0+B0	0	0	0	0	0	0	0	0	0	PASS
anti-B	A100+B300	+++	+++	+++	++	++	+++	+++	+++	++++	PASS
MoAb-B3	A50+B150	+++	++	+++	++	++	++	++++	+++	++++	PASS
	A25+B75	++	++	++	++	++	+	++	++	+++	PASS
	A12+B38	++	+	++	++	±	+	+	++	0	PASS x 6 FAIL x2
	A6+B19	+	±	+	+	0	±	0	+	0	PASS
	A3+B9	0	0	0	0	0	0	0	0	0	PASS
	A1.5+B4	0	0	0	0	0	0	0	0	0	PASS
	A0+B0	0	0	0	0	0	0	0	0	0	PASS

PASS= the same as, or no more than one grade different, to the reference grade

++++ = 4 , +++ = 3, ++ = 2, + = 1, ± = weak positive, 0 = negative

Table 9. Reproduciblity trial results of A+B kodecytes in CAT

Antisera	Kodecyte	Reference grade	Batch grades for each of 8 batches								PASS/FAIL
			Batch #								
			1	2	3	4	5	6	7	8	
anti-A	A100+B300	4(++++)	++++	++++	++++	++++	++++	++++	++++	++++	PASS
MoAb-A3	A50+B150	4(++++)	++++	++++	++++	++++	+++	++++	++++	++++	PASS
	A25+B75	3(+++)	++++	++++	++++	+++	++++	+++	+++	+++	PASS
	A12+B38	2(++)	+++	+++	+++	++	+++	++	++	++	PASS
	A6+B19	0/1(+)	0	++	++	0	+	±	0	0	PASS
	A3+B9	0	0	0	0	0	0	0	0	0	PASS
	A1.5+B4	0	0	0	0	0	0	0	0	0	PASS
	A0+B0	0	0	0	0	0	0	0	0	0	PASS
anti-B	A100+B300	4(++++)	++++	NT	++++	++++	++++	++++	++++	++++	PASS
MoAb-B3	A50+B150	4(++++)	++++	NT	++++	++++	++++	++++	++++	+++	PASS
	A25+B75	3(+++)	++++	NT	++++	+++	+++	++++	+++	++	PASS
	A12+B38	3(+++)	++	NT	+++	++	+++	++	0	0	FAIL x2
	A6+B19	0/1(+)	0	NT	++	0	+	0	0	0	PASS
	A3+B9	0	0	NT	0	0	0	0	0	0	PASS
	A1.5+B4	0	0	NT	0	0	0	0	0	0	PASS
	A0+B0	0	0	NT	0	0	0	0	0	0	PASS

PASS= the same as, or no more than one grade different, to the reference grade

++++ = 4 , +++ = 3, ++ = 2, + = 1, ± = weak positive, 0 = negative

Section B: Data Analysis

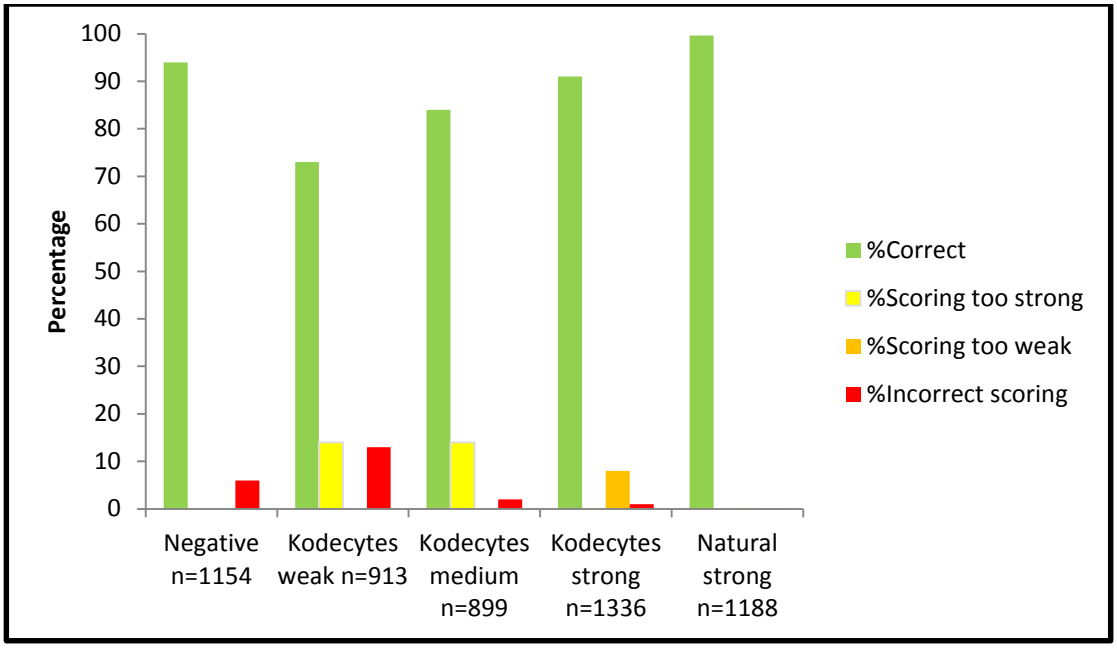
Statistical Analysis

Giving an overview of the total data from cohorts A, B, C and D, Table 10 and Figure 11 show the percentages of students correctly or incorrectly grading each category of natural cell or kodecyte in cohorts A, B, C and D. The data numbers reflect the number of results in each category, not the number of students. Where data numbers are different, this is because not all students in a cohort were present in all weeks. Additionally, different kodecytes were used each week.

Table 10. Results of student cohorts A, B, C, D grading all categories of kodecytes and natural cells

Cell type	Correct grading		Grading too strong		Grading too weak		Incorrect grading		
	n	%	n	%	n	%	n	%	Total
negative	1085	94					69	6	1154
kodecytes weak	667	73	125	14			121	13	913
kodecytes medium	753	84	128	14			18	2	899
kodecytes strong	1221	91			104	8	11	1	1336
natural strong	1184	99.6			2	0.2	2	0.2	1188

Figure 11. Students correctly or incorrectly grading each category of natural cell or kodecyte. The figure shows the data from Table 10 in graphical form. Definitions of scoring too strong, scoring too weak, and incorrect scoring are depicted in Figure 8.



In this large data set, problems were not apparent with strong, or negative reactions. Students graded both strongly agglutinating natural cells, and strongly agglutinating kodecytes correctly more than 90% of the time. Students correctly graded negatives 94% of the time.

Problems were revealed when grading weak and medium strength agglutination reactions. Students over-graded medium (2+) reactions 14% of the time, and graded them as negative 2% of the time. With weak reactions, they over-graded 14% of the time, and graded them as negative 13% of the time. As can be seen in Figure 11, the weaker the reaction, the lower the correct grading rate.

As stated in the methods, research aims were formulated. One of these aims was to ascertain if there was a measurable impact of kodecytes on grading outcomes in a university teaching environment. To address this aim, the statistical question was posed; *Is the difference between test 1 and test 2 in cohorts C and E statistically significant?* As stated earlier, the control group was presented with 12 kodecytes and natural cells to grade (test 1). They were then presented with a further 12 kodecytes and natural cells to grade (test 2). After both exercises had been completed the participants received their feedback. In the intervention group, participants also received 2 sets of 12 kodecytes and natural cells to grade. However, they received their feedback after performing test 1, and before performing test 2, and so were informed of the results, and had a chance to evaluate their skills before attempting the second set. Cohort C consisted of students, and cohort E of practitioners.

