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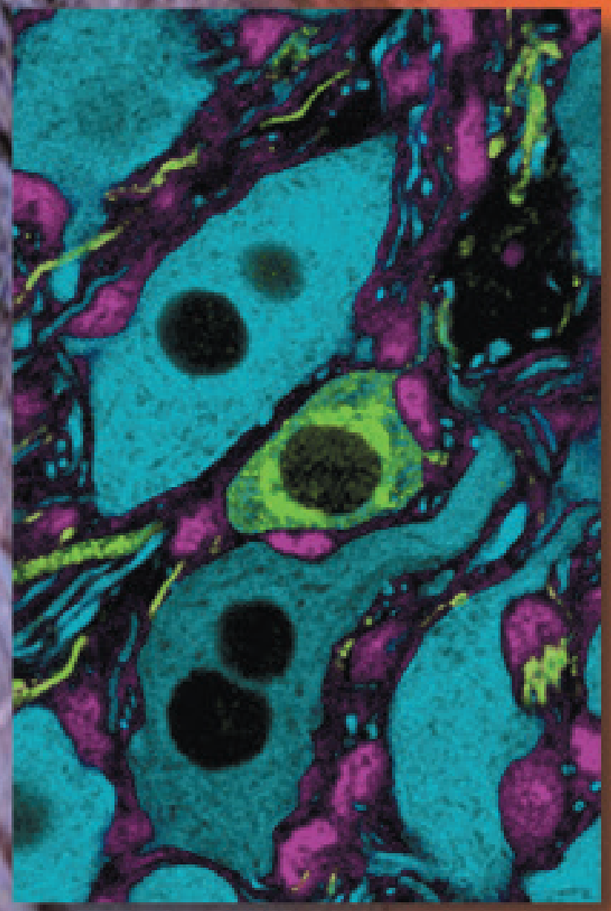

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Pre-analytical errors and their prevention in an emergency department setting

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Abstract

The pathology laboratory plays a key role in a patient's healthcare journey and the results from pathology testing can influence medication, hospital admission status and differential diagnoses. Errors in the pre-analytical phase may negatively impact the total testing process (TTP) and therefore patient outcomes. The pre-analytical phase involves multiple healthcare professionals from different disciplines and departments. An observational study of pre-analytical errors was conducted over two months at an Australian hospital emergency department (ED) and associated laboratory. This study was used to assess the type and number of pre-analytical errors occurring at this site. A Likert-Like survey targeting the ED staff was used to form a basis for the intervention phase of the experiment which aimed to reduce the overall number of pre-analytical errors occurring at this site. An intervention study focusing on educating the ED staff on pre-analytical errors from four bases (request-based, specimen-based, transport-based, and laboratory-based) was conducted over two months. The intervention was not successful in significantly reducing the number of pre-analytical errors at this site; however multiple suggestions have been made to help reduce the number of pre-analytical errors in the future. These include introducing a pneumatic tube system, running a monthly/bi-monthly training program, and implementing a training program targeting laboratory staff. An extended study with more frequent interventions is needed to assess the benefits of running an intervention training program focusing on pre-analytical errors.

Keywords: ***pre-analytical errors, medical errors, clinical laboratories, patient safety***

Introduction

Diagnostic testing in the pathology laboratory has a key role in the treatment and diagnosis of patients. Roughly 70% of all clinical decisions are based on laboratory results (Vanker *et al* 2010; Atay *et al* 2014; Cao *et al* 2016). The pathology laboratory is only one facet of the total testing process (TTP) as the ED, various information systems, and multiple healthcare professionals (e.g. clinicians, laboratory scientists nurses, phlebotomists, and orderlies) are also involved. The TTP heavily relies on communication, teamwork, experience, and knowledge to ensure the best outcome for the patient. The TTP for all pathology requests involves the pre-pre-analytical phase (the formation of a clinical question), pre-analytical phase (specimen collection), analytical phase (the analysis of a specimen), post-analytical phase (the reporting of results), and the post-post-analytical phase (the interpretation of laboratory results by a health professional) (Plebani 2012; Kaushik and Green 2014; Cao *et al* 2016). This study will consider the pre-pre and pre-analytical phase as a combined pre-analytical phase in the TTP.

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The pre-analytical phase has been suggested to have a higher error rate than the analytical and post-analytical phases, as the pre-analytical phase is not under direct laboratory control (Gunnur Dikmen *et al* 2015; Najat 2017). It has also been noted that pre-analytical errors compose the bulk of all laboratory errors (Marin *et al* 2014; Cao *et al* 2016; Osegbe *et al* 2016). Many of these pre-analytical errors are preventable, as seen in a study by Carraro and Plebani 2007, where 73% of the pre-analytical errors that occurred in their study were avoidable. Interventions to reduce pre-analytical errors have the greatest probability of impact on a patient's

outcome during their healthcare journey. This is due to the fact that any errors occurring in this phase have the potential to follow on to the analytical and post-analytical phases and affect all subsequent stages of the patient's healthcare journey (Lillo *et al* 2012; Plebani 2012; Al Saleem and Al-Surimi 2016). Many studies have focused on the potential risk and harm of these pre-analytical errors with methods that include training of laboratory staff, changes in pre-analytical methods/processes, the use of performance indicators, total quality management systems, and increased communication between different disciplines of health professionals and departments (Chawla *et al* 2010; Agarwal *et al* 2012; Kagan and Barnoy 2013; Sullivan *et al* 2013; Morias *et al* 2018).

Pre-analytical errors have the potential to cause a substantial burden on the healthcare system as they may lead to significant morbidity and mortality. They may also cause significant financial strain on the healthcare system through increased service costs, consumables and increased staffing requirements. Unnecessary patient follow-up investigations and poor patient outcomes can also be a direct result of pre-analytical errors (Le *et al* 2016; Tapper *et al* 2017; West *et al* 2017). This study attempts to reduce the number of pre-analytical errors seen within an Australian hospital site via an observational study, an ED staff survey and an intervention study to assess the effectiveness of the intervention. The outcome of this study will be used to determine if the interventions implemented were successful and if a significant reduction in pre-analytical errors had occurred.

Materials and Methods

Study population

The study population was comprised of pathology request forms and pathology samples from the hospital ED. Only ED pathology request forms that contained blood tests were analysed. A total number of 16955 pre-analytical errors were observed from 6692 pathology forms over two months. A survey regarding pre-analytical errors was distributed to the ED staff via Survey Monkey. The ED staff were given four months to complete this survey. The ULTRA laboratory information system (LIS) was used to assess laboratory-based errors. Ethics approval was obtained from the South Australian Local Health Network (SALHN) (OFR110.17) and Charles Sturt University Human Research Ethics Committees (CSU-HREC) (H17142).

Study overview

The study was composed of four phases. Phase One was an observational study data of the pre-analytical errors listed in Table 1, Phase Two was a Likert-Like survey sent to the hospital ED, Phase Three was when pre-intervention pre-analytical data were gathered, and Phase Four

Table 1. *List of measured pre-analytical errors during the observational study.*

Basis of error	Type of pre-analytical error
Request-based	No clinical notes provided
	No patient signature
	No specimen collection time provided
	No collector signature
	No doctor signature
	No sorter signature
	Duplicate request forms for the same patient episode and incomplete requests over multiple forms
	Duplicate test requests
	Test requested but not on EPAS
	Pathology test cancelled
	No Copy Doctor on request form but on EPAS
	Fasting status incorrect
	Inadequate information on request form regarding requesting doctor
	Inadequate information on request form regarding patient identification
	Illegible information on request form including tests requested, time of collection and clinical notes
	No drug regime information on request form
	Soiled pathology request form
Specimen-based	Haemolysed samples
	Transfusion samples rejected
	Blood gas sample type not specified
	Arterial specimen requested for blood gas but venous sample collected
	Inappropriately clotted sample
	Incorrect ant-coagulant ratio
	Lipaemic/icteric samples
	Insufficient sample volume
	Incorrectly labelled sample
	Unlabelled sample
	Sample contamination/poor quality of specimen
	Wrong sample for test requests
	Delay in specimen collection > 1 hour
Transport-based	Delay in specimen transport > 1 hour from collection
	Request received with no sample/s
	Samples received without request forms
	Inappropriate transport conditions
Laboratory-based	Avoidable delay in sample processing entering request forms > 1 hour
	Tests missed
	Misinterpretation of test requests
	Lost specimens
	Errors in patient demographics (age, sex, location, Medicare, gestation, etc.)
	Missed copy doctor
	Test added on by lab without EPAS request
	Clinical notes not typed
	Errors in patient billing

EPAS = Enterprise Patient Administration System

***Table 2**. Experimental overview.*

Experimental Phase	Outline	Duration of phase
Phase 1	Observational study data of the pre-analytical errors listed	Two months
Phase 2	A Likert-Like survey was sent to the ED staff at hospital	Four months
Phase 3	Pre-intervention pre-analytical data were gathered	One month
Phase 4	An intervention and the recording of the post-intervention pre-analytical errors	One month

comprised of the intervention and the recording of the post-intervention pre-analytical errors. Table 2 outlines the experimental phases. The errors that were identified in this study were resolved through the pre-existing channels used by SA Pathology (contacting the required medical professionals, the generation of amended reports and tracking changes through ULTRA).

The observational study was conducted for two months with 3775 pathology request forms and 6482 pathology specimens analysed for the presence of pre-analytical errors. The goal of the observation study was to establish a baseline number of pre-analytical errors occurring at the hospital ED and laboratory. The pre-analytical errors observed in this study were classified into request-based, specimen-based, transport-based, and laboratory-based errors (Table 1). The pathology specimen type included in this study is presented in Table 3.

***Table 3**. A list of pathology specimens observed in the observation and intervention studies.*

Specimen sample
Blood gas (G4) samples
Serum samples
K-EDTA samples
Coagulation samples
Lithium Heparin
Transfusion samples
Fluoride oxalate

K-EDTA = Potassium ethylenediaminetetraacetic acid

A participant information sheet with a Likert-Like survey link was sent to the hospital ED staff. The anonymous survey was conducted via Survey Monkey.

Table 4 presents the list of questions provided to the hospital emergency staff. Nine responses were submitted

***Table 4**. The Likert-Like survey questionnaire provided to the Hospital Emergency Staff.*

Survey Questionnaire	
Q1	I am aware that inappropriate collection, tube type, or anticoagulant ratio can significantly affect laboratory test results
Q2	The pathology laboratory regularly informs you of the reason/s why a sample has been rejected
Q3	I am aware that samples collected in a tube require a certain number of inversions to ensure the effectiveness of the clot activator or anti-coagulant when using a non-vacutainer collection method
Q4	I am aware that the use of an I.V. catheter devices (e.g. Jeleo IV catheters) for the collection of blood may lead to poor pathology sample quality including haemolysis, contamination, and the presence of intravenous fluid
Q5	I am aware of other pre-analytical factors that may affect laboratory test results including sample temperatures, patient identification, anti-coagulation ratio, and time delay
Q6	Would you benefit from a laboratory run pre-analytical training program?
Q7	Have you identified any areas or processes that may lead to pre-analytical errors? If so, what are they?
Q8	Do you have any suggestions to decrease the number of pre-analytical errors occurring in the Hospital Emergency Department and laboratory? If so, what are they?

by a potential 100 employees from the hospital ED. The participants were hospital ED employees (clinicians, nurses, and administrative staff) and were specifically targeted for the survey as they are directly involved in the pre-analytical process.

***Table 5**. A list of the pre-analytical errors observed during phases 3 and 4 (intervention).*

Basis of error	Type of pre-analytical error
Request-based	No clinical notes provided
	No patient signature
	No specimen collection time provided
	No collector signature
	No specimen sorter signature
	Duplicate request forms for the same patient episode and incomplete requests over multiple forms
	Duplicate test requests
	Fasting status incorrect
	Inadequate information on request from regarding patient identification
	No drug regime information on request form
	Wrong test selected
Specimen-based	Delay in specimen collection > 1hour
	Blood gas sample type not specified
	Arterial specimen requested for blood gas but venous sample collected
	Haemolysed samples
	Inappropriately clotted sample
	Incorrect anti-coagulant ratio
	Insufficient sample volume
	Incorrectly labelled sample
	Transfusion samples rejected
Transport-based	Delay in specimen transport > 1 hour from collection
	Request received with no sample/s
	Samples received without request forms
Laboratory-based	Avoidable delay in sample processing entering request forms > 1 hour
	Tests missed
	Misinterpretation of test requests
	Lost specimens
	Errors in patient demographics (age, sex, location, Medicare, gestation, etc.)
	Missed copy doctor
	Test added on by lab without EPAS request
	Clinical notes not typed
	Errors in patient billing
	Errors in patient demographics
	Missed copy doctor
	Clinical notes not typed
	Errors in patient billing
	Errors in patient identification between EPAS and ULTRA

EPAS = Enterprise Patient Administration System

The pre-analytical errors chosen for the intervention study in Phases 3 and 4 were based on the observational study. The number of pre-analytical errors was then compared between the pre-intervention and post-intervention phases. The intervention was in the form of a pre-analytical workshop targeting the hospital ED staff, which took place one day prior to the commencement of the post-intervention study. Staff members in attendance were issued with training documents pertaining to the various pre-analytical processes and were presented with some common scenarios in which pre-analytical errors arise.

The post-intervention phase was conducted over one month. This phase was to assess whether the intervention made a statistically significant impact on the number of pre-analytical errors observed. Thirty-two individual pre-analytical error categories were found in the pre-and post-intervention phase as seen in Table 5.

***Table 6**. Pathology specimens analysed in the observational study.*

Specimen sample	Number
Blood gas (G4) samples	249
Serum samples	2796
K-EDTA samples	2542
Coagulation samples	814
Lithium Heparin	1
Transfusion samples	66
Fluoride oxalate	14

K-EDTA = Potassium ethylenediaminetetraacetic acid

Only 32/44 categories were chosen for the intervention phase as the data could only be reliably obtained for each of these individual error types.

Statistical analysis

The pre-and post-intervention error rates were analysed using Graph Pad Prism. An unpaired t-test was used to analyse the data with significance representing a 95% confidence interval with $p < 0.05$ representing a significant result.

***Table 7**. The number and type of pre-analytical error observed in the observational study.*

Basis of error	Type of pre-analytical error	Number observed
Request-based	No clinical notes provided	2899
	No patient signature	2430
	No specimen collection time provided	1056
	No collector signature	964
	No doctors signature	1141
	No sorter signature	62
	Duplicate request forms for the same patient episode and incomplete requests over multiple forms.	157
	Duplicate test requests	26
	Test requested but not on EPAS	141
	Pathology test cancelled	276
	No Copy Doctor on request form but on EPAS	1851
	Fasting status incorrect	10
	Inadequate information on request from regarding requesting doctor	3
	Inadequate information on request from regarding patient identification	1
	Illegible information on request form including tests requested, time of collection and clinical notes	2
	No drug regime information on request form	10
	Soiled pathology request form	12

Specimen-based	Haemolysed samples	152
	Transfusion samples rejected	6
	Blood gas sample type not specified	45
	Arterial specimen requested for blood gas but venous sample collected	26
	Inappropriately clotted sample	34
	Incorrect anti-coagulant ratio	15
	Lipaemic/icteric samples	36
	Insufficient sample volume	10
	Incorrectly labelled sample	1
	Unlabelled sample	10
	Sample contamination/ poor quality of specimen	1
	Wrong sample for test requests	5
	Delay in specimen collection > 1hour	72
Transport-based	Delay in specimen transport > 1hour from collection	231
	Request received with no sample/s	28
	Samples received without request forms	30
	Inappropriate transport conditions	3
Laboratory-based	Avoidable delay in sample processing entering request forms > 1hour	37
	Tests missed	47
	Misinterpretation of test requests	5
	Lost specimens	1
	Errors in patient demographics (age sex location Medicare gestation)	172
	Missed copy doctor	46
	Test added on by lab without EPAS request	1
	Clinical notes not typed	45
	Errors in patient billing	125
	Errors in patient identification between EPAS and ULTRA	6

EPAS = Enterprise Patient Administration System

Results

Observational study results

An observational analysis of the pre-analytical errors at the hospital site was conducted over two months. 3775 pathology request forms and 6482 pathology specimens were analysed for the presence of pre-analytical errors. 12159 individual pre-analytical errors were observed during the study. The pathology specimens and errors analysed during the observational study are shown in Tables 6 and 7, with 44 different pre-analytical error categories established.

The request-based error type accounted for the highest number of pre-analytical errors. The error category with no clinical notes provided was the major reason why this error category was so predominant.

There was missing patient clinical information for 2899 out of 3775 pathology request forms from the ED. 1851 pathology requests did not have a general practitioner (GP) on the pathology request form but a GP was available on the Enterprise Patient Administration system (EPAS). The highest phlebotomy/collection error was sample haemolysis, with 152 individual pathology samples haemolysed during the observational study. A delay in specimen transport greater than an hour from the ED to the laboratory was the highest transport-based error, with 231 pathology requests being delayed by over an hour. Forty-seven pathology test requests were missed by the laboratory. Errors in patient demographics were observed in 172 instances making this pre-analytical error the highest laboratory-based error and these included five individual error categories. These categories were inadequate information on request form regarding

***Table 8**. The Likert-Like survey responses.*

Likert-Like Survey response						
	Question	**Strongly agree**	**Agree**	**Undecided**	**Disagree**	**Strongly agree**
Q1	I am aware that inappropriate collection, tube type, or anti-coagulant ratio can significantly affect laboratory test results	7	2			
Q2	The pathology laboratory regularly informs you of the reason/s why a sample has been rejected	3	6			
Q3	I am aware that samples collected in a tube require a certain number of inversions to ensure the effectiveness of the clot activator or anti-coagulant when using a non-vacutainer collection method	5	3			
Q4	I am aware that the use of an I.V. catheter devices (e.g. Jeleo IV catheters) for the collection of blood may lead to poor pathology sample quality including haemolysis, contamination, and the presence of intravenous fluid	3	6			

Q5	I am aware of other pre-analytical factors that may affect laboratory test results including sample temperatures, patient identification, anti-coagulation ratio, and time delay	4	4			
Q6	Would you benefit from a laboratory run pre-analytical training program?	1	4	3	1	
Q7	Have you identified any areas or processes that may lead to pre-analytical errors? If so, what are they?	**3 responses**				
		Human error. Incorrect documentation.				
		I do not have any concerns for my own ability. If I have any doubt, I refer to the collection handbook on the intranet or speak to someone in the lab.				
		No.				
Q8	Do you have any suggestions to decrease the number of pre-analytical errors occurring in the Hospital ED and laboratory? If so, what are they?	**2 responses**				
		Appropriate education for individuals who clearly have lack of knowledge of sample collection / transport conditions.				
		No.				

patient identification, insufficient sample volume, sample contamination/poor quality of specimens, lost specimens, and tests added on without EPAS requests.

Staff survey responses

A Likert-Like survey regarding pre-analytical errors was sent to the hospital ED staff before the intervention study. Eight questions were asked of the emergency staff and the survey responses can be seen in Table 8.

Two out of the eight questions did not receive a 100% response rate, with 'Have you identified any areas or processes that may lead to pre-analytical errors? If so, what are they?' only receiving three responses and 'Do you have any suggestions to decrease the number of pre-analytical errors occurring in the hospital ED and laboratory? If so, what are they?' receiving two responses.

The third survey question 'I am aware that samples collected in a tube require a certain number of inversions to ensure the effectiveness of the clot activator or anti-coagulant when using a non-vacutainer collection method' and the sixth question 'Would you benefit from a laboratory run pre-analytical training program?' received one negative response each showing the greatest uncertainty of the staff members surveyed.

Intervention results

An intervention analysis (phases 3 and 4) was conducted over two months. The pre-intervention and post-intervention data can be seen in Tables 9 and 10. 1492 pathology request forms and 2056 pathology specimens were analysed pre-intervention. 1544 pathology request forms and 2477 pathology specimens were analysed post-intervention. 3518 individual pre-analytical errors were identified pre-intervention, and 3779 pre-analytical errors were identified post-intervention.

The most frequent error in this period was the absence of clinical notes provided on pathology forms and this was the highest request-based error occurring in both the pre-and post-intervention phases. 1158 pathology request forms from the ED were observed without patient clinical information pre-intervention, and 1177 post-intervention. Sample haemolysis was the highest specimen-based error with 34 samples haemolysed pre-intervention compared to 51 samples haemolysed post-intervention. Delay in specimen transport greater than an hour was the highest transport-based error with 85 pathology requests delayed by over an hour pre-intervention compared to 97 pathology requests post-intervention. Errors in patient demographics were the highest laboratory-based error with 83 pathology requests with this error pre-intervention and 84 pathology requests post-intervention.

***Table 9**. The pathology specimens analysed in the pre- and post-intervention phase.*

Specimen sample	Number Pre	Number Post
Blood gas (G4) samples	67	96
Serum samples	1141	1109
K-EDTA samples	1029	986
Coagulation samples	248	266
Transfusion samples	17	12
Fluoride oxalate	4	8

K-EDTA = Potassium ethylenediaminetetraacetic acid

***Table 10**. The pre-analytical error rate pre- and post- intervention and the statistical impact of the intervention.*

Basis of error	Type of pre-analytical error	Pre	Post	Statistically significant impact (p-value)
Request-based	No clinical notes provided	1158	1177	0.8805 NS
	No specimen collection time provided	305	418	0.1291 NS
	No collector signature	269	376	0.1662 NS
	No sorter signature	25	48	0.3514 NS
	Duplicate request forms for the same patient episode and incomplete requests over multiple forms.	94	98	0.8866 NS
	Duplicate test requests	13	25	0.1406 NS
	Delay in specimen collection >1hour	38	50	0.2481 NS
	Fasting status incorrect	262	227	0.5107 NS
	Inadequate information on request from regarding patient identification	18	17	0.8952 NS
	No drug regime information on request form	2	3	0.6704 NS
	Wrong test selected	1	3	0.3903 NS

Specimen-based	Haemolysed samples	34	51	0.2059 NS
	Blood gas sample type not specified	2	0	0.134 NS
	Arterial specimen requested for blood gas but venous sample collected	1	7	0.235 NS
	Transfusion samples rejected	1	2	0.5370 NS
	Inappropriately clotted sample	11	6	0.2148 NS
	Incorrect anti-coagulant ratio	1	5	0.3559 NS
	Insufficient sample volume	0	5	0.0025
	Incorrectly labelled sample	8	2	0.134 NS
Transport-based	Delay in specimen transport >1hour from collection	85	97	0.5763 NS
	Request received with no sample/s	7	12	0.3559 NS
	Samples received without request forms	10	2	0.3262 NS
	Avoidable delay in sample processing entering request forms > 1hour	13	25	0.1578 NS
Laboratory-based	Tests missed	20	22	0.8262 NS
	Misinterpretation of test requests	7	9	0.5504 NS
	Lost specimens	1	0	0.3559 NS
	Errors in patient demographics	83	84	0.9706 NS
	Missed copy doctor	22	26	0.6540 NS
	Clinical notes not typed	39	45	0.6876 NS
	Errors in patient billing	72	54	0.3819 NS
	Errors in patient identification between EPAS and ULTRA	0	3	0.1682 NS

EPAS = Enterprise Patient Administration System

Five individual categories showed the greatest decrease in pre-analytical errors post-intervention with the first number the pre-intervention and the second the post-intervention total. These were no patient signature (916/ 880), incorrectly labelled samples (8/2), fasting status incorrect (262/227), samples received without request forms (10/2), and errors in patient billing (72/54). Seven individual categories showed the highest increase in pre-analytical errors post-intervention: no specimen collection time provided (305/418), no collectors signature (269/376), no sorter signature (25/48), duplicate test requests (13/25), delay in specimen collection greater than an hour (38/50), haemolysed sample (34/51), arterial specimen requested for blood gas but venous sample collected (1/7), and avoidable delay in sample processing over an hour (13/ 25). Insufficient sample volume showed the greatest change in error rates post-intervention (0/5). The other 31 pre-analytical error categories did not demonstrate significant changes.

Discussion

Observational data discussion

12159 pre-analytical errors from 3775 pathology request forms were observed during the observational study. Of the 3775 pathology request forms, 2899 did not contain clinical notes. Clinical notes are considered a primary method for clinicians to communicate effectively with laboratory staff. Poor communication may lead to compromised patient safety, inefficient use of valuable resources, and an increase in the number of pre-analytical errors (Atay *et al* 2014; Vermeir *et al* 2015). Clinical notes may also help laboratory staff interpret abnormal results and differentiate these abnormal results from possible sample contamination, patient mismatches, or analyser miss-sampling. A quality control step could also be introduced in the ED to identify request-based errors with a staff member checking the pathology request form for the presence of clinical notes, the inclusion of a general practitioner to review the pathology results, and the presence of a patient signature before the transportation of the pathology request to the laboratory (Al Saleem and Al-Surimi 2016).

1851 of the 3775 pathology requests did not include a GP to review the pathology results, however after further investigation, a portion of these pathology requests did have a GP associated with EPAS for patient clinical summaries. A patient's healthcare journey does not end once the patient has been discharged from the ED as it is often up to the GP to follow up with the patient and ensure that their healthcare needs are met. Additionally, the pathology testing performed while the patient was in the ED may not ever be acted upon by the GP if they are unaware of these results. This may lead to duplicate pathology testing and increased wait times for the patient before a clinical diagnosis can be made. A patient may also be discharged before all the pathology testing has been completed resulting in under-investigation of their results. It is recommended that the hospital information systems require additional contingencies in place so that whenever possible a GP be added to all pathology requests to allow for increased patient care and better handover from the ED.

The entry of 46 copy doctors onto the LIS was missed by the laboratory during the data entry of pathology request forms despite these names being present on the request form. It was noted that many were missed when they were present on the add-on pathology request form from EPAS. During the period of the study, the copy doctors on EPAS add-on request forms were appearing in a small font. This small font along with the unfamiliarity of the copy doctor location on add-on request forms may explain why these were missed by the laboratory staff on request form entry. When changes are made to the request form or ordering program (Oacis/EPAS), sufficient training needs to be provided to the staff using that system or type of pathology form.

157 of the 3775 pathology request forms contained a duplicate request form for the same patient episode with incomplete pathology requests present over the multiple forms. The duplicate request forms would be transported to the laboratory at the same time and associated with a single set of pathology samples. These duplicate request forms also contributed to the 46 copy doctors missed during data entry onto the LIS by the laboratory, as the copy doctor may have only appeared on a single form. The laboratory's procedure was to handwrite all test requests and copy doctors to a single form to ensure that they were not missed by the data entry operators. The multiple forms were all scanned onto the imaging system for viewing during data entry, but the copy doctors would still be missed if not present on the primary form/all of the forms presented to the laboratory for the same patient episode.

152 of the 6482 (2.34%) pathology samples were haemolysed. Haemolysis is known to interfere with many pathology assays such as troponin T and potassium (Sodi *et al* 2006). A haemolysed sample may lead to a delay in a critical pathology result, it may cause a patient to be unnecessarily re-bled, it may significantly increase a patient's length of stay occupying an ED bed and increase the consumables costs of the hospital (West *et al* 2017). A clinician may even abandon the pathology testing altogether due to collection difficulties or time constraints, consequently altering the patient's clinical management due to the lack of clinical information (Karcher and Lehman 2014; Le *et al* 2016). The sample haemolysis rate of 2.34% is higher than the mean rate of sample rejection found by Karcher and Lehman (2014) of ≤ 1% of the total number of specimens received in chemistry and haematology laboratories. The Karcher and Lehman (2014) error rate is consistent with the studies performed by Carraro and Plebani (2007), Atay *et al* (2014) and Kumar *et al* in 2014, with error rates of 1.23%, 0.65%, and 0.309% respectively. This higher error rate suggests that there is room for improvement regarding collection procedures at this site (Karcher and Lehman 2014; Carraro and Plebani 2007; Atay *et al* 2014: Kumar *et al* 2014).

Currently, the ED staff collect pathology samples from an intravenous (IV) catheter Jelco system. This system was designed to administer fluids to a patient and not for phlebotomy purposes. Due to the functional design of the system, it may lyse and shred red blood cells when they are being forced through the system as it was not designed for bi-directional flow. Ideally, pathology samples should

be collected directly from a vein via venepuncture, but in scenarios where this is not possible, switching from an IV catheter Jelco system to a system that is bi-directional may reduce the number of haemolysed samples. A pathology-based training program focused on phlebotomy may increase the confidence of emergency staff to collect pathology specimens through venepuncture and reduce the number of haemolysed samples from the ED.

231 of the 3775 (6.12%) pathology requests from the ED were delayed by over an hour before they reached the laboratory for testing. A delay in transport could be attributed to many reasons such as insufficient staff numbers, ED staff forgetting or be too busy to page an orderly to collect the pathology sample, the orderly may be unavailable to collect the pathology samples due to other responsibilities (e.g. moving a patient to a ward, cleaning a theatre suite) the orderly may not be available after hours or on weekends or the paging system may be unresponsive. A pneumatic tube system from the ED to the laboratory would be a cost-effective mechanism to drastically decrease the transport time of pathology specimens from the ED to the laboratory thereby reducing the overall turnaround time (TAT) of pathology requests (Hayes *et al* 2014; Le Quellec *et al* 2017). Reducing the overall TAT would help the ED staff to admit or discharge patients more promptly, in turn helping the ED to reach the target set by the National Emergency Access Time of four hours and reduce operating costs (Hayes *et al* 2014; Sullivan *et al* 2016). It would also allow the ED and orderly staff to conduct other duties.

Forty-seven pathology test requests were missed by the laboratory. This could result in the pathology tests not being performed, a significant delay in the clinician receiving the result and the result not being followed up by the clinician. The laboratory must ensure that a quality control program is in place which has the necessary capability to identify missed tests or has sufficient workflow processes to detect these errors.

172 errors in patient demographics were observed making this the highest laboratory-based error. This includes errors in a patient's date of birth, gender, Medicare number, gestation status and address. This may lead to multiple patient entries onto the laboratory LIS for the same patient, incorrect patient information displayed on the pathology reports, incorrect reference ranges, errors in pathology billing, and difficulty communicating with the patient if required. If a patient has multiple pathology entries it may significantly decrease a clinician's ability to access all the relevant pathology requests previously performed for their patient as these requests may be split over the duplicate patient entries. This may lead to duplicate pathology ordering, delayed treatment, and decreased patient care. An integrated hospital information system with the LIS might potentially help to reduce errors in patient demographics however the information provided to the hospital by the patient may not agree with the patient's Medicare records.

The avoidable delay in pathology sample processing greater than an hour as seen in Table 7 (37 out of 3775). Efficient workflow processes, prioritisation of staff and adequate resources are recommended to process ED pathology requests within a reasonable timeframe. This may include assigning a staff member to focus solely on the ED pathology requests including processing and data entry, finding clear ways to differentiate the ED requests from no non-ED requests and ensuring that add-on requests are easily visible to all staff.

Survey discussion

The survey responses show that the staff at the hospital ED were aware that inappropriate collection, tube type, and anticoagulant ratio can significantly affect laboratory results. The laboratory also informed the ED staff when a sample was rejected and the reason/s why it was rejected. Although the ED staff surveyed were aware of inappropriate collection methods this did not mean that specimen-based errors did not occur, as seen by the number of specimen/phlebotomy errors listed in Table 7.

The third survey question asked if staff were aware that samples required a certain number of inversions to ensure the effectiveness of the clot-activator or anticoagulant when using a non-vacutainer collection method. Five participants strongly agreed, two agreed, and one disagreed. The one response that disagreed shows that there is a potential lack of understanding by the ED staff regarding anticoagulants and clot-activators in pathology specimen tubes. If some of the ED staff did not understand how clot-activators or anticoagulants worked, it could explain the number of pre-analytical errors associated with inappropriately clotted specimens and the number of pathology specimens with an incorrect anticoagulant ratio for testing (Table 7). This survey question helped focus the training of staff during the intervention phase of the experiment.

The response to Q4 had nine responses with three responses that strongly agreed and six responses that agreed. All survey responses showed that staff who were surveyed understood the inherent risk to sample quality when collecting pathology samples through an IV catheter. This process was the standard for collecting pathology samples from a patient in the ED. The reasoning given by the ED staff was that it is easier to collect blood samples from the IV catheter Jelco system as the line was already attached to the patient. The staff were also reluctant to

take blood from a fresh venepuncture site if a line was already open to them. The observational study showed that 152 samples were haemolysed and one sample was contaminated (Table 7). The high rate of haemolysis seen in the observational study may in part be due to the use of the IV catheter Jelco collection process for pathology specimens.

Responses to Q5 showed that eight of the nine staff surveyed were aware of other pre-analytical factors that may affect pathology results with one response undecided. This suggests that some ED staff may be unsure how some pathology testing may be affected by temperature, time delay, etc.

Q6 showed a wide variance of responses. One response totally agreed, four responses agreed, three responses were undecided and one response disagreed. The wide variance of responses suggests that the ED staff surveyed were unsure of what a laboratory-run pre-analytical training program would entail or teach. The consensus from the survey results showed however that ED staff would be receptive to participating in a pathology-run training program. Training and education of staff involved in the pre-analytical process regarding pre-analytical errors and their potential impact is a crucial step in reducing these errors (Kumar *et al* 2014; Narang *et al* 2016).

Q7 was answered by three of the staff members surveyed. The responses to the question showed that one of the staff members surveyed had a good grasp on their abilities regarding pre-analytical errors as seen by their response 'I do not have any concerns for my own ability, if I have any doubt, I refer to the collection handbook on the intranet or speak to someone in the lab'. The response of 'Human error. Incorrect documentation' showed that this staff member understands that human error plays a role in all pre-analytical errors and that incorrect documentation may lead to errors in patient demographics.

The final survey question only had two responses with one response "Appropriate education for individuals who have lack of knowledge of sample collection/transport conditions" highlighting the need to educate staff who participate in the pre-analytical process.