Fisher's exact test applied to cohort C showed no statistical significance between test 1 and test 2 in the intervention group when grading positives. In the control group, there was a statistically significant improvement between test one and test two. However, this was not due to the kodecyte teaching intervention, as this group did not have the intervention. The improvement must have been due to chance or some other influence (proving the null hypothesis) (Table 11).

Table 11. Fisher's exact test to assess statistical significance between intervention and control groups when grading positives (cohort C)

Cohort C	Intervention group		Control group	
	Positives Correct [1]	Positives Incorrect[2]	Positives Correct [1]	Positives Incorrect[2]
Test 1	302 (82%)	68 (18%)	238 (79%)	62 (21%)
Test 2	304 (86%)	50 (16%)	243 (86%)	40 (14%)
	p = 0.13		p = 0.04	

1. number of times students graded correctly
2. number of times students graded too weak, too strong, or incorrectly

Therefore the conclusion is that for grading positives (1+ and 2+ and 3+ and 4+), the intervention did not make a difference to students' ability to grade.

However, we know from both published data (Henry, 2009) and unpublished observations in teaching the students that weak and medium grades of positive present more of a challenge to grade. Therefore the analysis above was repeated with only weak positives instead of all positives, and showed very significant improvement in the intervention group of cohort C students (p=0.01) and no significant improvement in the control group (p=0.38) (Table 12).

Table 12. Fisher's exact test to assess statistical significance between intervention and control groups when grading weak positives (cohort C)

Cohort C	Intervention group		Control group	
	Weak Positives[1] Correct	Weak Positives[1] Incorrect	Weak Positives[1] Correct	Weak Positives[1] Incorrect
Test 1	40 (54%)	34	34 (54%)	28
Test 2	95 (73%)	36	46 (65%)	27
	p = 0.01		p = 0.38	

1. kodecytes grading 1+

When weak and medium grades were combined very significant improvement was still demonstrated in the intervention group of cohort C (p=0.01), and no significant improvement in the control group (p=0.37) (Table 13)

Table 13. Fisher's exact test to assess statistical significance between intervention and control groups when grading weak and medium positives (cohort C)

Cohort C	Intervention group		Control group	
	Weak and medium positives[1] Correct	Weak and medium positives[1] Incorrect	Weak and medium positives[1] Correct	Weak and medium positives[1] Incorrect
Test 1	71	45	36	28
Test 2	133	41	75	41
	p = 0.01		p = 0.37	

[1] kodecytes grading 1+, 2+

Therefore the conclusion is that the intervention of teaching with kodecytes is effective in teaching students to grade weak and medium positive reactions.

With negatives there was no significant difference between test 1 and test 2 in either intervention or control group (Table 14).

Table 14. Fisher's exact test to assess statistical significance between intervention and control groups when grading negatives (cohort C)

Cohort C	Intervention group		Control group	
	Negatives Correct	Negatives Incorrect	Negatives Correct	Negatives Incorrect
Test 1	72 (97%)	2 (3%)	58 (97%)	2 (3%)
Test 2	93 (98%)	2 (2%)	68 (93%)	5 (7%)
	p = 1.000		P = 0.46	

In practitioners (cohort E) the intervention group showed significant increase in correct grading between test 1 and test 2 (p = 0.03) when grading positive reactions, whereas the control group did not show a difference between the two tests (p = 0.49) (Table 15).

This suggests that the intervention is effective in practitioners when grading positive reactions. Numbers were too small to separate weak positives from the total positives in this cohort.

Table 15. Fisher's exact test to assess statistical significance between intervention and control groups when grading positives (cohort E)

Cohort E	Intervention group		Control group	
	Positives Correct	Positives Incorrect	Positives Correct	Positives Incorrect
Test 1	36	13	46	3
Test 2	45	4	43	6
	p = 0.03		P = 0.49	

There was no significant difference between test 1 and test 2 in either control group (p = 0.75) or intervention group (p = 1.00) when practitioners graded negative reactions (Table 16).

Table 16. Fisher's exact test to assess statistical significance between intervention and control groups when grading negatives (cohort E)

Cohort E	Intervention group		Control group	
	Negatives Correct	Negatives Incorrect	Negatives Correct	Negatives Incorrect
Test 1	32	3	28	7
Test 2	32	3	30	5
	p = 1.00		P = 0.75	

In answer to the statistical question; *"Is there a statistically significant difference between students' ability to grade in individual students over time?"* data are presented in Table 17.

Table 17. Percentages of students correctly grading each category of natural cell or kodecyte at different time points. (cohorts A, B and D)

			negative		kodecyte weak		kodecyte strong		natural strong	
Students (n)	weeks		√	X	√	X	√	X	√	X
34	1 -2	n	198	10	91	37	86	20	101	1
		%	95	5	71	29	81	19	99	1
34	11 - 12	n	175	17	205	30	134	12	70	0
		%	91	9	87	13	92	8	100	0
27	13 - 14	n	109	11	68	40	108	6	63	0
		%	91	9	63	37	95	5	100	0
27	23-24	n	139	6	43	13	79	3	77	0
		%	96	4	77	23	96	4	100	0

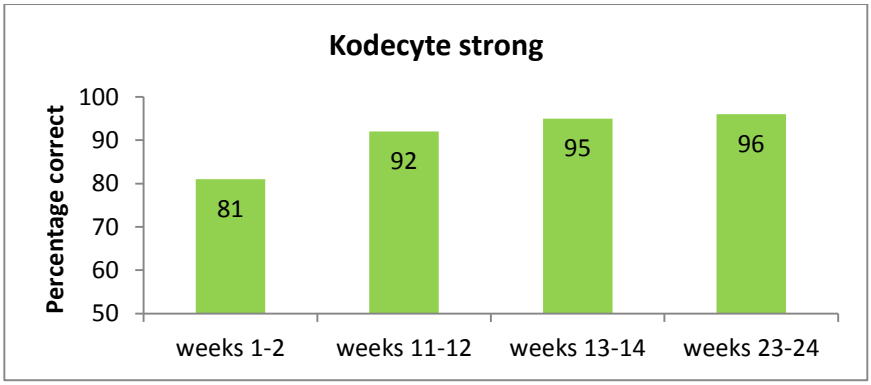
√ = correct, X = incorrect (too weak, too strong or incorrect)

As shown in Table 17 and Figure 12, students were grading correctly natural cells showing strong agglutination ≥99% of the time from week one, and negatives ≥90% of the time from week one.

Students showed steady improvement with grading of strong kodecytes, starting with a success percentage of 81% in weeks 1-2, and ending with a success percentage of 96% at

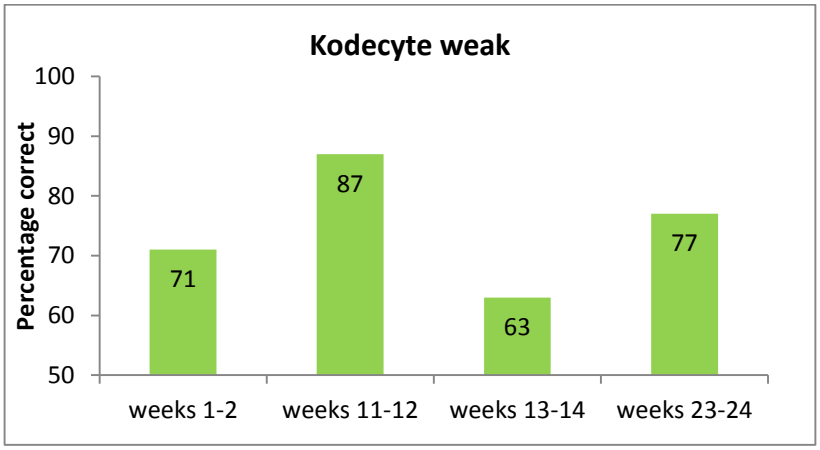
the end of the study (p=0.0014). Significant improvement was seen in the first 12 weeks (when comparing weeks 1-2 with weeks 11-12 p= 0.02). The improvement between weeks 11-12 and weeks 13-14, and between weeks 13-14 and weeks 23-24 was not significant (p values of 0.46 and 0.74). But the improvement overall was very significant (p=0.0014).