Nine survey responses would not constitute a standard distribution of responses from the ED staff. More ED staff members would need to complete the survey to ensure a more accurate picture of the questions asked. A survey targeting the laboratory should also be developed to assess the laboratory staff's understanding of pre-analytical errors and how to reduce them.

Intervention discussion

The intervention could not target all the pre-analytical errors listed in Table 1 as some of these errors would be unaffected by continuing education and instead are due to random/systematic errors such as patient fasting status. Laboratory-based errors were not specifically targeted by the intervention as the intervention targeted the ED staff members. Thirty-two pre-analytical error categories were analysed pre and post-intervention and of the 32 pre-analytical error categories analysed, only one category was shown to have a statistically significant change post-intervention. Insufficient sample volume was the only statistically significant result recorded post-intervention with a p-value of 0.0025. Zero pathology samples were observed to have insufficient sample volume for pathology testing pre-intervention versus five pathology samples post-intervention. This may be due to a variety of reasons including, difficult collection, inexperienced/new staff members, and a high number of tests requested per patient episode (Bhat *et al* 2012; Le *et al* 2016).

The intervention did not significantly reduce the highest occurring pre-analytical errors (request-based, specimen-based, transport-based, or laboratory-based). Five individual categories showed the biggest decrease in pre-analytical errors post-intervention and these categories were: no patient signature, incorrectly labelled pathology samples, incorrect fasting status, samples received without a request form, and errors in patient billing. No patient signature, incorrectly labelled pathology samples, and samples received without a request form were specifically targeted by the intervention. Incorrect fasting status would not have been altered by the intervention even though this was covered in the training provided, as patients who attend the ED are not generally in a fasting state. Errors in patient billing were not specifically targeted by the intervention either as the intervention did not target laboratory practices. Interventions tailored specifically for the laboratory, such as a laboratory information system training program, would be the next step in continuing education to reduce the number of laboratory-based pre-analytical errors. It has been previously demonstrated that a commitment to a pre-analytical training program can drastically reduce request-based errors and specimen-based errors (Carraro and Plebani 2007; Kumar *et al* 2014; Osegbe *et al* 2016).

Seven individual categories showed the biggest increase in pre-analytical errors post-intervention. These categories were no specimen collection time provided, no collector signature, no sorter signature, duplicate test requests, delay in specimen collection greater than an hour, sample haemolysis, arterial specimen requested but venous sample collected, and avoidable delay in sample

processing greater than an hour. All these categories were specifically targeted by the intervention training program. An increase in these pre-analytical errors suggests that the intervention training program was incomplete and unsuccessful. This may be because the intervention was conducted as a one-time session, thereby missing the night shift staff, on-duty staff, any staff not working that day, and the laboratory staff involved in the pre-analytical processes. As only one intervention session was provided to the hospital emergency staff any significant follow-up for the staff that attended the session was limited. Follow-up with individual staff members would allow for more targeted pre-analytical error reduction through targeted training. To improve the outcomes of the intervention, regular training interventions would need to be conducted. The interventions could take place on a monthly or bi-monthly basis, and they would target the ED and laboratory staff. A possible change in the ED staff members may have also reduced the effectiveness of the intervention as some staff may have left the hospital post-intervention and some staff may have started after the intervention phase was already complete.

A laboratory induction of new staff members working in the ED would allow the laboratory to educate these staff members on specimen collection issues, request forms, sample types, procedures, and other potential pre-analytical errors. This would help raise staff awareness regarding pre-analytical processes as well as inform the staff about common pre-analytical errors and their effect on the TTP (Atay *et al* 2014; Piva *et al* 2015; Tapper *et al* 2017). Gunnur Dikmen *et al* (2015) reasoned that healthcare sites that contain a high turnover and an inadequate training/induction system would increase the number of pre-analytical errors seen. An intervention program targeting specific laboratory-based pre-analytical errors would need to be developed to ensure that the laboratory staff are also involved in the process (Gunnur Dikmen *et al* 2015).

A continuation of this study with increased interventions is warranted to reduce the total number of pre-analytical errors occurring at the hospital ED and laboratory. A reduction in the number of pre-analytical errors would increase patient safety, decrease TATs for pathology results, reduce patient length of stay, reduce duplication in workflow, decrease the time spent by staff fixing pre-analytical errors, and reduce overall hospital operational costs (Da 2010; Kaushik and Green 2014; West *et al* 2017; Morias *et al* 2018). A study by Kaushik and Green (2014) estimated that 0.23% to 1.2% of hospital operating costs can be attributed to pre-analytical errors (Kaushik and Green 2014). Pre-analytical errors should be prevented, but the cost associated with preventing the error may outweigh the benefits. If this is the case, then the resources should be focused on preventing the errors that may cause the greatest patient harm or may provide the greatest benefit to the hospital/laboratory (Carraro and Plebani 2007; Lippi and Guidi 2007).

Limitations

The pre-analytical error data gathered from the observational and intervention studies were only from one site and therefore it would not be practical to infer that the error rates are reflective of other EDs and their associated laboratories. Repeating the observational study and intervention study at multiple sites would allow for the observation of trends and a baseline number of pre-analytical errors to be established. Some errors detected in this study were not detected by the normal quality procedures of the laboratory. The current laboratory quality system is inadequate for detecting all the pre-analytical errors that occurred during this studyand some of the errors detected may have been inconsequential.

Conclusion

The observational study found 12159 unique pre-analytical errors over four different categories suggesting that an intervention targeting these pre-analytical errors was warranted. The survey response data showed that the hospital ED staff were open to a laboratory training program targeting pre-analytical errors. Although almost no statistically significant difference was observed in the number of pre-analytical errors pre- and post-intervention, this study has identified key areas of future intervention. These interventions would include a monthly/bi-monthly laboratory-run intervention program targeting the ED and laboratory staff, the installation of a pneumatic tube system to decrease TATs for laboratory testing, upgrading to bi-directional IV catheters, and improved laboratory workflows. These interventions would lead to better patient outcomes, decreased ED operating costs, and a better working relationship between the ED and the pathology laboratory.

Conflicts of interest and funding disclosure

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References

Agarwal R, Chaturvedi S, Chhillar N, Goyal R, Pant I, Tripathi CB 2012. Role of intervention on laboratory performance: Evaluation of quality indicators in a tertiary care hospital. *Indian J Clin Biochem* 27: 61-68.

Al Saleem N, Al-Surimi K 2016. Reducing the occurrence of errors in a laboratory's specimen receiving and processing department. *BMJ Open Quality* 5: u211474. w214624.

Atay A, Demir L, Cuhadar S, Saglam G, Unal H, Aksun S, Arslan B, Ozkan A, Sutcu R 2014. Clinical biochemistry laboratory rejection rates due to various types of preanalytical errors. *Biochemia medica* 24: 376-382.

Bhat V, Tiwari M, Chavan P, Kelkar R 2012. Analysis of laboratory sample rejections in the pre-analytical stage at an oncology center. *Clin Chim Acta* 413: 1203-1206.

Cao L, Chen M, Phipps RA, Del Guidice RE, Handy BC, Wagar EA, Meng QH 2016. Causes and impact of specimen rejection in a clinical chemistry laboratory. *Clin Chim Acta* 458: 154-158.

Carraro P, Plebani M 2007. Errors in a stat laboratory: types and frequencies 10 years later. *Clin Chem* 53: 1338-1342.

Chawla R, Goswami B, Singh B, Chawla A, Gupta VK, Mallika V 2010. Evaluating laboratory performance with quality indicators. *Lab Med* 41: 297-300.

Da RG 2010. Pre-analytical workstations as a tool for reducing laboratory errors. *J Med Biochem* 29: 315-324.

Gunnur Dikmen Z, Pinar A, Akbiyik F 2015. Specimen rejection in laboratory medicine: Necessary for patient safety? *Biochemia Medica* 25: 377-385.

Hayes KJ, Reed N, Fitzgerald A, Watt V 2014. Applying lean flows in pathology laboratory remodelling. *J Health Org Manag* 28: 229-246.

Kagan I, Barnoy S 2013. Organizational safety culture and medical error reporting by Israeli nurses. *J Nurs Scholarsh* 45: 273-280.

Karcher DS, Lehman CM 2014. Clinical consequences of specimen rejection: a College of American Pathologists Q-Probes analysis of 78 clinical laboratories. *Arch Pathol Lab Med* 138: 1003-1008.

Kaushik N, Green S 2014. Pre-analytical errors: their impact and how to minimize them. *MLO Med Lab Obs* 46: 22-24.

Kumar SA, Jayanna P, Prabhudesai S, Kumar A 2014. Evaluation of quality indicators in a laboratory supporting tertiary cancer care facilities in India. *Lab Med* 45: 272-277.

Le Quellec S, Paris M, Nougier C, Sobas F, Rugeri L, Girard S, Bordet J-C, Négrier C, Dargaud Y 2017. Pre-analytical effects of pneumatic tube system transport on routine haematology and coagulation tests, global coagulation assays and platelet function assays. *Thromb Res* 153: 7-13.

Le RD, Melanson SE, Petrides AK, Goonan EM, Bixho I, Landman AB, Brogan AM, Bates DW, Tanasijevic MJ 2016. Significant reduction in preanalytical errors for nonphlebotomy blood draws after implementation of a novel integrated specimen collection module. *Am J Clin Pathol* 146: 456-461.

Lillo R, Salinas M, López Garrigós M, Naranjo Santana Y, Gutiérrez M, Marín MD, Miralles M, Uris Sellés J 2012. Reducing preanalytical laboratory sample errors through educational and technological interventions. *Clin Lab* 58: 9-10.

Lippi G, Guidi GC 2007. Risk management in the preanalytical phase of laboratory testing. *De Gruyter* 45: 720-727.

Marin AG, Rivas-Ruiz F, del Mar Pérez-Hidalgo M, Molina-Mendoza P 2014. Pre-analytical errors management in the clinical laboratory: a five-year study. through educational and technological interventions. *Biochemia medica* 24: 248-257.

Morias C, Palmer G, Santhakumar A 2018. Pre-analytical errors and patient outcomes in the total testing process. *Aust J Med Sci* 39: 14-27.

Najat D 2017. Prevalence of pre-analytical errors in clinical chemistry diagnostic labs in Sulaimani city of Iraqi Kurdistan. *PLoS One* 12: e0170211.

Narang V, Kaur H, Selhi PK, Sood N, Singh A 2016. Preanalytical errors in hematology laboratory-an avoidable incompetence. *Iran J Pathol* 11: 151.

Osegbe I, Afolabi O, Onyenekwu C 2016. The effectiveness of clinician education on the adequate completion of laboratory test request forms at a tertiary hospital. *Ann Med Health Sci Res* 6: 90-94.

Piva E, Tosato F, Plebani M 2015. Pre-analytical phase: The automated ProTube device supports quality assurance in the phlebotomy process. *Clin Chim Acta* 451: 287-291.

Plebani M 2012. Quality indicators to detect pre-analytical errors in laboratory testing. *Clin Biochem Rev* 33: 85.

Sodi R, Darn SM, Davison AS, Stott A, Shenkin A 2006. Mechanism of interference by haemolysis in the cardiac troponin T immunoassay. *Ann Clin Biochem* 43: 49-56.

Sullivan C, Staib A, Khanna S, Good NM, Boyle J, Cattell R, Heiniger L, Griffin BR, Bell AJ, Lind J 2016. The National Emergency Access Target (NEAT) and the 4-hour rule: time to review the target. *Med J Aust* 204: 354-354.

Sullivan P, Cruise K, Callahan CD 2013. Lean six sigma methods reduce and sustain troponin turnaround time (TAT) for emergency department patients. *Am J Clin Pathol* 140: A237-A237.

Tapper MA, Pethick JC, Dilworth LL, McGrowder DA 2017. Pre-analytical errors at the chemical pathology laboratory of a teaching hospital. *Journal of clinical and diagnostic research: JCDR* 11: BC16.

Vanker N, van Wyk J, Zemlin AE, Erasmus RT 2010. A Six Sigma approach to the rate and clinical effect of registration errors in a laboratory. *J Clin Pathol* 63: 434-437.

Vermeir P, Vandijck D, Degroote S, Peleman R, Verhaeghe R, Mortier E, Hallaert G, Van Daele S, Buylaert W, Vogelaers D 2015. Communication in healthcare: a narrative review of the literature and practical recommendations. *Int J Clin Pract* 69: 1257-1267.

West J, Atherton J, Costelloe SJ, Pourmahram G, Stretton A, Cornes M 2017. Preanalytical errors in medical laboratories: a review of the available methodologies of data collection and analysis. *Ann Clin Biochem* 54: 14-19.

Effectiveness of oral iron supplementation for treatment of iron deficiency anaemia

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Abstract

Iron deficiency anaemia affects approximately 25% of the world's population. It is a major cause of morbidity and financial burden on healthcare provision, both in Australia and worldwide. Iron deficiency without anaemia even more common. Treatment often begins with supplementation of oral iron tablets to replenish the total iron stores in the body and to correct anaemia. There is limited evidence about the benefits of giving iron to people who do not have anaemia. Some patients, including those who have not responded to oral supplements may benefit from intravenous iron and further investigations are needed if the iron deficiency has not been corrected. The efficacy of iron supplementation is dependent on numerous factors and this has been a field of interest for researchers. This literature review explores the effectiveness of oral iron supplementation for treatment of iron deficiency anaemia and factors affecting its efficacy.

Keywords: ***iron deficiency anaemia, oral iron supplementation, iron sulphate, elemental iron, intravenous infusion, bioavailability***

Introduction

Iron deficiency is one of the most common causes of anaemia globally, particularly in developing countries such as Africa and some parts of Asia. Iron deficiency anaemia (IDA) is a major cause of morbidity and financial burden on healthcare provision, and is the cause of approximately 17% of the anaemias prevalent in the world's population (Pasricha *et al* 2021). The World Health Organization (WHO) estimates that 8% of preschool children, 12% of pregnant women and 15% of non-pregnant women of reproductive age in Australia have anaemia, with iron deficiency being the major cause (Pasricha *et al* 2010). Anaemia is highly prevalent in indigenous communities and is an ongoing health issue. Studies of an Aboriginal community in Western Australia identified anaemia among 55% of women and 18% of men (Hopkins *et al* 1997). More than 2 decades later again in Far North Queensland and elsewhere in remote northern Australia, it was again shown that 55% of pregnant indigenous women had anaemia (Leonard *et al* 2018). The prevalence of IDA is higher in indigenous populations, especially adolescent females, with approximately 25% of adolescent indigenous females having IDA in comparison to 8-10% non-indigenous adolescent females suffering from IDA (Department of Health 2019). Although it is three times as common as iron deficiency anaemia, iron deficiency without anaemia is an under-recognised and undertreated condition (Ioannou *et al* 2002; Balendran 2021).

IDA disproportionately affects children and adult females (Coad 2014). Numerous treatment options are available but for many decades oral iron supplementation has been the first choice of treatment. Advancements in other treatment options such as intravenous iron infusion has brought into question the efficacy of oral iron supplementation. The effectiveness of oral iron is dependent on bioavailability which is the proportion of elemental iron that is absorbed from the tablet. The bioavailability of oral iron is affected by various factors such as diet, choice of the iron compound, and dosage, as well as dosing frequency, and will be discussed in this review with the aim to determine the most effective treatment protocol. The scope of this research is only on the effectiveness of oral iron supplementation and therefore other treatment options will not be reviewed in detail and only be referred to as appropriate.

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Background

Iron deficiency

Iron deficiency is categorised into three stages: iron depletion, iron deficient erythropoiesis, and iron deficiency anaemia (Brittenham 2018; Short and Domagalski 2013). Iron depletion occurs when the iron stored in the body is reduced but has little effect on the level of functional iron compounds. Iron deficient erythropoiesis is the restriction of haemoglobin production due to exhaustion of iron storage, which also limits the production of metabolically active compounds that require iron (Brittenham 2018; Abbaspour *et al* 2014). At this stage, haemoglobin and haematocrit concentration will start to decrease gradually, but the morphology of erythrocytes is unaffected (Means 2020). In addition, other measures such as concentration of serum iron, transferrin saturation, and ferritin also start to decline, while serum transferrin concentration and TIBC increases. Further reduction of total iron content will lead to a reduction of haemoglobin by up to 2 mg/dL (20 g/L) or more, and eventually the development of iron deficiency anaemia (Means 2020).

Causes of iron deficiency anaemia

IDA is usually an acquired condition where it can occur as an isolated disorder, secondary to a single primary cause or even because of multiple pathological disorders (Camaschella 2019). The primary cause of IDA is due to insufficient intake of bioavailable iron, along with increased demand for iron due to growth, pregnancy, and menstruation. The popularisation of vegan or vegetarian diets further contributes to decreased iron intake, especially among young females (Abbaspour *et al* 2014). Blood loss is another common cause of IDA, from heavy menstruation in premenopausal women or chronic gastrointestinal bleeding caused by pathologic infection such as *Helicobacter pylori* infection commonly seen in the elderly (Department of Health 2019). Excess blood loss requires immediate attention, as each millilitre of blood lost results in approximately 0.5mg of iron loss (Abbaspour *et al* 2014; Short and Domagalski 2013). In some cases, particularly in postmenopausal women and men, chronic gastrointestinal bleeding with the coexistence of intestinal diseases such as coeliac disease and cancer is seen. This results in dysfunction of gastric acid secretion, and impaired iron absorption, consequently decreasing iron uptake (Department of Health, 2019; Short and Domagalski 2013).

From infancy to adolescence, iron deficiency is mainly due to the increase in blood volume and lean body mass required for growth (Department of Health, 2019; Abbaspour *et al* 2014). Infants up to four months old are in a state of “iron feast” (Subramaniam and Girish 2015) where the iron requirement is highest due to rapid growth and unless approximately 30% (about 0.9-1.3 mg/kg body weight) of iron is supplied from the diet (Domellof 2011) iron deficiency may develop resulting in decreased haemoglobin from approximately 170 g/L at birth to 110 g/L at two months of age (Subramaniam and Girish 2015). But the diet of this age group though is usually low in iron and therefore iron deficiency is common. Iron therapy at an early stage is critical in this age group as iron deficiency can lead to irreversible neurodevelopmental and cognitive defects (Subramaniam and Girish 2015).

The prevalence of IDA is higher in women than men, particularly in younger populations including children, adolescents, and women of reproductive age, as well as pregnant females (Abbaspour *et al* 2014). Each age group experiences different physiological changes and therefore their iron requirement will also differ (Table 1).

***Table 1**. Iron requirement in different age groups (National Health and Medical Research Council 2014).*

Age group	Estimated daily requirement (mg/day)	Recommended daily intake (mg/day)
Adolescent (9-18 years old)	6-8	6-15
Adult males (19-50 years old)	8	18
Adult males (51-70 years old)	5	8
Menstruating women (14-50 years old)	8	11-18
Pregnant women	22	27
Postmenopausal women	5	8

In menstruating females the average daily iron loss is increased to approximately 2 mg each day (National Health and Medical Research Council 2014). Depending on the patient's haemoglobin and haematocrit concentration, as well as their menstruation pattern, iron loss during menstruation can vary from 25-50 mg per cycle (Means 2020).

Pregnancy results in a significant increase in daily iron requirement to compensate for placental growth, rise in the red cell mass of the mother, and fetal erythropoiesis (Department of Health 2019; Abbaspour *et al* 2014). In comparison, postmenopausal women require a relatively low amount. In adults, only 5% of iron replenishment is required from the diet, as the breaking down of old red blood cells by macrophages act as a recycling system of iron, and therefore this age group is at relatively low risk (Subramaniam and Girish 2015; Abbaspour *et al* 2014).

IDA diagnosis

IDA is defined as iron stores and haemoglobin levels of two standard deviations below the normal level (Short and Domagalski 2013). Diagnosis of IDA is usually confirmed by full blood examination and serum ferritin concentration. Serum ferritin concentration is reflective of the total iron stores and is considered the most accurate measure of iron status (Department of Health 2019). Iron is stored in insoluble forms in the liver, spleen, and bone marrow. It should be noted that ferritin concentration can increase during infection or inflammation, regardless of a change in the level of iron stores as it is an acute-phase protein (Abbaspour *et al* 2014). This increase in ferritin due to infection or inflammation may mask the iron deficiency in the patient.

The normal level of iron stored in the body is confirmed by the concentration of serum ferritin of > 15 µg/L. In contrast, a low level of 12 µg/L for children under 5, and < 15 µg/L for over 5 years old is indicative of iron depletion (Abbaspour *et al* 2014). As stated by WHO, an individual is diagnosed with anaemia when the hemoglobin level is 110 g/L in men and non-pregnant females and < 70-100 g/L in pregnant women (World Health Organization 2011). In conjunction, a blood iron concentration of < 12 µmol/L confirms the diagnosis of IDA (National Heart, Lung, and Blood Institute 2018; Camaschella 2015a). In case of iron deficiency without anaemia, the serum ferritin level is usually < 30 µg/L with a normal hemoglobin level (Camaschella 2015b).

In the elderly including men and postmenopausal women, routine screening, and endoscopic examination can be performed to confirm the other causes of iron deficiency such as coeliac disease and gastrointestinal bleeding or lesions (Pasricha *et al* 2021).

IDA treatment

The aim of treatment for IDA is to manage the underlying condition causing anaemia and iron loss, as well as to replenish iron stores (Elstrott *et al* 2020). Treatment options for IDA will differ in each patient depending on their gender, age, and the presence or absence of primary pathology leading to anaemia. The severity of anaemia or iron deficiency and symptoms, as well as the time available for correction of the iron store, should also be considered (Cappellini *et al* 2020).

Treatment options for IDA include oral iron supplementation, intravenous iron therapy, and red cell transfusion (Department of Health 2019; Elstrott *et al* 2020). In most cases, patients with IDA are treated with oral iron supplementation first. Other treatment options such as IV infusion (Ning 2011) should be considered when the patient is nonresponsive or intolerant of oral iron therapy.

The effectiveness of oral iron supplementation is dependent on various factors as these affect the bioavailability, which is the proportion of absorbable iron content in the oral supplement that can be utilized for erythrocytosis (Stoffel *et al* 2020). In this review, the choice of the iron compound, dosage, and dosing frequency, as well as dietary interference with iron absorption is discussed.

Choice of the iron compound

In recent years, the number of supplement preparations has increased, with more than 100 formulations available over the counter in Australia. Only a handful however contain sufficient elemental iron to treat IDA (Department of Health 2019). The most common formulations used are ferrous sulfate and ferrous fumarate. Their bioavailability, effect on the regeneration of haemoglobin and the possible side effects are similar but differ in the elemental iron content. Ferrous sulfate contains the highest amount of elemental iron with 350 mg (Stoffel *et al* 2020; Santiago 2012). It is given as first-line treatment in most cases due to its high bioavailability and efficacy, along with its low cost (Abbaspour *et al* 2014; Santiago 2012). Ferrous sulfate is however one of the compounds that often cause adverse side effects, especially when given in high dosage. Common side effects include damage to the gastrointestinal lumen resulting in epigastric pain, nausea, and constipation. This leads to reduced compliance with the treatment by 30-70% (Stoffel *et al* 2020). In addition, it has also been shown that plasma malondialdehyde may be increased by ferrous sulfate, which is indicative of lipid peroxidation (Zhu *et al* 2016; Marks *et al* 2014). This can damage tissues by increasing oxidative stress in organs, particularly in the liver, where iron metabolism takes

place. To compensate for this problem, the use of ferric sodium ethylenediaminetetraacetic acid (FeNa-EDTA) in replacement of ferrous sulfate has been proposed by the International Nutritional Anaemia Consultative Group (Zhu *et al* 2016). FeNa-EDTA is an iron compound that can be metabolised without interfering with phytates, an iron inhibitor found in cereals and legumes (Department of Health 2019; Zhu *et al* 2016). This allows a 2-3 times increase in absorption in comparison with other iron compounds and is regarded as the most favourable iron compound, particularly in developing countries where a plant-based diet is common (Zhu *et al* 2016). Recent studies have found that hematological status in pregnant women has been improved with moderate dosing of FeNa-EDTA (Wagner and Baran 2010).

Carbonyl iron contains over 98% of elemental iron and has traditionally been used for food additives as well as a part of mineral supplementation (Zhu *et al* 2016). A study conducted by Marks *et al* (2014) has shown that over the course of eight weeks, 45 mg of carbonyl iron supplementation successfully replenished haemoglobin concentration and was successful at treating IDA (Marks *et al* 2014). Due to its low bioavailability and its efficacy being high-dose dependent, it is not suited for daily use.

Other iron compounds, such as ferric iron-polysaccharide complexes, are also used for treatment, which is claimed to be more palatable and with reduced side effects, but there are no sufficient studies that show concrete evidence of efficacy (Stoffel *et al* 2020).

Dosage and dosing frequency

The current recommended dose of oral iron for the treatment of IDA widely varies, ranging from 60-220 mg/day (Department of Health 2019; Stoffel *et al* 2020; Camaschella 2019). However, numerous studies have shown that lower doses are more effective than the recommended higher dosing, exhibit fewer side effects in women and children, and are therefore better tolerated (Department of Health 2019; Camaschella 2019). In children, this ranges from 3-6 mg elemental iron per day and 20-50 mg daily for adults (Department of Health 2019; Stoffel *et al* 2020). There is no current evidence to show that the presence of side effects is dose dependent.

Even though some iron compounds are effective at high dosage, the fraction of iron that is absorbed from high-dose supplementation is shown to be low, which indicates that a large proportion will be unabsorbed (Stoffel *et al* 2020). Unabsorbed iron poses problems as it can form free radicals in the body. The excess unabsorbed iron and free radicals damage tissues and disrupt the microbiota of the gut, altering the composition of protective bacteria (Abbaspour *et al* 2014; Stoffel *et al* 2020; Rusu *et al* 2020). This reduces the population of beneficial bacteria such as Lactobacillus and Bifidobacterium (Camaschella 2019). It also enhances opportunistic pathogens such as Enterobacteriaceae, which can cause gut irritation, inflammation, and dysbiosis (Abbaspour *et al* 2014; Stoffel *et al* 2020). Upon contact with oxygen, unabsorbed iron in the body can also form insoluble oxides that can accumulate in the body (Abbaspour *et al* 2014).

The recommended dosing frequency also varies widely, with some suggesting single-day dosing and others recommend divided dosing (Department of Health 2019; Stoffel *et al* 2020). A low dose of approximately 30-60 mg of elemental iron is usually prescribed in adults either daily or intermittently (varies from every second day to weekly). Though intermittent dosing is recommended by WHO, especially in developing countries, both are shown to be effective in mild IDA patients who show side effects at higher dosing. There is no oral supplement formulation that contains the recommended amount of iron in Australia currently, and therefore this approach is only available in some countries (Department of Health 2019).

The relationship between dosage of oral tablet dosing frequency and impact on hepcidin stimulation has been an area of interest for researchers. Hepcidin is a peptide hormone secreted by hepatocytes in the liver. Figure 1 illustrates the relationship between the level of iron and hepcidin secretion in the liver.

Hepcidin regulates iron absorption as well as its usage and storage by binding to ferroportin (Abbaspour *et al* 2014; Brittenham 2018). This binding stimulates ferroportin metabolism that reduces cell surface ferroportin (Figure 1B). Decreased ferroportin limits the entry of iron from sites such as macrophages, hepatocytes, and intestinal enterocytes into plasma resulting in low transferrin saturation and restricting the delivery of iron to erythrocyte precursors (Abbaspour *et al* 2014; Brittenham 2018). It should be noted that even a mild increase in serum iron can activate hepcidin (Camaschella 2019). One study has shown that high oral iron administration of 60-100 mg in IDA patients can stimulate an acute increase in circulating hepcidin, especially when given on consecutive days (Stoffel *et al* 2020; Elstrott *et al* 2020). Consequently, an increase in hepcidin concentration caused by iron supplementation reduces the bioavailability of elemental iron and restricts the absorption of iron from the supplement, and therefore high dose and frequent dosing can be ineffective (Elstrott *et al* 2020; Pasricha *et al* 2021; Camaschella 2019). It was concluded that alternate dosing of oral iron of >60 mg should be administered to maximise iron absorption (Stoffel *et al* 2020).

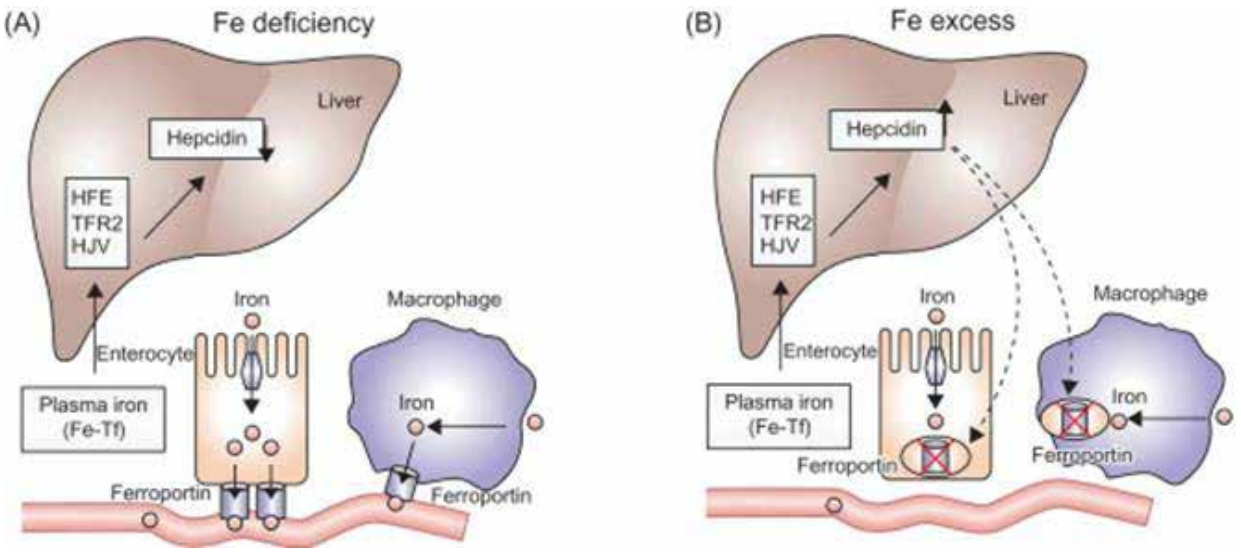

Figure 1. *Relationship between the level of iron and hepcidin secretion in the liver. From Human Biochemistry (1st ed, p. 601), by G. Litwack, 2018, Elsevier. (https://www.sciencedirect.com/science/article/pii/B9780123838643000193)*

Another study conducted by Stoffel *et al* (2017) has focused on the dosing frequency. Forty women were divided into two groups of 20, one group with high frequent dosing (1 set dose of supplementation for 14 consecutive days), and the other with low-frequency dosing (the same number of doses but dosing frequency spread across 28 days) (Figure 2A). The total and fractional iron absorption was measured in both groups and the group with less frequent dosing had improved iron absorption, both fractional and total (Stoffel *et al* 2017). When the scope of research was shifted to the dosage number, where the timeframe was set to 2 weeks for both groups, higher total iron absorption was achieved by the group with high dosing frequency (Figure 2B) (Stoffel *et al* 2017). From this study, it is apparent that oral supplementation is most effective when given intermittently (every second day) in high dosage.

While numerous studies have been conducted to show efficacy for both low and high dosages and frequencies, there is still no definite recommendation that can be applied to all patients. While treatment of IDA by oral iron therapy has been the gold standard for many years, the standardization of treatment protocol has been challenging due to lack of evidence (Elstrott *et al* 2020).

Effects of diet

The bioavailability of oral iron supplementation can vary greatly depending on whether it is taken with or without a meal as various dietary elements can interact with iron absorption. Generally, oral iron supplements should be taken on an empty stomach, at least an hour before a meal to maximise iron absorption and to avoid interference with food or beverages which lead to the formation of nonabsorbable complexes in the gut. Consumption of iron supplementation when fasting has a relatively high fractional iron absorption of 5-28%, whereas only 2-13% absorption is achieved when consumed with food (Abbaspour *et al* 2014; Stoffel *et al* 2020). Though it is ideal to take oral iron without food, it is not realistic as many patients experience side effects such as nausea and epigastric pain, especially when given a high iron dosage (Abbaspour *et al* 2014). Whole grains or pulses are one of the common foods that interfere with iron absorption. They are commonly incorporated into a plant-based diet and have high phytic acid and phytate content which inhibits iron absorption even when at low concentrations (Stoffel *et al* 2020; Abbaspour *et al* 2014). Polyphenol, found in various beverages including coffee, black tea, herbal tea, red wine, and hot chocolate, as well as in some plant food, also impairs iron absorption in the stomach (Abbaspour *et al* 2014; Lynch *et al* 2018). While many iron inhibitors interfere with nonheme iron absorption, calcium, mainly contained in dairy products, can inhibit both heme and nonheme iron absorption (Abbaspour *et al* 2014).

Iron supplements taken with food or other supplements rich in ascorbic acid can enhance iron absorption. Ascorbic acid, (Vitamin C) is able to reduce ferric (Fe3+) to ferrous (Fe2+) iron in the gastrointestinal lumen. It also has the potential to chelate iron from iron absorption inhibitors including phytate, polyphenols and calcium (Abbaspour *et al* 2014; Stoffel *et al* 2020). This effect however is dose dependent.