Figure 12. Percentage of students correctly grading strong kodecytes at different time points. (cohorts A, B and D)



With weak kodecytes, students improved from 71% correct (weeks 1-2) to 87% (weeks 11-12), then declined to 63% (weeks 13-14), then improved again to 77% (weeks 23-24). (Table 17 and Figure 13)

Figure 13. Percentage of students correctly grading weak kodecytes at different time points. (cohorts A, B and D)



The improvement between weeks 1-2 and weeks 11-12 was significant (p=0.0002). The decline between weeks 11-12 and weeks 13-14 was also significant (p=<0.0001). There was no significant change when comparing weeks 1-2 with weeks 23-24 (p=0.48) (the start of the study compared to the end of the study).

Medium kodecytes are presented in Table 18, (and not Table 17) as only cohort A used them in weeks 1-2. Nor did cohort A use medium kodecytes in large enough numbers beyond week 12 to be included in the data analysis. These were oversights in study design. Therefore data is presented only from cohort A in weeks 1-12, and weeks 11-12.

Table 18. Percentage of students correctly grading medium kodecytes at two time points. (cohort A, 20 students)

	kodecytes medium			
Weeks	Correct		Incorrect	
	n	%	n	%
1 - 2	88	86	14	14
11 - 12	32	89	4	11

Students showed no significant change from 86% correct (weeks 1-2) to 89% (weeks 11-12) ($p=0.57$).

Research Aim 3) Can use of kodecytes highlight grading problems in students?

Seven of 20 students (35%) in cohort A were found to have made three or more errors in the first two weeks. Their progress across either 12 weeks (students # 89 and 98) or 24 weeks (students #5, 43, 44, 83, 84 and 98) are presented in Table 19.

Table 19. Subset of students with early grading difficulties

			Number and percentages of errors						
			Student anonymised #						
			5	43	44	83	84	89	98
Initial error type			↑→	↑→	↑↓←	↑	↑↓→	↑↓	↓
weeks	1-2	n	6/24	3/24	4/24	4/24	3/24	4/24	3/24
		%	25	13	17	17	13	17	13
	11-12	n	3/24	2/24	4/24	2/24	4/24	3/22	2/24
		%	12	8	17	8	17	14	8
	13-14	n	4/23	1/23	5/23	23/23	5/23	NA	NA
		%	17	4	22	0	22		
	23-24	n	1/22	3/22	2/22	1/22	1/22	NA	NA
		%	4	14	9	4	4		
Outcome			√	=	=	=	=		
p value weeks (1-2) vs weeks (23-24)			0.09	0.70	0.67	0.35	0.61		

↑ grading too strong
↓ grading too weak
↑↓ gradings both too strong and too weak
→ grading negative as positive
← grading positive as negative
√ outcome: grading improvement
= outcome: no change
NA Data not available

Of the seven students with initial grading problems, the five who stayed in the study for 24 weeks, all improved their performance. However, as individuals, their improvement did not reach statistical significance (Table 19).

To test the power of the tool to effect improvement in the group of students with initial grading problems, Fisher's exact test was applied to data from weeks 1-2, versus data from weeks 23-24 for all five students. Results are shown in Table 20. The p value of 0.02 supports the result that the tool is effective in improving performance in a group of students when their results are considered collectively.

Table 20. Fisher's exact test on combined data for five students with initial grading problems

Period	Number correct	Number incorrect
Weeks 1-2	98	22
Weeks 23-24	102	8
	p=0.02	

Thirteen of the 20 students did not have grading problems in weeks 1-2 (that is they made less than three errors in the first two weeks). Nine of these 13 maintained their correct grading rate at 90% or greater throughout the 24 weeks of the study. Of the four students with no initial grading problems, who developed grading problems in later weeks, two showed a significant decline in their grading ability (Table 21).

Table 21. Grading trends of students without initial problems, who developed grading problems over 24 weeks.

Number and percentages of errors						
			Student anonymised #			
			470	420	530	570
Error type developed			↑	↑	↓←	↑
Weeks	1-2	n	2/13	1/13	2/13	0/13
		%	15	8	15	0
	11-12	n	0/24	2/24	5/24	1/24
		%	0	8	21	4
	13-14	n	3/20	0/20	1/20	2/20
		%	15	0	5	10
	23-24	n	5/16	7/16	2/16	5/16
		%	31	44	13	31
Outcome			=	-	=	-
p value weeks (1-2) vs weeks (23-24)			0.41	0.04	1.00	0.05

↑ grading too strong
↓ grading too weak
← grading positive as negative
= outcome: no change
- outcome: grading decline

Questionnaire Analysis

Research Aim 4. What are participants' perceptions of the impact of kodecytes?

In all participants, self- perceptions of both technical performance and confidence improved after using kodecytes. Figure 14 and 15 show the graphed response to the questionnaire instruction *"Please reflect on your technical performance before and after the exercises with the kodecytes. Indicate on the scale your perceived ability to grade serologic tube reactions before and after the kodecyte exercises. A score of 1 means you think your grading ability is poor. A score of 5 means you think your grading ability is excellent. You may mark yourself anywhere on the scale".*

Figure 16 and 17 show the graphed response to the questionnaire instruction *"Please reflect on your confidence level in grading before and after the exercises with the kodecytes. Indicate on the how confident you felt about grading serologic tube reactions before and after the kodecyte exercises. A score of 1 means you have low confidence in your ability. A score of 5 means you have high confidence in your ability".*

Figure 14. Perception of own **technical performance** by **students** before and after using kodecytes. Perception was self-measured on a Likert scale, where 1 = poor, and 5 = excellent self-perceived grading ability.

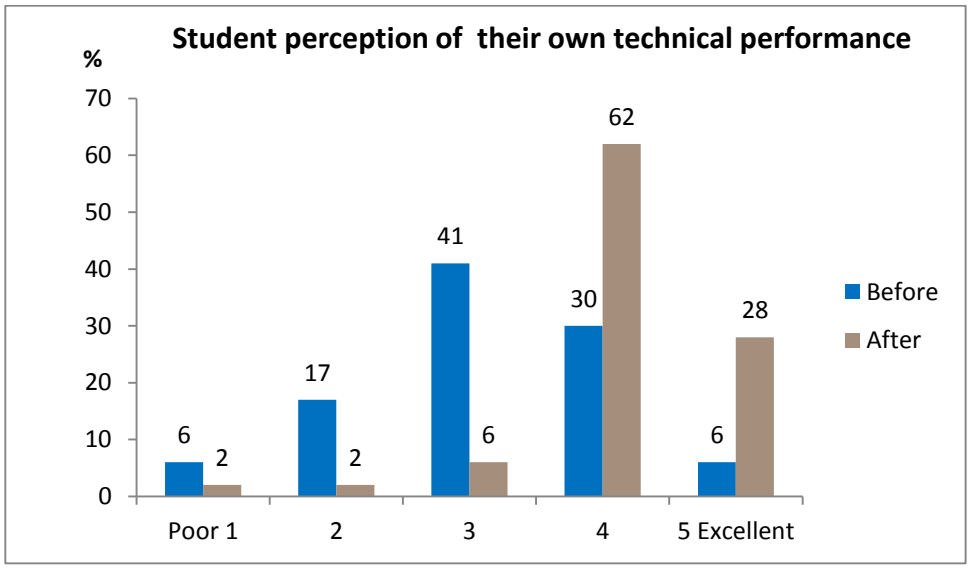


Figure 15. Perception of **technical performance** in **practitioners** before and after using kodecytes. Perception was self- measured on a Likert scale, where 1 = poor, and 5 = excellent self-perceived grading ability.