On top of diets, medications that reduce gastric acidity usually prescribed to treat conditions such as heartburn and gastritis gastroesophageal reflux disease, should also be avoided as they may impair iron absorption (Ning and

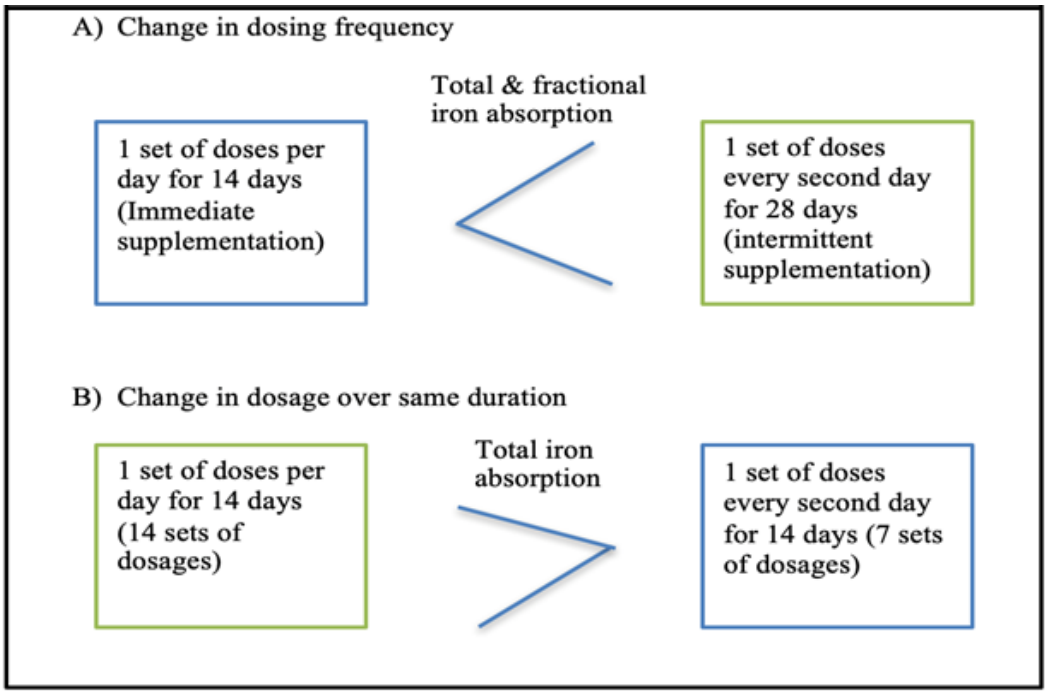

***Figure 2**. Summary of results presented by Stoffel et al (2017).*

Zeller 2019). These include antacids, histamine-2 blockers, and proton pump inhibitors that are readily available over the counter (U.S. Food and Drug Administration 2021).

Intravenous iron infusion

Intravenous (IV) iron infusion is another common treatment option for IDA and its efficacy has been researched for many years. IV iron therapy is often administered for patients who are nonresponsive to oral iron supplementation despite increase in dosage and dosing frequency or change in iron compounds (Cappellini *et al* 2020). Patients who are intolerant to iron and experience severe side effects should also be transitioned to IV infusion. A meta-analysis conducted by Moore *et al* (2011) comparing oral supplementation and IV iron has reported that adverse gastrointestinal side effects were experienced by only 13% of patients who received IV iron in comparison to 32% by patients treated with oral iron supplementation (Moore 2011). In addition, IV iron may be preferred over oral iron in patients with a known condition causing gastrointestinal malabsorption, such as inflammatory bowel disease, chronic kidney disease, renal failure, and in some cases, heavy chronic blood loss (Elstrott *et al* 2020).

In recent studies, Elstrott (2020) and Ning (2019) have shown that IV iron infusion can provide faster resolution to IDA compared to oral supplementation and therefore are considered more effective (Elstrott *et al* 2020: Ning 2019). IV iron therapy is found to be particularly effective for pregnant females as it can achieve a more complete correction of haemoglobin concentration (Department of Health 2019; Means 2020). In addition, it has been found that IV iron can rapidly correct haemoglobin and haematocrit levels synchronously and are able to produce a sustained haemoglobin response (Means 2020; Okam 2016).

In the past, IV iron therapy has been avoided mainly due to the risk of causing anaphylaxis in patients (Deloughery 2019). Newer and safer formulations have been developed and can be administered at a relatively low cost (Department of Health 2019). The current formulations of IV iron available in Australia are iron polymaltose and iron sucrose (Pasricha *et al* 2010). Iron polymaltose is the most preferred formulation as it is the only formulation that allows a "total-dose" infusion of 1000-2500 mg of elemental iron, which can replenish the iron store in a single treatment (Pasricha *et al* 2010; Baird-Gunning and Bromley 2016). There are several limitations that should be considered though. Intravenous iron infusion requires preparation time and administration time that can be up to five hours depending on the formulation given which limits its use outside of hospital settings (Baird-Gunning and Bromley 2016). Patients should also be aware of the risk of hypersensitivity and serious infusion reactions which can be fatal. Hypersensitivity reactions can occur with any formulation but is rarely observed with non-dextran formulations such as iron polymaltose, iron sucrose and ferric carboxymaltose (Baird-Gunning and Bromley 2016).

Monitoring of treatment

After therapeutic doses of oral iron of approximately 60 mg of elemental iron per day in adults, reticulocytosis should occur within 72 hours. A successful response will show a rise in haemoglobin of 1 g/dL (10 g/L) after a month (Short and Domagalski 2013). From then, haemoglobin levels should rise by approximately 20 g/L every 3 weeks. The minimum time required to correct anaemia and total iron store to a normal level is 3 months, and this can be up to 6 months in adults and 2-3 months in children (Department of Health 2019). While there are no standards regarding follow-up after the initial therapy for IDA, regardless of treatment choice, full blood count, reticulocytes, reticulated-haemoglobin content, and iron status should be evaluated every 3 months for a year until the values have increased to the normal level (Cappellini *et al* 2020; Ning and Zeller 2019). This is to determine whether the continuation of oral supplementation is required and to establish the optimal dosage and dosing frequency of the treatment for the patient. In pregnant females, haemoglobin and serum ferritin concentration should be measured 2-4 weeks after the initial treatment and should be monitored each trimester (Mirza *et al* 2018). If the indices of full blood examination are normal, the monitoring can be ceased after one more additional full blood count after 12 months (Short and Domagalski 2013).

Measuring the efficacy of treatment

Determination of the efficacy of oral iron supplements is crucial for the management of IDA. Assessment of response to iron therapy is usually done by a full blood examination and measuring the response of haemoglobin level after 14 days of oral iron treatment helps to evaluate whether the treatment has been effective or not, and if not, when to transition the patient from oral iron to other treatment options (Okam 2017). A study conducted by Okam *et al* (2017) reported that patients who have responded to the oral iron treatment have shown an increase in their haemoglobin level from baseline to ≥ 10 g/L on day 14. Alternatively, non-responders have shown an increase of < 10 g/L. In addition, it has been found that it is unlikely for nonresponsive patients that were identified on the 14th day of treatment to show further increase in haemoglobin level (greater than or equal to 20 g/L) with the continuation of oral iron therapy (Okam 2017). In clinical practice, nonresponsive patients should consider IV infusion as an alternative treatment option.

Different treatment strategies for different age groups

Infants

The therapeutic dosage of oral iron differs by age. The treatment of IDA for infants begins with iron therapy by administration of oral ferrous sulfate. The recommended dose is 3-6 mg/day of elemental iron as this is sufficient for the treatment and associated with fewer adverse effects (Subramaniam and Girish 2015; Means 2020). In addition to iron therapy, dietary changes should be made. For children older than 12 months, consumption of milk should not exceed over 500 mL/day to limit the interference of iron by calcium (Subramaniam and Girish 2015).

Children and non-pregnant adolescent females

The current recommendation set by the World Health Organization for treatment of IDA in children from age of 5 to 12, and for menstruating non-pregnant adolescent females is 30-60 mg/day of elemental iron supplementation daily (World Health Organization 2016).

Pregnant females

The dosage of elemental iron supplied is dependent on the estimated iron stores by measuring serum ferritin concentration (Means 2020). Depletion in iron store is indicated with low serum ferritin of < 30 µg/L (Api *et al* 2015). At this level, patients are usually administered 80-100 mg of elemental iron (Means 2020). In cases of severe anaemia, intolerance to oral iron or in an emergency, IV iron is preferred as it provides rapid correction of haemoglobin level. However, IV iron can only be administered during the second and third trimesters (Api *et al* 2015).

Elderly

In older patients with IDA, improvement in diet and a low dose of oral iron supplementation is sufficient to treat the condition (Abbaspour *et al* 2014; Lanier 2018). Low dosing and intermittent supplementation have been shown to be effective with minimal adverse effects.

Australian guidelines

The National Health and Medical Research Council has determined that oral iron therapy should be the first line of treatment in Australia (Department of Health 2019). As stated in the Australian Medicines Handbook, the recommended dose for adults with IDA is 100-200 mg of elemental iron administered daily in divided doses (Blood Safe 2018). Depending on the formulation of supplementation prescribed, this is usually obtained from 1-2 iron tablets per day. It also advises that the supplementation should be taken without food consumption, either an hour before or 2 hours after a meal to maximize the iron absorption. Intravenous iron infusion is offered to patients who are intolerant or nonresponsive to oral iron therapy.

Conclusion

Various studies have identified the benefits of oral iron supplementation in different dosages and dosing frequencies, yet the debate on the efficacy of oral iron is still ongoing. Though administration of high dosage oral iron may provide faster resolution in correcting anaemia, the disadvantages of intolerance and adverse effects outweigh the benefits. At this time, as stated in the Australian guideline, high dosing of approximately 100-200 mg of elemental iron supplied in the form of ferrous sulfate taken daily seems to be the best way to treat IDA with minimal side effects. The treatment should be continued for a minimum of 2-3 months, or until the hemoglobin and serum ferritin levels are restored. Current research has shown the advantages and disadvantages of oral supplementation, however the results are still insufficient and vague to propose a defined treatment protocol. Further studies need to be conducted to establish a standard for monitoring as well as treatment strategies for different age groups to effectively treat IDA.

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References

Abbaspour N, Hurrell R, Kelishadi R 2014. Review on iron and its importance for human health. Journal of research in medical sciences: *The Official Journal of Isfahan University of Medical Sciences* 19(2):164–174.

Api O, Breyma C, Çetiner M, Demir C, Ecder T 2015. Diagnosis and treatment of iron deficiency anemia during pregnancy and the postpartum period: Iron deficiency anemia working group consensus report. *Journal of Turkish Society of Obstetrics and Gynecology* 12(3):173-181.

Baird-Gunning J, Bromley J 2016. Correcting iron deficiency. *Australian Prescriber* 39(6): 193-199.

Balendran S, Forsyth C 2021. Non-anaemic iron deficiency. *Australian Prescriber* 44:193-196.

Blood Safe 2018. *Oral Preparations for Treatment of Iron Deficiency in Australia*, Government of South Australia. <https://www.sahealth.sa.gov.au/wps/wcm/connect/81d0f6804f7202a8b7aef774733d1f2b/BloodSafe_Iron_tablets_chart_TP-L3 410+v2.0+Jul+2018_Final+for+web.pdf?MOD=AJPERES>.

Brittenham GM 2018. Pathophysiology of iron homeostasis, In: R Hoffman, LE Silberstein, JI Weitz, ME Salama, EJB Jr, HE Heslop, J Anastasi & SA Abutalib, (eds). *Hematology*, edition 7: Elsevier;478-490.

Camaschella C 2015a. Iron-Deficiency Anemia. *New England Journal of Medicine* 372(19): 1832-1843.

Camaschella C 2015b Iron deficiency: new insights into diagnosis and treatment. *Hematology* 2015(1): 8-13.

Camaschella C 2019. Iron deficiency. *Blood* 133(1): 30-39.

Cappellini MD, Musallam KM, Taher AT 2020. Iron deficiency anaemia revisited. *Journal of Internal Medicine* 287(2): 153-170.

Coad J, Pedley K. 2014. Iron deficiency and iron deficiency anemia in women. *Scand J Clin Lab Invest Suppl* 244:82-89.

Deloughery TG 2019. Safety of Oral and Intravenous Iron. *Acta Haematologica* 142(1): 8-12.

Department of Health 2019. *Anaemia*. <https://www.health.gov.au/resources/pregnancy-care-guidelines/part-f-routine-maternal-health-tests/anaemia>.

Domellöf M 2011. Iron requirements in infancy. *Ann Nutr Metab* 59(1):59-63.

Elstrott B, Khan L, Olson S, Raghunathan V, Deloughery T, Shatzel JJ 2020. 'The role of iron repletion in adult iron deficiency anemia and other diseases. *European Journal of Haematology* 104(3): 153-161.

Hopkins RM, Gracey MS, Hobbs RP, Spargo RM, Yates M, Thompson RC 1997. The prevalence of hookworm infection, iron deficiency and anaemia in an aboriginal community in north-west Australia. *Med J Aust* 166:241-244.

Ioannou GN, Rockey DC, Bryson CL, Weiss NS 2002. Iron deficiency and gastrointestinal malignancy: a population-based cohort study. *Am J Med* 113:276-280.

Lanier JB, Park JJ, Callahan RC 2018. Anemia in Older Adults. *American Family Physician* 98(7): 437-442.

Leonard D, Buettner P, Thompson F, Makrides M, McDermott R 2018. Anaemia in pregnancy among Aboriginal and Torres Strait Islander women of Far North Queensland: a retrospective cohort study. *Nutrition and Dietetics* 75: 457- 467.

Litwack, G. 2017. *Human Biochemistry*, Elsevier, <https://www.elsevier.com/books/human-biochemistry/litwack/978-0-12-383864-3>.

Lynch S, Pfeiffer CM, Georgieff MK, Brittenham G, Fairweather-Tait S, Hurrell RF, Raiten DJ 2018. Biomarkers of Nutrition for Development (BOND) – Iron Review. *The Journal of Nutrition* 148(1): 1001S-1067S.

Marks DC, Speedy J, Robinson KL, Brama T, Capper HR, Mondy P, Keller AJ 2014. An 8-week course of 45 mg of carbonyl iron daily reduces iron deficiency in female whole blood donors aged 18 to 45 years: Results of a prospective randomized controlled trial: Iron replacement in blood donors. *Transfusion (Philadelphia, Pa)* 54(3.2): 780-788.

Means RT 2020. Iron Deficiency and Iron Deficiency Anemia: Implications and Impact in Pregnancy, Fetal Development, and Early Childhood Parameters. *Nutrients* 12(2): 447.

Mirza FG, Abdul-Kadir R, Breymann C, Fraser IS, Taher A 2018. Impact and management of iron deficiency and iron deficiency anemia in women's health. *Expert Review of Hematology* 11(9): 727-736.

Moore RA, Gaskell H, Rose P, Allan J 2011. Meta-analysis of efficacy and safety of intravenous ferric carboxymaltose (Ferinject) from clinical trial reports and published trial data. *BMC Blood Disorders* 11(1): 4.

National Health and Medical Research Council. 2014. *Iron: Nutrient Reference Values for Australia and New Zealand*.

National Heart, Lung, and Blood Institute. 2018. *Iron-Deficiency Anemia*. <https://www.nhlbi.nih.gov/health-topics/iron-deficiency-anemia>.

Ning S, Zeller MP. 2019. Management of iron deficiency. *Hematology* 2019(1): 315-322.

Okam MM, Koch TA, Tran MH 2017. Iron Supplementation, Response in Iron-Deficiency Anemia: Analysis of Five Trials. *The American Journal of Medicine* 130(8): 991. e991-991. e998.

Pasricha SR, Flecknoe-Brown SC, Allen KJ, Gibson PR, McMahon LP, Olynyk JK, Roger SD, Savoia HF, Tampi R, Thomson AR, Wood EM, Robinson KL 2010. Diagnosis and management of iron deficiency anaemia: a clinical update. *Med J Aust* 193:525-532.

Pasricha SR, Tye-Din J, Muckenthaler MU, Swinkels DW 2021. Iron deficiency. *The Lancet* 397(10270): 233-248.

Rusu IG, Suharoschi R, Vodnar DC, Pop CR, Socaci SA, Vulturar R, Istrati M, Moroșan I, Fărcaș AC, Kerezsi AD, Mureșan CI, Pop OL 2020. Iron Supplementation Influence on the Gut Microbiota and Probiotic Intake Effect in Iron Deficiency-A Literature-Based Review. *Nutrients* 12(7): 1993.

Santiago P 2012. Ferrous versus Ferric Oral Iron Formulations for the Treatment of Iron Deficiency: A Clinical Overview. *The Scientific World Journal* 2012: 1-5.

Short MW, Domagalski JE 2013. Iron deficiency anemia: evaluation and management. *American Family Physician* 87(2): 98-104.

Stoffel NU, Von Siebenthal HK, Moretti D, Zimmermann MB 2020. Oral iron supplementation in iron-deficient women: How much and how often?. *Molecular Aspects of Medicine* 75:100865.

Stoffel NU, Cercamondi CI, Brittenham G, Zeder C, Geurts-Moespot AJ, Swinkels DW, Moretti D, Zimmermann MB 2017. Iron absorption from oral iron supplements given on consecutive versus alternate days and as single morning doses versus twice-daily split dosing in iron-depleted women: two open-label, randomized controlled trials. *The Lancet Haematology* 4(11): e524–e533.

Subramaniam G, Girish M 2015. Iron Deficiency Anemia in Children. *The Indian Journal of Pediatrics* 82(6): 558-564.

World Health Organization. 2011. *Hemoglobin concentrations for the diagnosis of anaemia and assessment of severity*. <https://apps.who.int/iris/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf>.

World Health Organization. 2016. *Daily iron supplementation in infants and children*. <https://apps.who.int/iris/bitstream/handle/10665/204712/9789241549523_eng.pdf>.

Zhu Q, Qian Y, Yang Y, Wu W, Xie J, Wei D 2016. Effects of carbonyl iron powder on iron deficiency anemia and its subchronic toxicity. *Journal of Food and Drug Analysis* 24(4): 746-753.

Accuracy and precision of a point of care SARS-CoV-2 antigen rapid diagnostic test assessed in an Australian cohort

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Abstract

The aims of this study was to assess the clinical performance of the investigational product (IP) EuGeni SARS-CoV-2 antigen-detecting (Ag) rapid diagnostic test (RDT) and AX-2X-S reader in an Australian cohort. This prospective study, conducted at The Alfred and Macfarlane Burnet Centre, recruited a total of 200 participants presenting for SARS-CoV-2 testing. Two samples were provided – anterior nasal or combined nose/throat. The data was stratified by specimen type, days post onset of symptoms or viral load, and analysed using R (4.2.0) software. The sensitivity and specificity of the IP test against the gold-standard test, real-time reverse transcription (RT) polymerase chain reaction (RT-PCR) was calculated and between data subsets. A cohort of samples with different viral loads and days post onset of symptoms were sequenced to confirm the detected variants.

The IP detected SARS-CoV-2 with a sensitivity of 80.9% and 87.8% for anterior nasal and combined nose/throat samples, respectively, and 100% specificity. These increased when collecting within the first 3 days of symptom onset (92.0% and 94.1%, respectively). Three circulating lineages of the last variant of concern (VOC), Omicron were also identified by the EuGeni SARS-CoV-2 Ag RDT confirming its effectiveness in a clinical context. The EuGeni research test is the first particle based fluorescent SARS-CoV-2 test designed and trialed in Australia.

Keywords: ***SARS-CoV-2, COVID-19, point of care, Omicron, lateral flow immunoassay, variants of concern***

Introduction

The ongoing SARS-CoV-2 pandemic, which cause the COVID-19 disease, has had widespread impact on healthcare, both in Australia and globally. As of February 2023, SARS-CoV-2 has infected more than 757 million people worldwide and resulted in more than 6.8 million fatalities globally (WHO 2023). Substantial overhaul of public health strategy has been seen across the globe, and some of them are still applicable today such as social distancing measures, mask-wearing recommendation, and, most importantly, anti-SARS-CoV-2 vaccinations. Evidence suggests that these policies have substantially reduced the impacts of the global pandemic (Chu *et al* 2020), however immunity derived from vaccination or previous infection do not entirely prevent viral transmission and the burden of SARS- CoV-2 in the community is likely to remain impacting health settings.

The gold standard for SARS-CoV-2 detection is widely recognized as being real-time reverse transcription polymerase chain reaction (RT-PCR). Although a high sensitivity and specificity method of detection most of the RT-PCR is limited to laboratory-based procedures

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and can take hours to produce a result. An alternative is isothermal PCR which can reduce the time to results but require expensive cartridges and specialized equipment. To accelerate diagnosis, reduce the burden on pathology laboratories and clinics as well as reducing test costs rapid antigen tests (RATs) gained popularity with these tests often being of high specificity but of variable sensitivity (Scheiblauer *et al* 2021). With the arrival of Omicron into the community In Australia, the government invested in these test systems, highlighting their widespread acceptance as first line of COVID-19 diagnosis (Australian Bureau of Statistics 2022).

The latest variant of concern (VOC), Omicron, was first reported in 2021. Omicron and some of its subvariants have shown to significantly reduce the performance of readily available RATs (Leuzinger *et al* 2022; Cocherie *et al* 2022; Bayart *et al* 2022) or provided highly variable results (Bekliz *et al* 2022) when compared to ancestral or Delta SARS-CoV-2 variants. Although several interpretations for this have been proposed, namely lower viral load in vaccinated individuals (Woodbridge *et al* 2022), sample site collection (Schuit *et al* 2022) or high number of mutations in the Spike protein (Chakraborty *et al* 2022; Osterman *et al* 2022), although evidence for the latter is contentious (Raïch-Regué *et al* 2022). Nevertheless these observations highlight how emergent variants can affect the performance of these widely used tests.

The EuGeni SARS-CoV-2 Ag RDT and associated EuGeni AX-2X-S fluorescent reader was developed at AnteoTech (Brisbane, Australia) to overcome these limitations. The present clinical performance study is aimed at assessing this immunochromatographic RAT in a clinical setting while challenged with different sample collection sites and circulating VOCs.

Materials and Methods

Ethical approval

The ethical aspects of this research project were approved by the Human Research Ethics Committee (HREC) of Alfred Hospital Ethics Committee (EC00315) with all participants providing informed consent. The study was conducted in compliance with ICH-GCP and in accordance with the Declaration of Helsinki.

Study details and population

This prospective clinical performance study was conducted between February 18, 2022, and May 31, 2022 at The Alfred Health, Department of Infectious Diseases and The Macfarlane Burnet Institute for Medical Research and Public Health in Melbourne, Australia. Participation in the study was voluntary and participants filled out a short questionnaire, specifying current respiratory symptoms, time from onset of symptoms, close contact status, and demographics amongst other demographic details.

Specimen collection

Two upper respiratory swabs per participant were obtained by self-sampling under the guidance of trained healthcare professionals. The first swab was taken by either bilateral anterior nasal sampling or combined bilateral mid-turbinate and oropharyngeal (throat) sampling for analysis with the EuGeni SARS-CoV-2 Ag RDT. The second followed the standard of care bilateral mid-turbinate and throat swab which was then placed into viral transport media (VTM) and transported to the specified laboratory for reference RT-PCR testing as per the standard of care. Recruitment continued until a target of 50 positive and 50 negative participants was obtained for each of the two sampling methods.

Diagnostic testing

Routine SARS-CoV-2 RT-PCR testing (Roche Diagnostics) was performed at the Department of Microbiology at the Alfred Hospital, as per NATA procedures, using the cobas SARS-CoV-2 Test (Roche) on the cobas 6800 System (Roche). The residual swab sample from the SARS-CoV-2 RT-PCR test was stored at -80°C for further sequencing.

The EuGeni SARS-CoV-2 Ag RDT is an immunofluorescent assay for the rapid (15 minutes) qualitative detection of SARS-CoV-2 nucleocapsid antigen in upper respiratory samples in a POC setting. The test was performed as per manufacturer's instructions (AnteoTech Ltd.). Collected swabs were transferred to pre-filled tubes of proprietary extraction buffer, dropped onto the EuGeni SARS-CoV-2 Ag RDT cassettes, and incubated before being read by the EuGeni AX-2X-S Reader. The results can be "positive", "negative" for the presence of SARS-CoV-2 antigen or "invalid". If the latter, a new sample collection and read is recommended. All samples were stored at 2-8°C then transferred to lysis within 2.5 hours of collection and tested within the timeframe specified in the manufacturer's IFU.

Next generation sequencing

Thirty patient samples across a range of viral loads (Cycle Threshold, Cts) were submitted for SARS-CoV-2 sequencing by whole genome sequencing. RNA was extracted from samples using the DSP Virus/Pathogen Mini Kit (Qiagen) and sequencing performed using the Nextera XT DNA Library Preparation Kit (Illumina) on the NextSeq 2000 (Illumina) platform as per manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using R (4.2.0) software, with population characteristics analysed as descriptive statistics. Sensitivity and specificity with 95% confidence intervals (CIs), and positive and negative predictive values were calculated using the RT-PCR results as the gold-standard reference test to determine the percent positive or negative agreement (PPA/PNA). Sensitivity was also evaluated by days post onset of symptoms and reference RT-PCR Ct range. For genotyping data, consensus genomes were generated using ivar 1.3.1 with the 29903nt Wuhan-1 as reference, lineages determined using Pangolin v3.1.20, and variants called using minimap2, paftools call, and bcftools csq. The authors had full access to all the data used in the study.

Results

Swabs were collected from a total of 200 symptomatic and asymptomatic participants, with 100 anterior nasal and 100 combined nose and throat swabs tested on the EuGeni SARS-CoV-2 Ag RDT. Population demographics are presented in Table 1.

No invalid results were recorded during the study, with one anterior nasal swab producing an equivocal reference RT-PCR test result and thus was removed from analysis. Examples of EuGeni SARS-CoV-2 Ag RDT strip images across a range of viral loads are shown in Figure 1A, along with relative fluorescence results from the EuGeni AX-2X-S reader raw data in Figure 1B-D.

EuGeni SARS-CoV-2 Ag RDT Performance

Out of 96 RT-PCR positive samples, 81 (84.4%) were detected by the EuGeni SARS-CoV-2 Ag RDT, with overall

Table 1. *Participant demographics from recruited participants.*

	Anterior nasal swab		Combined swab[1]	
Number of patients	99		100	
Gender (no of female)	61	61.6%	66	66.0%
Age (years) (SD)	41.6	±14.5	46.1	±13.1
Vaccine doses:				
0	1	1.0%	0	0.0%
2	17	17.2%	7	7.0%
3	81	81.8%	89	89.0%
4	0	0.0%	4	4.0%
Asymptomatic participants	52	52.5%	52	52.0%
Days post onset of symptoms:				
0-3	25	53.2%	17	35.4%
4-7	22	46.8%	31	64.6%
Mean (SD)	4.0	(1.9)	3.8	(1.4)
Symptoms at collection:				
Sore throat	43	34.4%	38	38.0%
Cough	40	32.0%	35	35.0%
Headache	40	32.0%	28	28.0%
Runny nose	33	26.4%	37	37.0%
Muscle aches	29	23.2%	17	17.0%
Fever	20	17.6%	14	14.0%
Chills or sweats	14	11.2%	14	14.0%
Shortness of breath	14	11.2%	16	16.0%
Loss or change in sense of smell or taste	8	6.4%	4	4.0%

[1]Combined bilateral mid-turbinate and oropharyngeal (throat); SD: standard deviation

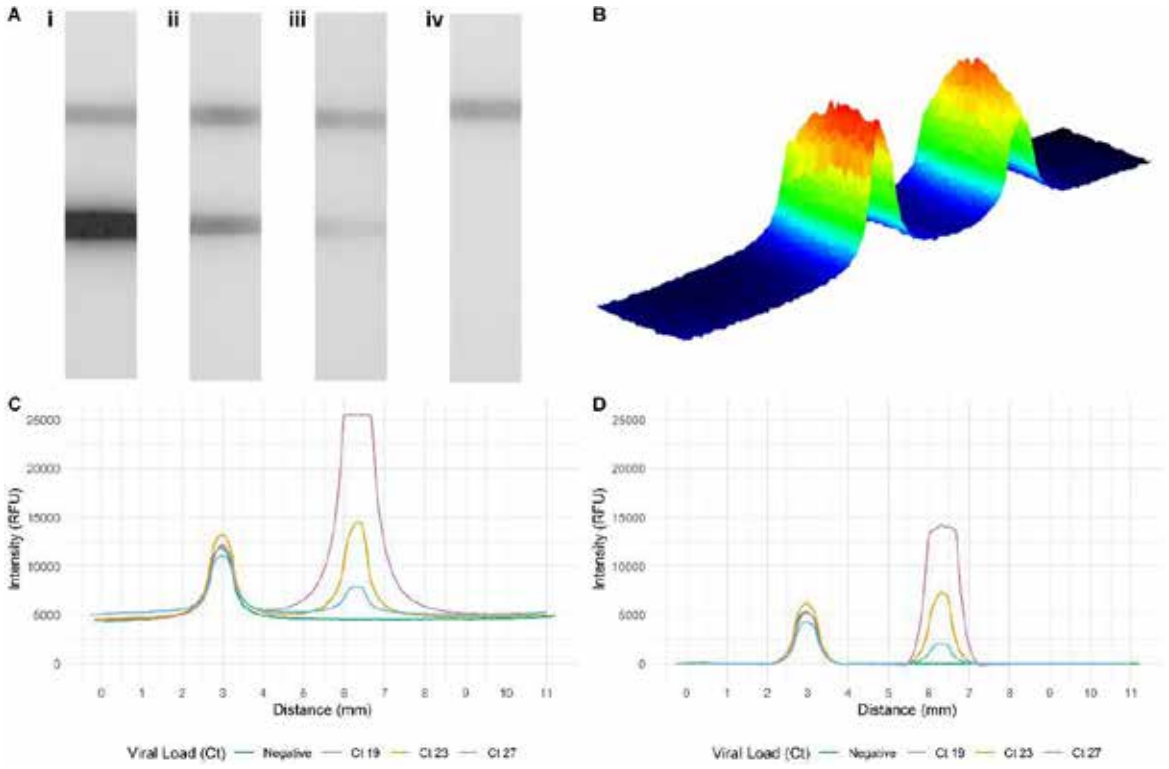

Figure 1. *Presentation of raw data from the EuGeni SARS-CoV-2 Ag RDT. (A) Raw strip images of patient samples, corresponding RT-PCR results of Ct 19 (i), Ct 23 (ii), and Ct 27 (iii), along with a negative patient sample (iv). (B) An interpolated and normalized three-dimensional histogram of patient sample A-ii from analysis with the EuGeni AX-2X-S reader software (C) Two-dimensional histogram of peak relative fluorescence (RFU) for samples Ai-Aiv, measured in 25um intervals, and (D) the normalised and corrected RFU signal used for diagnosis.*

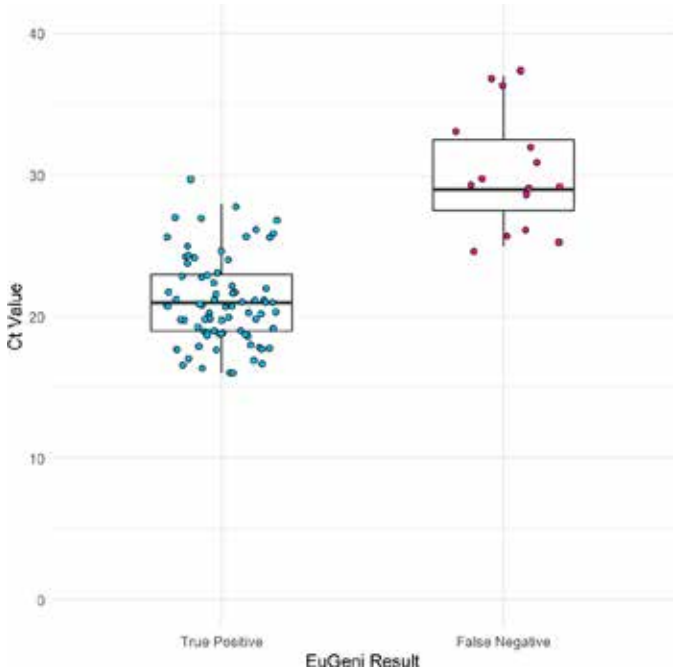

Figure 2. *Overall EuGeni performance according to viral load (Ct) for RT-PCR positive samples, combining both the anterior nasal sampling and combined bilateral mid-turbinate and throat nasal sampling methods. Viral load is measured by Ct value, with colours highlighting true positives (Blue) and false negatives (Red).*

***Table 2**. EuGeni SARS-CoV-2 Ag RDT performance by sample location.*

Sample type	EuGeni Result	RT-PCR +	RT-PCR -	Sensitivity (95% Confidence Interval)	Specificity (95% Confidence Interval)
Anterior nasal	+	38	0	80.9% 67.5% - 89.6%	100.0% 93.1% - 100.0%
	-	9	52	N/A	N/A
Combined Nose throat	+	43	0	87.8% 75.8% - 94.3%	100.0% 93.0% - 100.0%
	-	6	51	N/A	N/A

RT-PCR: reverse transcriptase polymerase chain reaction.