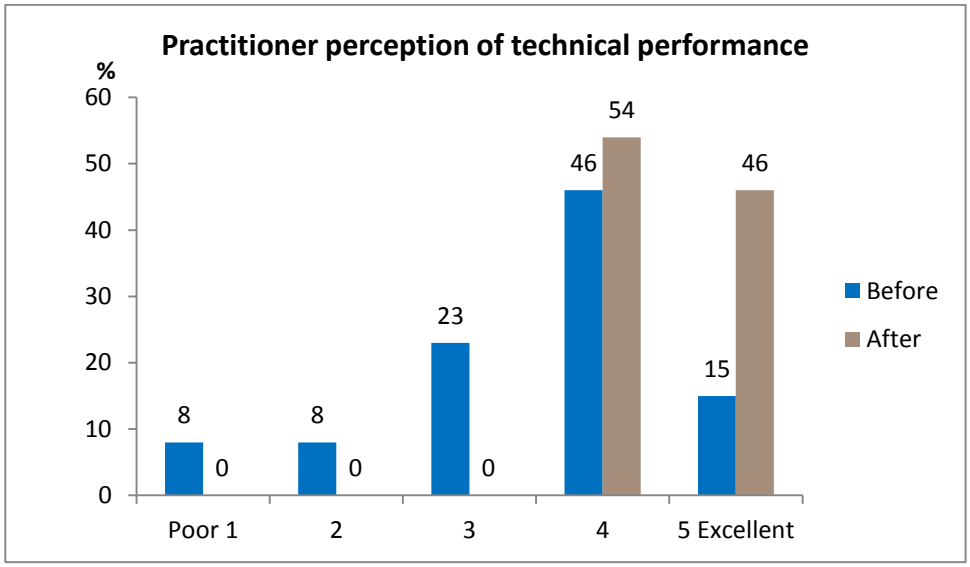


Figure 16. Perception of **confidence level** in **students** before and after using kodecytes. Perception was self- measured on a Likert scale, where 1 = poor, and 5 = excellent self-perceived confidence in grading.

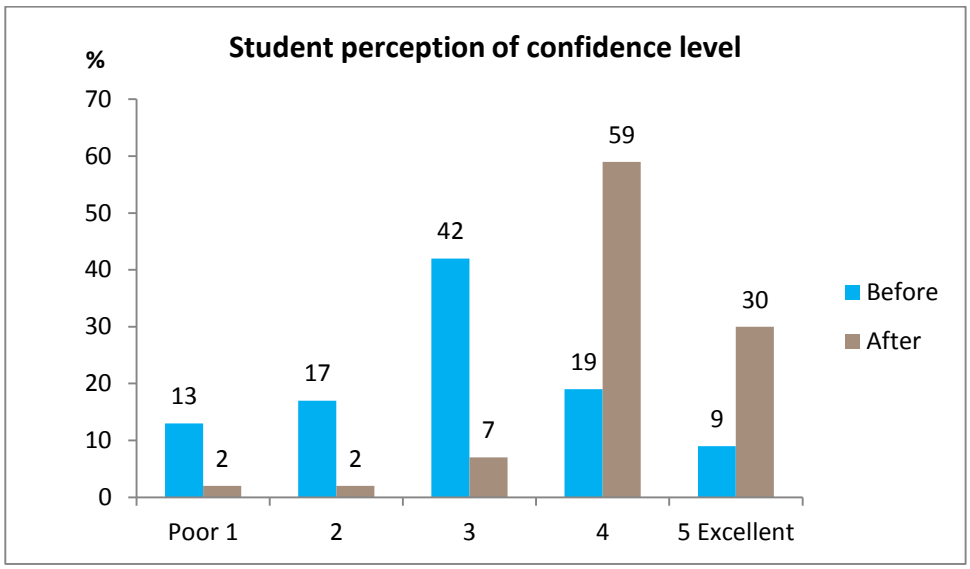
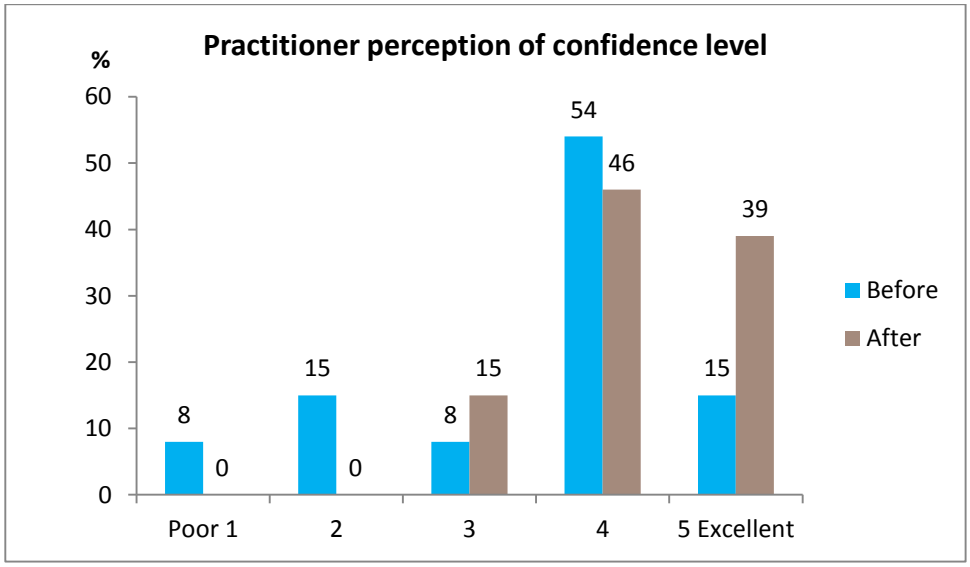


Figure 17. Perception of **confidence level** in **practitioners** before and after using kodecytes. Perception was self- measured on a Likert scale, where 1 = poor, and 5 = excellent self-perceived confidence in grading.



44 students and 12 practitioners completed the qualitative question “Do you consider kodecytes to be a valuable teaching tool?”: 42 students (95%) answered Yes, and two (5%) No, to this question.