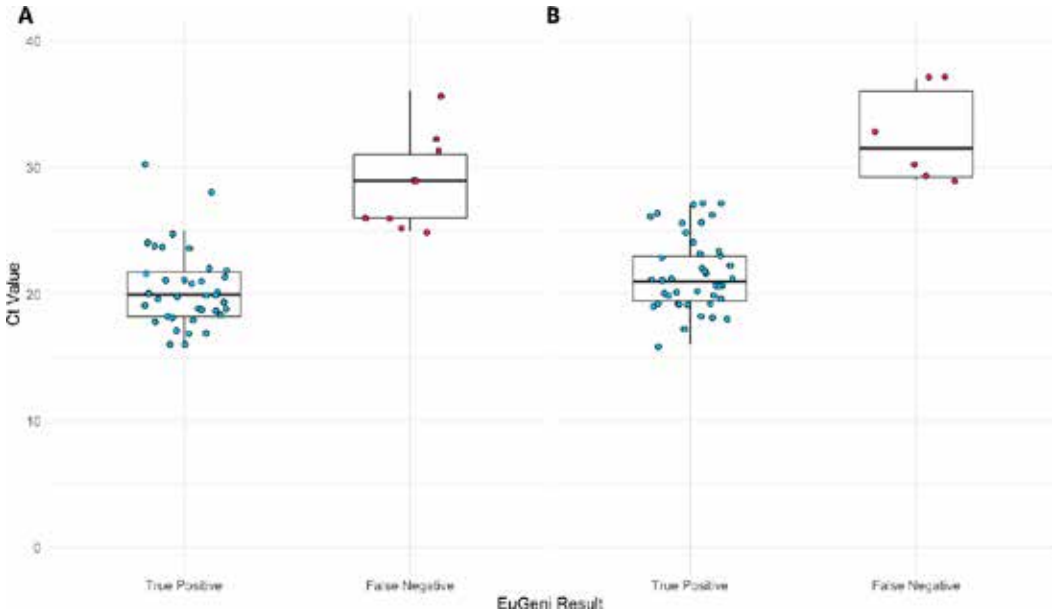

***Figure 3**. EuGeni results per sample type and viral load for (A) bilateral anterior nasal sampling or (B) combined bilateral mid-turbinate and throat nasal sampling according to viral load for RT-PCR positive samples. Viral load is measured by Ct value, with colours highlighting true positives (Blue) and false negatives (Red).*

***Table 3**. EuGeni SARS-CoV-2 Ag RDT performance by sample site, Ct range, and days post onset of symptoms. One sample with unknown days post-onset of symptoms and was excluded from the stratification below.*

	Ct	Symptomatic 0 - 3		Symptomatic 4 - 7	
		Sensitivity (95% CI)	*n/N*	*Sensitivity (95% CI)*	*n/N*
Anterior nasal	All	92.0% (75.0% - 97.8%)	23/25	68.2% (47.3% - 83.6%)	15/22
	≤Ct25	100.0% (85.7% - 100.0%)	23/23	86.7% (62.1% - 96.3%)	13/15
	>Ct25	0% (0.0% - 65.8%)	0/2	28.6% (8.2% - 64.1%)	2/7
Combined Nose Throat	All	94.1% (73.0% - 99.0%)	16/17	87.1% (71.2% - 94.9%)	27/31
	≤Ct25	100.0% (77.2%-100%)	13/13	100.0% (85.1%-100%)	22/22
	>Ct25	75.0% (30.1% - 95.4%)	3/4	55.6% (26.7% - 81.1%)	5/9

Ct: Cycle Threshold; n/N: detected/total samples.

performance according to viral load (Ct) shown in Figure 2. Sensitivity for the anterior nasal samples was 80.9% (95% CI: 67.5%-89.6%), and the combined nose and throat 87.8% (95% CI: 75.8%-94.3%) (Table 2). Distribution of sample viral loads for the contrasting sampling locations is presented in Figure 3. Sensitivity for patients at 0-3 and 4-7 days post onset of symptoms, along with stratification by viral load detected during RT-PCR (measured by Ct value) is presented in Table 3. Sensitivities of 92.0% (CI: 75.0%-97.8%) and 94.1% (CI: 73.0%-99.0%) observed for the first 3 days of symptom onset in anterior nasal and combined nose throat samples, respectively, dropping to 68.2% (CI: 47.3%-83.6%) and 87.1% (CI: 71.2%-94.9%) after 4 days since symptom onset. These results are presented in Figure 4, with viral loads stratified by sample type and days post onset of symptoms. Specificity was observed to be 100% across all samples, along with a 100% positive predictive value.

SARS-CoV-2 variants detected by EuGeni SARS-CoV-2 Ag RDT

Genotyping of EuGeni positive samples identified that 21.1% (4/19) and 73.7% (14/19) of samples were Pango Omicron lineages BA.1 and BA.2, respectively. Additionally one sample was identified as lineage BA.5.2.1. The variants detected are displayed as follows in Table 4, along with the mean viral loads, measured in Ct, and days post onset of symptoms.

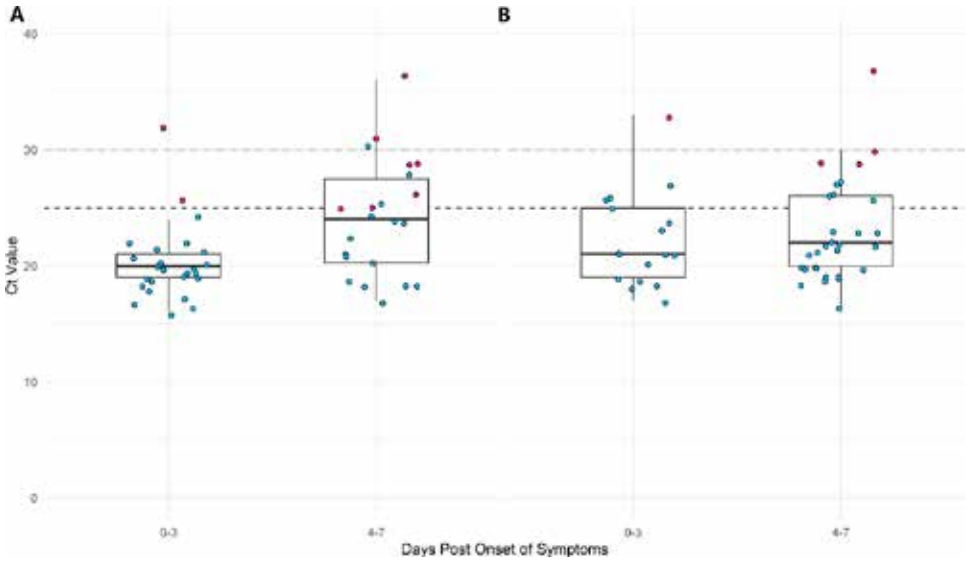

***Figure 4**. EuGeni results based on days post onset symptoms, sample type and viral load (A) bilateral anterior nasal sampling or (B) combined bilateral mid-turbinate and throat nasal sampling according to viral load for RT-PCR positive samples, stratified by days post onset of symptoms. One sample with unknown days post-onset of symptoms and was excluded from the stratification below. Viral load is measured by Ct value, with colours highlighting true positives (Blue) and false negatives (Red). Cut- offs of Ct 25 and Ct 30 are annotated.*

***Table 4**. Pango Omicron lineage of samples identified by whole genome sequencing.*

Variant	n	Mean Ct	Mean Symptom Onset (Days)
BA.1.1.14	1	21.0	3.0
BA.1.17	2	21.5	2.0
BA.1.17.1	1	18.0	3.0
BA.2	10	19.7	3.1
BA.2.10.1	2	20.5	3.5
BA.2.12.1	1	16.0	4.0
BA.2.3	1	22.0	5.0
BA.5.2.1	1	21.0	5.0

n: number of samples, Ct: cycle threshold

In addition, the EuGeni test has also demonstrated to detect Delta and Wild Type variants (data not shown).

Discussion

This study assessed the diagnostic performance of the EuGeni SARS-CoV-2 Ag RDT and EuGeni AX-2X-S in anterior nasal or combined nose throat sampling, both common standard of care sampling procedures. The test effectively detected three circulating lineages of the Omicron variant of SARS-CoV-2, and up to 8 Omicron subvariants. The IP test also outperformed some COVID-19 approved RATs in terms of sensitivity (Goodall *et al* 2022; Schuit *et al* 2022) when testing patients within 3 days post onset of symptoms, for both single and combined specimens (92% and 94.1% sensitivity, respectively). From the 200 participants the overall sensitivity (78.6% and 87.8% for single and combined samples, respectively) was comparable to published data (Goodall *et al* 2022; Schuit *et al* 2022). In all instances, a 100% specificity was reported.

These results suggest the EuGeni test and fluorescent reader may detect circulating variants at greater sensitivity than colorimetric approved lateral flow RATs. It is important to note that certain factors are known to influence the predictive value of an RAT/RDT such as specimen quality, site of swabbing, patient viral load, and sample handling (McCulloch *et al* 2020). Because the Australian government guidelines required that self-sampling be performed in this study, it is possible greater sensitivity could have been reported across the participants if health care professionals would have collected the samples, which is ultimately the intended use of the EuGeni test.

This clinical study also identified that the sampling location may be a contributing factor in test performance, as reinforced by similar studies (Desmet *et al* 2021; Goodall *et al* 2022; LeBlanc *et al* 2020; Vlek *et al* 2021), with greater sensitivity in combined nose and throat samples when compared to anterior nasal samples.

After 4 days from symptom onset the test lost sensitivity for both collections. If this was due to the shedding kinetics' nature of the Omicron variant, which has shorter incubation periods (3-3.4 days) than ancestral and Delta variants (Puhach *et al* 2023), self-sampling inconsistencies exacerbated by lower viral loads after 3 days or other variables could be further explored. Interestingly, combined collections performed similarly between 0-3 and 4-7 days post onset of symptoms than single sampling (median Cts), while fewer false negatives were observed at 4-7 days for the former when compared to the latter.

Some limitations of the present study include the relatively small cohort, lack of identification of asymptomatic SARS-CoV-2 patients and the small subset of patients with lower viral loads and later stages of disease. If these could be identified then knowledge on test performance would improve.

In summary, the EuGeni SARS-CoV-2 Ag RDT and EuGeni AX-2X-S fluorescence reader have shown superior sensitivity in the diagnosis of COVID-19 in the early stages of the disease than currently approved RATs in Australia and similar performance after 3 days post onset symptoms. This europium-based COVID-19 rapid tests could widen the available portfolio of high sensitive SARS-CoV-2 tests in Australia, potentially improving diagnostic streaming and patient care management.

Conflicts of Interest

The authors declare the following potential conflicts of interest: EE, LH, CYH and RS are employees of the study sponsor Anteotech Ltd.

Acknowledgement

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Author contributions

EE designed and oversaw the study. JM and HD oversaw clinical trial site operations, recruitment, and laboratory testing. RP coordinated study. TM oversaw QA/QC at Burnet Institute. SZ, SH, ZW, TM and HD performed testing. LH performed statistical analysis. LH and EE wrote the manuscript. JM, HD, RP, TM, ZW, SZ, CH, RS reviewed and edited the manuscript. EE approved the manuscript.

References

Australian Bureau of Statistics. Effects of COVID-19 strains on the Australian economy. https://www.abs.gov.au/articles/effects-covid-19-strains-australian-economy. Accessed February 28, 2023.

Bayart JL, Degosserie J, Favresse J, Gillot C, Didembourg M, Djokoto HP, Verbelen V, Roussel G, Maschietto C, Mullier F, Dogné JM, Douxfils J 2022. Analytical Sensitivity of Six SARS-CoV-2 Rapid Antigen Tests for Omicron versus Delta Variant. *Viruses* 14(4):654.

Bekliz M, Adea K, Puhach O, Perez-Rodriguez F, Marques Melancia S, Baggio S, Corvaglia AR, Jacquerioz F, Alvarez C, Essaidi-Laziosi M, Escadafal C, Kaiser L, Eckerle I 2022.

Analytical Sensitivity of Eight Different SARS-CoV-2 Antigen-Detecting Rapid Tests for Omicron-BA.1 Variant. *Microbiol Spectr* 10(4):e0085322.

Chakraborty C, Bhattacharya M, Sharma AR, Mallik B 2022. Omicron (B.1.1.529). A new heavily mutated variant: Mapped location and probable properties of its mutations with an emphasis on S- glycoprotein. *Int J Biol Macromol* 219: 980-997.

Chu DK, Duda S, Solo KY, Schunemann H 2020. Physical Distancing Face Masks and Eye Protection to Prevent Person-to-Person Transmission of SARS-CoV-2 and COVID-19: A Systematic Review and Meta-Analysis. *J Vasc Surg* 72(4) 1500.

Cocherie T, Bastide M, Sakhi S, Zafilaza K, Flandre P, Leducq V, Jary A, Burrel S, Louet M, Calvez V, Marcelin AG, Marot S 2022. Decreased Sensitivity of Rapid Antigen Test Is Associated with a Lower Viral Load of Omicron than Delta SARS-CoV-2 Variant. *Microbiol Spectr* 10(5):e0192222.

Desmet T, Paepe P, Boelens J, Coorevits L, Padalko E, Vandendriessche S, Leroux-Roels I, Aerssens A, Callens S, Braeckel EV, Malfait T, Vermassen F, Verhasselt B 2021. Combined oropharyngeal/nasal swab is equivalent to nasopharyngeal sampling for SARS-CoV-2 diagnostic PCR. *BMC Microbiol* 21(1) 31.

Goodall BL, LeBlanc J, Hatchetter TF, Barett L, Patriquin G 2023. Investigating the Sensitivity of Nasal or Throat Swabs: Combination of Both Swabs Increases the Sensitivity of SARS-CoV-2 Rapid Antigen Tests. *Microbiol Spectr* 10(4):e00217-22.

LeBlanc JJ, Heinstein C, MacDonald J, Pettipas J, Hatchette T, F, Patriquin G 2020. A combined oropharyngeal/nares swab is a suitable alternative to nasopharyngeal swabs for the detection of SARS-CoV-2. *J Clin Virol* 128 104442.

Leuzinger K, Roloff T, Egli A, Hirsch HH 2022. Impact of SARS-CoV-2 Omicron on Rapid Antigen Testing Developed for Early-Pandemic SARS-CoV-2 Variants. *Microbiol Spectr* 10(4) e0200622.

McCulloch DJ Kim AE Wilcox NC Logue JK Greninger AL, Englund JA, Chu HY 2020. Comparison of Unsupervised Home Self-collected Midnasal Swabs With Clinician-Collected Nasopharyngeal Swabs for Detection of SARS-CoV-2 Infection. *JAMA Netw Open* 3(7) e2016382.

Osterman A, Badell I, Basara E, Stern M, Kriesel F, Eletreby M, Öztan GN, Huber M, Autenrieth H, Knabe R, Späth PM, Muenchhoff M, Graf A, Krebs S, Blum H, Durner J, Czibere L, Dächert C, Kaderali L, Baldauf HM, Keppler OT 2022. Impaired detection of omicron by SARS-CoV-2 rapid antigen tests. *Med Microbiol Immunol* 211(2-3):105-117.

Puhach O, Meyer B, Eckerle I 2023. SARS-CoV-2 viral load and shedding kinetics. *Nat Rev Microbiol* 21:147–161.

Raïch-Regué D, Muñoz-Basagoiti J, Perez-Zsolt D, Noguera-Julian M, Pradenas E, Riveira-Muñoz E, Giménez N, Carabaza A, Giménez F, Saludes V, Martró E, Robert N, Blanco I, Paredes R, Ruiz L, Ballana E, Clotet B, Blanco J, Izquierdo-Useros N 2022. Performance of SARS-CoV-2 Antigen-Detecting Rapid Diagnostic Tests for Omicron and Other Variants of Concern. *Front Microbiol* 13:810576.

Samsunder N, de Vos M, Ngcapu S, Giandhari J, Lewis L, Kharsany ABM, Cawood B, de Oliveira T, Karim QA, Karim SA, Naidoo K, Escadafal C, Sivro A 2022. Clinical Evaluation of Severe Acute Respiratory Syndrome Coronavirus 2 Rapid Antigen Tests During the Omicron Wave in South Africa. *J Infect Dis* 226(8):1412–1417.

Scheiblauer HF, Filomena A, Nitsche A, Puyskens A, Corman VM, Drosten C, Zwirglmaier K, Lange C, Emmerich P, Müller M, Knauer O, Nübling CM 2021. Comparative sensitivity evaluation for 122 CE-marked rapid diagnostic tests for SARS-CoV-2 antigen Germany September 2020 to April 2021. *Euro Surveill* 26(44) 2100441.

Schuit E, Venekamp RP, Hooft L, Veldhuijzen IK, van den Bijllaardt W, Pas SD, Zwart VF, Lodder EB, Hellwich M, Koppelman M, Molenkamp R, Wijers CJH, Vroom IH, Smeets LC, Nagel-Imming CRS, Han WGH, van den Hof S, Kluytmans JAJW, van de Wijgert JHHM, Moons KGM 2022. Diagnostic accuracy of covid-19 rapid antigen tests with unsupervised self-sampling in people with symptoms in the omicron period: cross sectional study. *BMJ* 378:e071215.

Schuit E. Venekamp RP, Hooft L, Veldhuijzen IK, van den Bijllaardt W, Pas SD, Zwart VF, Lodder EB, Hellwich M, Koppelman M, Molenkamp R, Wijers C, Vroom IH, Smeets LC, Nagel- Imming C, Han W, van den Hof S, Kluytmans J, van de Wijgert J, Moons K 2022. Diagnostic accuracy of covid-19 rapid antigen tests with unsupervised self-sampling in people with symptoms in the omicron period: cross sectional study. *BMJ* 378:e071215.

Vlek A, Wesselius TS, Achterberg R, Thijsen S 2021. Combined throat/nasal swab sampling for SARS-CoV-2 is equivalent to nasopharyngeal sampling. *Eur J Clin Microbiol Infect Dis* 40(1) 193– 195.

Woodbridge Y, Amit S, Huppert A, Kopleman NM 2022. Viral load dynamics of SARS-CoV-2 Delta and Omicron variants following multiple vaccine doses and previous infection. *Nat Commun* 13:6706.