Theme analysis of “Why or why not?” yielded the following quotes, grouped in themes:

Theme: Experienced improvement through repetition/practise (n = 13)

Quotes:

“not knowing what the reaction should be really forces you to look carefully. Often it is easy to just be like “oh yeah I see it” when you haven’t had enough practise”

“practising many times improved my grading ability”

“It is a good practise tool, by grading kodecytes we were able to grade tube techniques really well”

“trains the eyes regularly”

“it gives students more practise we can get good at grading”

“gained experience from each grading, when you practise more, you can see the results easily”

“practising kodecytes enabled us to read weak positive tubes”

“Self-doubt could be minimised through repetition and practise”

“being able to practise more is helpful”

“means we are able to get valuable practise/experience”

“makes grading clearer rather than relying on pictures”

“provide good practise in grading technique, giving more confidence”

“we got more practice to build up confidence”

Theme: Helps with grading a range of reactions, including weak reactions (n = 11)

Quotes:

“Before the introduction to kodecytes I’ve been used to grading strong reactions so the kodecytes helped me to learn to recognise weak reactions”

“they give the ability to grade weaker reactions”

“allows students to visualise weak reactions”

“Helps recognise weak reactions”

“enables students to observe what weaker reactions may look like instead of just 4+ and 0”

“practising kodecytes enables us to read weak positive tubes”

“It will be very helpful for grading results in transfusion science; knowing how to differentiate between the negatives and the positives”

“it will help improve the quality of student grading”

“enables students to differentiate more clearly between weaker reactions and negative reactions”

"provides an excellent teaching tool to grade different levels of agglutination from 4+ to 1+"

"better to know the weak reaction rather than just positive or negative"

Theme: Improved confidence (n = 3)

Quotes:

" I gained confidence and became better at grading reactions"

"not knowing (the order of the panel) really helps increase confidence when you do get it right"
"provide good practise in grading technique, giving more confidence"

"we got more practice to build up confidence"

"good self-assessment; saw own improvement throughout the semester"

"grading is a daily task blood bank personnel have to deal with. Having confidence in their ability to perform and grade these reactions reflects on the end result"

Theme: Like the appearance of kodecytes (n=6)

Quotes

"the results were no different from real blood cells"

"gives you clear results of reactions that occur"

"better visualisation of what results might occur"

"reactions were pretty clear, made it easy to grade them"

"better and clearer observation"

"kodecytes give more sensitive test results"

From students answering negatively:

"too subjective"

" I did not find consistency in my ability to grade the tubes; too much variation in appearance of agglutination".

All 12 practitioners (100%) answered YES and 11 provided qualitative feedback under a common theme of relevance to their workplace. The quotes were:

"It is very difficult to teach manual reading techniques as the weaker reactions are just not available. Also difficult to compare what one person sees with what another person sees. I would like to get every person that passes through blood bank to be trained with kodecytes"

"They actually read very much like unmodified cells. This allows confidence in obtaining an expected result across many samples, while showing very clearly reaction and technical differences. With CAT technology its easy to demonstrate reactions differences; this allows that confidence in tube methods"

"Bulk exposure and repetition is beneficial. Having expected results to compare to gives confidence in ability, and can also expose weakness or need to improve"

"Helps me to re-sharpen the skill in agglutination serology"

"Exposure to weak reactions is really important, to learn to recognise and grade, as it may be clinically significant"

"There is no other source of sensitivity control. Practitioners rarely see some weakly reacting patient samples so this provides experience and confidence and the ability to ensure individuals are proficient to a satisfactory level"

"kodecytes offer the opportunity to generate weak positive reactions, which would otherwise be seen only rarely"

"Easier to find weaker reactions to test with. Not sure of the price so cannot comment if it is cost effective using this technology"

[kodecytes] "are useful in creating method to make control cells"

"Specific and easy to learn from and the method does increase the confidence level for students who have never done the work before"

"Extremely valuable teaching tool. Stable, replicable and gain confidence in a standardised system. Need kodecytes in our lab!"

Discussion

Haemagglutination remains one of the gold standard *in vitro* methods for predicting *in vivo* compatibility of transfused blood. The advent of nationalised electronic blood management systems for keeping and searching records of patients' blood groups and antibody histories has undoubtedly made blood transfusion safer, since it is well known that computers make fewer errors than human beings, and that the majority of computer software errors are those introduced to the software by humans (Duffuaa & Raouf, 1997). Similarly, the advent of the electronic crossmatch, where the computer relies on the record of the blood group of the patient and the donor to select ABO compatible red cells, has added a layer of safety (Chapman, Milkins, & Voak, 2000). However, the computerised record is only as good as the technology and skill (including grading) used to generate the ABO blood group. If errors are made in grading and interpreting the blood group reaction, the wrong information may be entered in the blood management system. The software will then be acting on the wrong information in selecting blood.

Skills in recognising a range of grades of haemagglutination are therefore critical to safe practice in the preparation of blood for transfusion. Before entering the workplace, graduates need to master this skill, along with many others, at the university. The university programmes bring much strength to the graduate and the profession in terms of theory and practical-based skills. But, with the change from apprenticeship-style training to a university-based degree there has been a loss of ability to teach all serological grades to students. Even in the workplace, there is a scarcity of weak positive reactions (as stated by the practitioners who participated in the study). It is also known from an international survey that working practitioners in blood banks have problems correctly grading weak agglutination (Henry, 2009). Weak positive reactions need to be recognised as they indicate a positive *in vitro* reaction has occurred, be that in phenotyping or the crossmatch. The danger in missing the weak positive reaction is that patients may be misgrouped, or have blood wrongly classified as compatible. They may then be issued with incompatible red cells for transfusion. Both these scenarios potentially have very serious transfusion consequences for the patient. Antibody titration, used to monitor antibodies during pregnancy between Rh incompatible mother and fetus, or levels of ABO system antibodies in blood donors, is another laboratory technique where variation in results is seen (AuBuchon, de Wildt-Eggen, & Dumont, 2008). This can be due to a large number of variables, of which the ability of the operator to grade the tube reaction is one. Since the end point of a titre is locally determined by the last dilution to show a positive reaction at a particular grade (AuBuchon et al, 2008), the ability to recognise that grade should control, at least to some extent, this particular variable. Grading in ABO phenotyping is important in transplantation settings, particularly haemopoietic transplant, where ABO donor-receipient chimerism is seen (Klein & Anstee, 2013).

The purpose of the study was to create a robust and reproducible teaching tool for grading, and to evaluate the impact of this tool. The design of the study was based on KODE technology, with its unique aspects of attaching synthetic blood group antigens with controlled quantitative expression on the red cell surface. It is difficult to make quantitative control cells for the reasons discussed in the introduction, with the fundamental problem being that biological variation exists between individuals, and low antigen expressions are rare, and it is difficult or impossible to find every reaction grade. KODE technology overcomes these difficulties to produce red cells with controlled amounts of synthetic blood group antigens on the cell surface.

By using A+B kodecytes of different concentrations to produce red cells with a range of grading strengths with a standardised anti-A reagent, and giving these to students in blind panels to repeatedly evaluate their grading ability, a tool was created to teach grading to students.

The first aim was to create a teaching tool which was robust and consistent. This was achieved, with the technology found to be extremely robust. The production of kodecyte panels that stored normally in red cell preservative solution, and agglutinated at the same or within 1 grade in each batch, 98% of the time, gave a high degree of control to the teaching tool. The consistency of labelling with the construct, and the grading results produced, testify to the robustness of the tool. It was also very technically easy to prepare, which is important in a busy teaching environment.

The results were highly reproducible. The small number of discrepancies seen in reproducibility tests (see (Table 8 and Table 9. Reproduciblity trial results of A+B kodecytes in CAT are likely due to the fact that both the A6+B19, and A12+B38 kodecytes are very weak, with the A6+B19 being right on the limit of tube detection. It seems likely that the cell was labelled with construct, but that the researcher was unable to see it. The subjectivity of the tube technique may be the limitation here, rather than variability with the labelling technique. Alternatively, there may have been a problem with the operator's technique in the preparation of that kodecyte (for example pipetting error). It is also acknowledged that other variables such as lighting, magnification and centrifuge calibration could also have influenced results. The A12+B38 kodecyte failed to come within grade of the reference reaction twice, producing a negative reaction when a positive was expected with the anti-B. The series of concentrations of kodecytes also fell too rapidly on one of those occasions, with a drop from 3+ to zero within one tube. This points to an operator error rather a problem with kodecyte reproducibility. Other studies have shown the consistency of A+B kodecytes prepared in the same way to be very high (bioCSL, n.d). The batches of kodecytes containing these preparation failures were still able to used without adversely affecting results, as the participant grades were always compared to those gained by the experienced serologist, not to the expected result for a particular kodecyte.

The second aim was to assess the measurability of impact of the teaching tool. The impact of the tool was measurable, with statistical tools able to be applied to data sets to produce meaningful results.

Whilst students did not have problems grading natural strong, or negative reactions, they did have problems with grading weak and medium strength reactions, and, to a lesser extent, strong kodecytes (which are weaker in antigen strength than natural cells). The weaker the grade, the lower the correct grading rate fell. The technology in this study was standardised to tube serology with one anti-A reagent, whereas the Henry (2009) survey across many laboratories utilised many technologies and many antisera. Therefore it is difficult to make comparisons with the survey findings. However, the trend that the weaker the agglutination grade, the lower the correct grading rate seen in this study, is also borne out in Henry's study.

It was proven that intervention with kodecytes is effective in improving the ability of students to grade weak and medium positives at one time point, as shown by the cross-sectional results with cohort C; the students who used kodecytes once, in intervention and control groups.

Over time, the ability to grade weak and medium positives was variable. The reasons for this are unknown. In hindsight, it would have been valuable to collect data at more time points with more students. However, this would have been logistically difficult, as the study had to fit into the teaching of the class, rather than vice-versa.

Over time, the ability to grade strong kodecytes improved. Whilst this suggests benefit from kodecytes, care must be taken in interpreting this result, as it is well known that time can act as a confounding variable (Platt, Schisterman, & Cole, 2009).

Practitioners showed improvement in grading positives. It must be acknowledged however that this cohort was very small, and therefore the results should be interpreted with caution. It would have been ideal to run the trials with further participants, but attempts to recruit further practitioners within the timeframe of the study were unsuccessful

The third aim was to assess whether kodecytes could be used to highlight grading problems in students. When all student data was combined, the high number of students without grading problems masked the small number of students with grading problems. When the subset of students with grading problems was separated, the kodecytes proved to be a powerful tool in both identifying these students, and in those students showing improvement as a group over the course of the study. This will be most beneficial in the teaching setting, where early identification and intervention with kodecytes will allow lecturers to identify and monitor these students. Previously, with only access to strong positive and negative grades, it was not possible to identify these students as 98% of students in cohort A graded natural cells correctly. Being able to identify students with grading difficulties early could result in improved performance, and ultimately patient safety, when these students reach the hospital blood bank environment.

It was interesting to note the four students who did not have grading problems initially, but who developed them over time. Of these four, two showed significant decline in the last two weeks of the study (Table 21). The reason for this decline is unknown, but one can speculate that these students may have become disinterested in the exercise. It was unfortunate that at this stage of the project, ethical constraints to prevent identification of the students meant that these students could not receive further remedial training before being released to clinical placement. If the students had known this could be a barrier to entering clinical placement, they may have maintained their interest.

One difficulty in data analysis was the number of variables that had to be recorded and then analysed in the data spreadsheets. Data variables included weeks of enrolment in the programme, type of kodecyte or natural cell used in a particular week and student absences in a particular week. It was always time-consuming, and sometimes difficult to always accurately count data sets.

The final aim was to find out how valuable the students and practitioners found the kodecytes. The results in the participant questionnaire were quite powerful, showing marked upward movement in perception of both technical performance and confidence in ability to grade. Both students and practitioners commented on the power of the teaching tool to demonstrate and practise the grading of weak reactions, and to improve their confidence and skill. This will be valuable in both the university setting, where students are learning skills before they go to placement and then into the workforce, and in the workplace where qualified practitioners' competency can decrease through lack of ability to practise a skill. In the student setting, the grading exercises fit easily as a 10-minute preamble into a four- hour teaching laboratory session, complementing the teaching time.

Kodecytes mean the university is able to produce panels of cells of varying grades of agglutination at any time, on demand. This is a very different picture to that seen in previous years, when it was impossible to teach students the weak positive grade. The weekly grading exercise has been implemented at AUT as an addition to all laboratory manuals for Transfusion Science papers. As evidenced by their qualitative feedback, the majority of students enjoyed using the technology, and found benefit through weekly practise. This is a good fit Emson's aim for AUT of graduates being "adequately prepared to utilise and apply their knowledge and skills on completion of a programme", as the grading skill is critical to competence in the workplace.

In addition to the weekly grading exercise, the tool has far wider potential; to mimic patients with anomalous ABO groups (for example weak subgroups of A), to mimic dosage in saline antibody identification panels, and to be used as an appropriately weak control in antisera evaluation exercises.

Practitioners also commented collectively on how beneficial more regular exposure to the weak grade would be to them, as this is not encountered frequently, but is critical to patient safety.

The technology will also help AUT overcome the increasing difficulty from securing donations of human sera containing anti-D.

Both students and practitioners also confirmed the observation that kodecytes behave like natural cells, with comments “they actually read very much like unmodified cells” and “the results were no different from real blood cells”. Comments from all cohorts were unsolicited, and therefore the more powerful.

In the diagnostic laboratory, the tool can be purchased as a commercial product (KODE FSL-A+B (tri) serologic teaching kit), and would fulfil the accreditation quality requirements for internal surveys, to demonstrate staff consistency in grading, and to highlight problems amongst staff. Practitioner comments such as *“I would like to get every person that passes through blood bank to be trained with kodecytes”* suggest that the tool would be well accepted by practising blood bankers.

Conclusion

This research has resulted in the production and successful trial of a new teaching tool in the university setting. The highly robust and reproducible nature of the KODE technology ideally lent itself to the development of such a tool.

All the data collected was able to produce a measurable outcome, albeit with limitations. Students with grading deficiencies were identified, and in all cases demonstrated improvement. The conclusion is that the tool should continue to be used in the university.

Future perspectives

Further data collection will allow more robust statistical analysis and conclusions. Development of a computer programme to count the data sets would enhance the accuracy and speed of analysis.

The development of other teaching tools using KODE technology is planned. It is hoped to develop kodecytes which react with IgG antibodies in the antiglobulin test. This should allow development of a better range of grading strengths and flexibility than the current policy of using anti-D with D positive and D negative cells. A construct able to allow demonstration and measurement of antibody induced haemolysis would also further enhance the university teaching tool kit.

It is planned to implement the tool to assess students, and to ensure they pass the assessments with kodecytes to a satisfactory level before being released to clinical placement.

Appendices

Appendix 1: Ethics approval

AUT UNIVERSITY

MEMORANDUM

Auckland University of Technology Ethics Committee (AUTEC)

To: Stephen Henry
From: **Dr Rosemary Godbold** Executive Secretary, AUTEC
Date: 19 January 2012
Subject: Ethics Application Number 11/323 **Kodecytes: A novel teaching tool in transfusion science.**

Dear Stephen

Thank you for providing written evidence as requested. I am pleased to advise that it satisfies the points raised by the Auckland University of Technology Ethics Committee (AUTEC) at their meeting on 12 December 2011 and I have approved your ethics application. This delegated approval is made in accordance with section 5.3.2.3 of AUTEC's *Applying for Ethics Approval: Guidelines and Procedures* and is subject to endorsement at AUTEC's meeting on 13 February 2012.

Your ethics application is approved for a period of three years until 18 February 2015.

I advise that as part of the ethics approval process, you are required to submit the following to AUTEC:

- A brief annual progress report using form EA2, which is available online through http://www.aut.ac.nz/research/research-ethics/ethics. When necessary this form may also be used to request an extension of the approval at least one month prior to its expiry on 18 February 2015;
- A brief report on the status of the project using form EA3, which is available online through http://www.aut.ac.nz/research/research-ethics/ethics. This report is to be submitted either when the approval expires on 18 February 2015 or on completion of the project, whichever comes sooner;

It is a condition of approval that AUTEC is notified of any adverse events or if the research does not commence. AUTEC approval needs to be sought for any alteration to the research, including any alteration of or addition to any documents that are provided to participants. You are reminded that, as applicant, you are responsible for ensuring that research undertaken under this approval occurs within the parameters outlined in the approved application.

Please note that AUTEC grants ethical approval only. If you require management approval from an institution or organisation for your research, then you will need to make the arrangements necessary to obtain this.

When communicating with us about this application, we ask that you use the application number and study title to enable us to provide you with prompt service. Should you have any further enquiries regarding this matter, you are welcome to contact me by email at ethics@aut.ac.nz or by telephone on 921 9999 at extension 6902.

On behalf of AUTEC and myself, I wish you success with your research and look forward to reading about it in your reports.

Yours sincerely

Dr Rosemary Godbold
Executive Secretary
Auckland University of Technology Ethics Committee

Cc: Holly Perry hperry@aut.ac.nz

From the desk of ...
Dr Rosemary Godbold
Executive Secretary
AUTEC

Private Bag 92006, Auckland 1142
New Zealand
E-mail: ethics@aut.ac.nz

Tel: 64 9 921 9999
ext 8860
Fax: 64 9 921 9902

Appendix 2; Information sheets

Student Participant Information Sheet

Date Information Sheet Produced:

16 Jan. 12

Project Title

Designer red cells; a novel teaching tool in Transfusion Science.

An Invitation

I am Holly Perry; the principal researcher in this study. I am a senior lecturer in Transfusion Science in the School of Applied Sciences at AUT.

This research will contribute to my MPhil. I hope to publish results in an international Transfusion journal, and to disseminate the results at Transfusion conferences in New Zealand and internationally.

If you agree to participate in my study, your participation is voluntary you may withdraw at any time prior to the completion of data collection.

As a student of AUT and you choose to participate or not participate, this will neither advantage nor disadvantage your study at AUT.

What is the purpose of this research?

You are invited to participate in a research project to assess the validity of designer red cells as a teaching tool in Transfusion Science.

The ability of transfusion scientists in the laboratory to recognize and grade reactions used to make clinical decisions is paramount to their competency. But with the international movement away from clinical apprenticeship style training, to contrived practical training in academic institutions, there is less opportunity for learning this skill. There is also limited access to patient samples in Universities, which significantly limits resources for training purposes. Recently a technique for making a 'designer red cell' which mimics a range of natural cells has been reported. These so-called kodecytes have also been used in quality control systems. The aim of this study is to examine if kodecytes can be used as a tool for training serologists to accurately grade reactions.

As a student of AUT, you will be using these kodecytes each week as part of your normal class training. These exercises are not optional and are part of teaching, not research. The research is the subsequent analysis and reporting of the data. Your participation in the research is completely voluntary. If you choose not to participate in the research, there will be no disadvantage to your study or grades at AUT.

Holly Perry is your paper leader and also your programme leader. You may have some concerns that due to Holly's position, you are obliged to participate in the research. This is not the case. To ensure you feel comfortable about your decision to participate or not, AUT has put the following procedures in place:

John Prince, a lecturer from the Faculty of Engineering, with no involvement in approving Science students' results or academic progression, is presenting you with this information in class today. You will have 1 week to consider whether you wish to participate or not. Next week, John will take a few minutes of our class time to answer any questions you may have, and to ask you to sign consent forms for those of you who have decided to participate. Holly Perry will not know who has consented to participate, or who has not consented to participate.

Each week you will complete the training exercises with the kodecytes. In class each week, Holly Perry will make available her results with the same kodecytes, so that you can assess and monitor your performance as a student. You will give a copy of your results to Holly Perry with your student number on your worksheets.

At the end of the semester Holly Perry will give all worksheets to John Prince. John will de-identify your data by replacing your student number with a study number. Your identity as a research participant will be known only to John, not to Holly. Holly will then use the study numbers to analyse the data for the research. Receipt of the de-identified research data, and analysis of the data, will begin only after Undergraduate Examination Board has confirmed your final grade in your Transfusion Science paper.

Your class data will be combined with the data of up to 60 other students in different classes. It will be impossible to identify individuals.

How was I identified and why am I being invited to participate in this research?

You have been invited to participate as a current student using the research tools in our regular Transfusion classes.

Practising medical laboratory scientists have also been invited to participate so that we can compare the performance of the teaching tool in the hands of students as compared to more experienced practitioners.

What will happen in this research?

Your results from grading a set of kodecytes will be analysed in comparison to the results gained by other participants. On page 4, you can see the worksheet you will be completing each week.

As a BMLS student, this testing forms a compulsory part of your normal practical work. The research, in which your participation is completely voluntary, is allowing your results to be used in the analysis and reporting of the data.

What are the discomforts and risks?

It is not anticipated you would experience any discomfort or risks.

What are the benefits?

It is hoped that this research will validate the existing teaching kits of kodecytes as a teaching tool and thus improve practice. For the researcher (Holly Perry), the benefit would be completing a post graduate qualification.

How will my privacy be protected?

Your identity will not known to the researchers, only to an independent individual (John Prince) as a code number which you will be assigned at the commencement of the study. Your results will be confidential and you will not be identified through any publication of data.

What are the costs of participating in this research?

You will not incur any financial costs through participation.

As a student, you will not need to devote any extra time to the project.

What opportunity do I have to consider this invitation?

Please consider whether you would like to participate over the next week.

How do I agree to participate in this research?

Please sign the Consent Form attached to this information sheet and return to John Prince

Will I receive feedback on the results of this research?

As a BMLS student, you will receive regular feedback on your performance as part of the normal class teaching procedures. This is not the research.

You will have the opportunity to receive a copy of a summary of the research at the end of the study.

Declared conflict of interest:

The researcher's primary supervisor Professor Stephen Henry has developed a kodecytes teaching kit and this product is for sale to diagnostic laboratories. The research has the potential to increase sales of the kit. AUT is a shareholder in the company which owns the rights to this kit.

What do I do if I have concerns about this research?

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor, Stephen Henry shenry@aut.ac.nz, phone 09 921 9999 extn 9708.

Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC, Dr Rosemary Godbold, rosemary.godbold@aut.ac.nz , 921 9999 ext 6902.

Whom do I contact for further information about this research?

Researcher Contact Details

Holly Perry. hperry@aut.ac.nz, phone 09 921 9999 extn 8033

Supervisor Contact Details

Stephen Henry shenry@aut.ac.nz, phone 09 921 9999 extn xxxx.

Approved by the Auckland University of Technology Ethics Committee on *type the date final ethics approval was granted,* AUTEC Reference number *11/323*.

Practitioner
Information Sheet

Date Information Sheet produced: 16 Jan. 12

Project Title: Designer red cells; a novel teaching tool in Transfusion Science.

An Invitation

I am Holly Perry; the principal researcher in this study. I am a senior lecturer in Transfusion Science in the School of Applied Sciences at AUT.

This research will contribute to my MPhil. I hope to publish results in an international Transfusion journal, and to disseminate the results at Transfusion conferences in New Zealand and internationally.

If you agree to participate in my study, your participation is voluntary you may withdraw at any time prior to the completion of data collection.

What is the purpose of this research?

You are invited to participate in a research project to assess the validity of designer red cells as a teaching tool in Transfusion Science.

The ability of transfusion scientists in the laboratory to recognize and grade reactions used to make clinical decisions is paramount to their competency. But with the international movement away from clinical apprenticeship style training, to contrived practical training in academic institutions, there is less opportunity for learning this skill. There is also limited access to patient samples in Universities, which significantly limits resources for training purposes. Recently a technique for making a 'designer red cell' which mimics a range of natural cells has been reported. These so-called kodecytes have also been used in quality control systems . The aim of this study is to examine if kodecytes can be used as a tool for training serologists to accurately grade reactions.

Students of AUT Transfusion Science classes have also been invited to participate in this research.

How was I identified and why am I being invited to participate in this research?

You have been invited to participate as a practising medical laboratory scientist or technician. You have been invited so that we can compare the performance of the teaching tool in the hands of students as compared to more experienced practitioners.

What will happen in this research?

You would be asked to grade a set of kodecytes (strength unknown to you as a participant) against 1 or 2 antisera. Your results will be analysed in comparison to the results gained by other participants. You will be asked to complete a questionnaire.

What are the discomforts and risks?

It is not anticipated you would experience any discomfort or risks.

What are the benefits?

It is hoped that this research will validate the existing teaching kits of kodecytes as a teaching tool and thus improve practice. For the researcher (Holly Perry), the benefit would be completing a post graduate qualification.

How will my privacy be protected?

Your identity will be known only to the researchers as a code number which you will be assigned at the commencement of the study. Your results will be confidential and you will not be identified through any publication of data.

What are the costs of participating in this research?

You will not incur any financial costs through participation.

As a student, you will not need to devote any extra time to the project.

As a practitioner, participation in the project would require your participation and time at an AUT workshop.

What opportunity do I have to consider this invitation?

Please consider whether you would like to participate over the next week.

How do I agree to participate in this research?

Please sign the Consent Form attached to this information sheet and return to Holly Perry

Will I receive feedback on the results of this research?

You will have the opportunity to receive a copy of a summary of the research at the end of the study.

Declared conflict of interest:

The researcher's primary supervisor Professor Stephen Henry has developed a kodecytes teaching kit and this product is for sale to diagnostic laboratories. The research has the potential to increase sales of the kit. AUT is a shareholder in the company which owns the rights to this kit.

What do I do if I have concerns about this research?

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor, *Stephen Henry shenry@aut.ac.nz, phone 09 921 9999 extn 9708*.

Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC, Dr Rosemary Godbold, *rosemary.godbold@aut.ac.nz* , 921 9999 ext 6902.

Whom do I contact for further information about this research? Researcher Contact Details

Holly Perry. hperry@aut.ac.nz, phone 09 921 9999 extn 8033

Supervisor Contact Details

Stephen Henry shenry@aut.ac.nz, phone 09 921 9999 extn 9708.

Approved by the Auckland University of Technology Ethics Committee on *type the date final ethics approval was granted*, AUTEC Reference number *11/323*.

Appendix 3: Consent forms

Consent Form for students

Project Title: ***Kodecytes; a novel teaching tool in Transfusion Science***

Project Supervisor: ***Professor Stephen Henry***

Researcher: ***Holly Perry***

- O I have read and understood the information provided about this research project in the Information Sheet dated 21 November 2011.
- O I have had an opportunity to ask questions and to have them answered.
- O I understand that my results will be included in analysis for the purposes of this research
- O I understand that the exercises in which data is generated is part of my normal teaching programme and this research only uses my results for analysis
- O I understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way.
- O If I withdraw, I understand that all relevant information will be destroyed.
- O I agree to take part in this research.
- O I wish to receive a copy of the report from the research (please tick one): YesO NoO

Participant's signature: .....

Participant's name: .....

Participant's Contact Details (if appropriate):

.....

.....

.....

.....

Date:

Approved by the Auckland University of Technology Ethics Committee on *type the date on which the final approval was granted* ***AUTEC Reference number*** *type the AUTEC reference number*

Note: The Participant should retain a copy of this form.

Consent Form for practitioners

Project Title: ***Kodecytes; a novel teaching tool in Transfusion Science***

Project Supervisor: ***Professor Stephen Henry***

Researcher: ***Holly Perry***

- O I have read and understood the information provided about this research project in the Information Sheet dated 21 November 2011.
- O I have had an opportunity to ask questions and to have them answered.
- O I understand that my results will be included in analysis for the purposes of this research
- O I understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way.
- O If I withdraw, I understand that all relevant information will be destroyed.
- O I agree to take part in this research.
- O I wish to receive a copy of the report from the research (please tick one): YesO NoO

Participant's signature: .....

Participant's name: .....

Participant's Contact Details (if appropriate):

.....

.....

.....

.....

Date:

Approved by the Auckland University of Technology Ethics Committee on *type the date on which the final approval was granted* ***AUTEC Reference number*** *type the AUTEC reference number*

Note: The Participant should retain a copy of this form.

This version was last edited on 13 October 2010

Appendix 4: Questionnaire

Questionnaire

Project Title: ***Kodecytes; a novel teaching tool in Transfusion Science***
Project Supervisor: ***Professor Stephen Henry***
Researcher: ***Holly Perry***

1. Please reflect on your **technical performance** before and after the exercises with the kodecytes.

   Indicate on the scale your perceived ability to grade serologic tube reactions before and after the kodecyte exercises.

   A score of 1 means you think your grading ability is poor. A score of 5 means you think your grading ability is excellent. You may mark yourself anywhere on the scale.

   Before Kodecytes

   Poor ——|——|——|——|—— Excellent

   1 2 3 4 5

   After Kodecytes

   Poor ——|——|——|——|—— Excellent

   1 2 3 4 5

2. Please reflect on your **confidence level** in grading before and after the exercises with the kodecytes.

   Indicate on the scale how confident you felt about grading serologic tube reactions before and after the kodecyte exercises.

   A score of 1 means you have low confidence in your ability. A score of 5 means you have high confidence in your ability.

   Before Kodecytes

   Low ——|——|——|——|—— High

   1 2 3 4 5

   After Kodecytes

   Low ——|——|——|——|—— High

   1 2 3 4 5

PLEASE TURN OVER TO PAGE 2 OF THE QUESTIONNAIRE

This version was last edited on 21 June 2012

Do you consider kodecytes to be a valuable teaching tool? Please explain why or why not.

Date:

Approved by the Auckland University of Technology Ethics Committee on 19/01/12 AUTEC Reference number 11/323

This version was last edited on 21 June 2012

Appendix 5: Standard protocol for kodecyte trials

Introduction

Kodecytes are group O donor cells which have had functional spacer lipid (FSL) chemical constructs inserted into them. A range of FSLs can be inserted. The ones you are using here have had A and B antigens inserted into their surface, so they will react with anti-A.

The amount of anti-A added to the cell surface is controlled during manufacture and is different on each of the cells you are going to receive. Grading the kodecytes will allow you to get used to grading strong, medium, weak and negative reactions.

Instructions

1. Locate your set of 12 tubes labelled 1-12, and your set of control tubes, labelled A_1B, A_2, B and O. Each of these contains 1 drop of 5% cell suspension which the lecturer has dispensed for you
2. Add 1 drop of CSL anti-A into each tube
3. Incubate the tubes for 5 minutes at room temperature
4. Record the manufacturer, batch number and expiry date of the anti-A
5. Spin all tubes for 15 seconds at 2000rpm in a Diacent 12 centrifuge.
6. Grade and record your reactions in the table below using the 0,1,2,3,4 grading system

Tube	1	2	3	4	5	6	7	8	9	10	11	12
Student Grade												
Lecturer grade												

Tube	A_1B	A_2	B	O
Student Grade				

Antisera Specificity_____

Antisera Manufacturer_____

Antisera Batch Number_____

Antisera Expiry Date_____

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