World Health Organisation (WHO). WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int/. Accessed February 28, 2023.

Immunohistochemistry and basal cell carcinoma in the era of personalised medicine

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Abstract

Basal cell carcinoma (BCC) is the most common human malignancy of skin and Australia has the highest incidence of BCC in the world. The number of advanced BCC (aBCC) cases in Australia is increasing. However, new personalised medicines are now available to treat aBCC and are highly effective. Laboratory diagnosis of aBCC can be challenging using routine histology. Molecular genetic tests for routine use in the diagnostic lab are not yet available. Innovative immunohistochemistry panels can help correctly identify this group of patients and thereby allow them access to effective personalised medicine. In this article, we review the epidemiology, genetics and treatment of aBCC with particular focus on the role of immunohistochemistry in laboratory diagnosis of this increasingly common cancer.

Keywords: ***carcinoma, basal cell, immunohistochemistry, precision medicine***

Background

BCC is exceedingly common in Australia and the majority are easy to diagnose and easy to treat (Goldenberg *et al* 2016; Seidl-Philipp *et al* 2021). BCC is often trivialised and not recorded in cancer registries (Lomas *et al* 2012). Furthermore, BCC is often grouped together with cutaneous squamous cell carcinoma (cSCC) and referred to as non-melanoma skin cancer (NMSC) or keratinocyte cancer (KC). We do know the incidence of BCC is increasing around the world (Lomas *et al* 2012). aBCC can be defined as a BCC where complete resection (also referred to as R0 resection) does not appear feasible and includes locally advanced BCC (laBCC) and metastatic BCC (mBCC). The age-adjusted incidence of aBCC in the US was estimated as 1.83 in a recent study (Goldenberg *et al* 2016). In Australia, deaths from NMSC have almost doubled over the last 20 years, the majority of which are likely to cSCC but we do not have more granular data (Figure 1).

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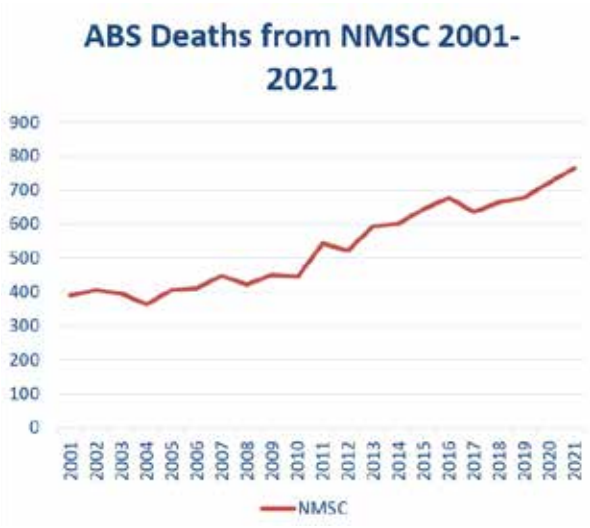

Figure 1*. Deaths from non-melanoma skin cancer in Australia over the last 20 years – Australian Bureau of Statistics data (https://www.abs.gov.au/ accessed December 2022).*

Incidence of advanced BCC

BCC is the most common human malignancy globally but the true incidence is not known as most cancer registries do not record it (Rubin *et al* 2005; Messina *et al* 2018;

Ciążyńska *et al* 2021). Approximately 2 in 3 Australians are diagnosed with a skin cancer before age 70 (Lomas *et al* 2012; Cameron *et al* 2019; Ciążyńska *et al* 2021). BCC is more common in Caucasian people (Niculet *et al* 2022). Australia has a large Caucasian population, many of whom live in a subtropical climate (Richmond-Sinclair *et al* 2009). The incidence of BCC increases inversely with latitude (Lomas *et al* 2012) and Queensland (located

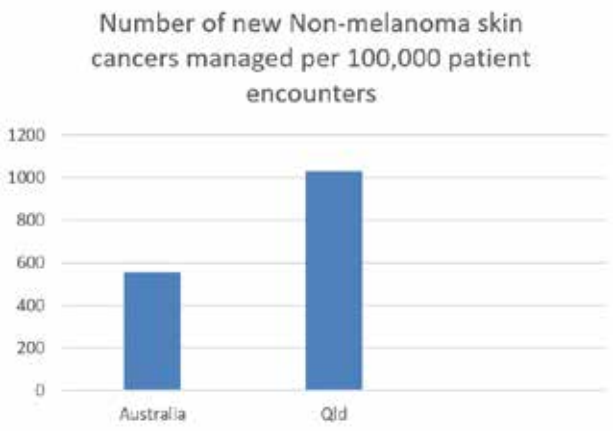

Figure 2. *Incidence of non-melanoma skin cancers in Queensland vs Australian national average - modified from (Pollack et al 2014).*

closest to the equator) has the highest incidence of skin cancer compared to other Australian states and territories and therefore the highest incidence in the world (Perera *et al* 2015) (Figure 2).

The incidence of primary BCC is high but metastatic BCC (mBCC) is considered a very rare event. The global incidence of mBCC is most often quoted as between 0.0028% (28 mBCC cases per million) and 0.55% (1 mBCC case per 182) of histologically examined BCCs (Snow *et al* 1994). Pathologists tend not to consider mBCC as a possible differential diagnosis because it is so rare (Messina *et al* 2018). However, these global statistics were originally derived in 1961 and are likely outdated with the true incidence of mBCC likely to be higher than currently believed (Snow *et al* 1994; Wadhera *et al* 2006). A recent large population based study from the US found an age adjusted incidence rate of 0.04 (Goldenberg *et al* 2016). In Australia and New Zealand, studies have reported considerably higher rates of primary and mBCC (Tang *et al* 2017).

Sunshine Coast in Queensland has an almost unique combination of outdoor lifestyle (UV exposure), high average age, low latitude, and a predominantly Caucasian population, increasing absolute risk of skin cancer. The burden of skin cancer on the Sunshine Coast is among the highest anywhere in the world and the diagnosis of aBCC/mBCC is not a rare event – the Anatomical

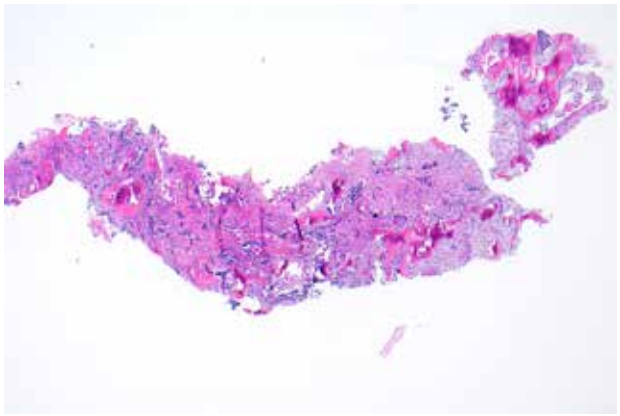

Figure 3. *Core biopsy showing metastatic BCC invading bone (H&E, original magnification x40).*

Pathology laboratory at the largest hospital in the region, the Sunshine Coast University Hospital, has seen at least 6 cases over the last 5 years (personal communication) (Figure 3). However, separating true cases of mBCC from its many mimics, most of which are more common, is an everyday problem.

Risk factors for aBCC include male sex (~2:1), primary tumour site (head and neck, "mask" area, genitals), large/neglected tumours, recurrent lesions, deep invasion (beyond the subcutis) and previous radiotherapy (Bisceglia *et al* 2020).

Genetics and personalised treatment of BCC

A great leap forwards was made in the understanding of the molecular pathology underpinning the world's most common malignancy with the discovery of mutations affecting the human homolog of Drosophila patched (PTCH1) in tumours from patients with the naevoid basal cell carcinoma syndrome (Gorlin Goltz Syndrome). The human PTCH1 gene codes for the hedgehog receptor patched, which normally binds and inhibits smoothened (SMO). SMO in turn sends a signal via suppressor of fused (SUFU) and glioma associated oncogene proteins (GLI1). The SUFU-GLI1 complex upregulates DNA transcription. In the context of BCC, PTCH1 functions as a tumour suppression gene and SMO as a proto-oncogene (Hoashi *et al* 2022).

Sonic hedgehog (SHH) is a ligand that binds to PTCH1 and results in dis-inhibition of SMO (Cameron *et al* 2019; Kim *et al* 2019). Thus, this signalling pathway is known as the hedgehog pathway (HH pathway) (Figure 4). The HH pathway is crucial in normal embryo development and has been found to be important in carcinogenesis of a number of tumours including brain, liver, gallbladder, pancreas, stomach, colon, breast, lung, prostate and haematological malignancies (Jeng *et al* 2020). It is now well established that the majority of all BCCs (familial

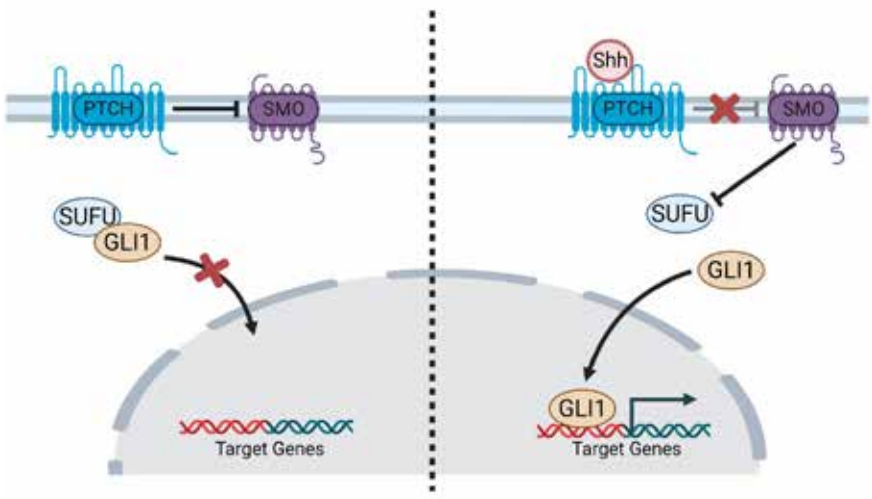

Figure 4. *HH Pathway functioning normally: Sonic hedgehog (Shh) ligand binds to patched1 (PTCH) which disinhibits smoothened (SMO) and signals suppressor of fused (SUFU)/glioma-associated oncogene (GLI1) mediated transcription of genes.*

and sporadic) have mutations of the SHH/PTCH/SMO pathway (Bonilla *et al* 2016).

The discovery of the role the HH pathway plays in human carcinogenesis has led to the development of treatments that target the that pathway. SMO inhibitors are already available in Australia and have entered routine practice. The 2 approved drugs are Vismodegib (Erivedge, Roche) and Sonidegib (Odomzo, Novartis). Vismodegib was first approved by the US Food and Drug Administration in 2012 and Sonidegib in 2015. Vismodegib and Sonidegib are effective in treating laBCC and mBCC (Sekulic and Von Hoff 2016). A pooled analysis of response to Vismodegib from 8 studies showed objective response for laBCC had a weighted average of 65% and complete response 31%; mBCC objective response was 34% and complete response 4% (Jacobsen *et al* 2016). Other HH Pathway inhibitors are under development. Parenthetically, immunotherapy (Cemiplimab) is also available for aBCC and is effective (Stratigos *et al* 2021).

Laboratory diagnosis of BCC

Basal cell carcinomas show remarkable morphological variability with over 60 different varieties previously described. BCCs are extremely common and despite the remarkable histological variability, the histological features of the vast majority of cases are so distinctive and familiar that simple H&E stained sections can diagnose most primary lesions (Swanson *et al* 1998). Entities that can mimic primary BCC include benign skin tumours (examples include trichoepithelioma, syringoma, tumour of the follicular infundibulum) (Figure 5) and other cutaneous malignancies (examples include squamous cell carcinomas (SCC), Merkel cell carcinoma, microcystic adnexal carcinoma, sebaceous carcinoma) (Katona *et al* 2008; Clements and Khachemoune 2021) (Figure 6).

The differential diagnosis of mBCC is different and includes the primary skin malignancies listed above but also malignancies from non-cutaneous sites, principally on the head and neck (examples include mucosal SCC, adenoid cystic carcinoma, other salivary gland carcinomas and nasopharyngeal carcinoma). The principal histological mimic of mBCC is SCC (with basaloid features) as these entities are both common and overlap histologically (Figure 7). SCC can be from either a head and neck mucosal primary or a cutaneous primary. The risk of misdiagnosis is increased with small biopsies (Villada *et al* 2018). Misdiagnosis has the potential to cause both overtreatment and undertreatment.

Most primary skin cancer cases are diagnosed on skin biopsies through routine H&E staining. However, diagnosis of metastatic skin cancer including BCC is usually achieved by core biopsy or cytology of lymph nodes and requires further investigation with immunohistochemistry to identify the epitopes and proteins of interest that are undetectable through routine staining.

Molecular genetic analysis of BCC has not yet entered routine pathology practice. A rapid and economical technique is currently lacking. There are 2 principal reasons for this. One, the majority of BCCs are easy to diagnose on routine H&E, therefore little emphasis has been placed on developing such tests for routine use. Two, the mutations found in BCC are highly variable, therefore tests which cast a broad net are necessary and these tend to be slow, expensive and difficult to interpret.

Immunohistochemistry and BCC

Perhaps cheap and reliable molecular test will arrive and transform how we classify and diagnose aBCC. In the meantime, an efficient and simple immunohistochemistry

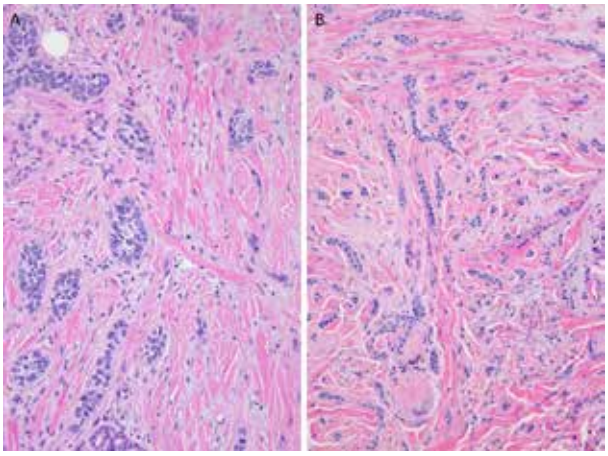

Figure 5. *Histological mimics of primary BCC: A. Infiltrative/micronodular BCC; B. Desmoplastic Trichoepithelioma (both H&E original magnification x200).*

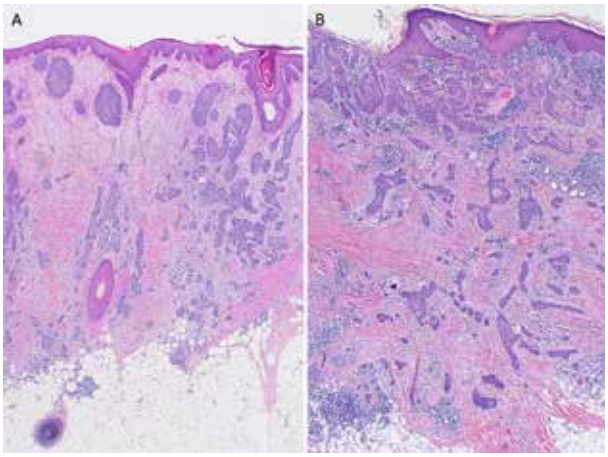

Figure 6. *Histological mimics of primary BCC: A. Infiltrative/micronodular BCC; B. cSCC (with basaloid features) (both H&E, original magnification x40).*

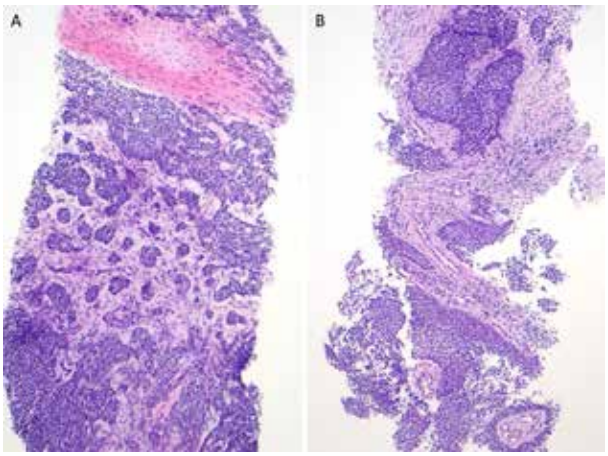

Figure 7. *Histological mimics of metastatic BCC: A. metastatic BCC in a lung biopsy (note bronchial cartilage at top); B. metastatic mucosal SCC (from tonsil) in cervical lymph node (both H&E, original magnification x200).*

panel for routine practice can enable accurate diagnosis and thereby gain access to personalised treatment for aBCC patients.

Previous studies have described several antibodies that show promise for the diagnosis of mBCC. BerEP4 is an antibody to epithelial cell adhesion molecule and was one of the first and perhaps most consistently used antibodies for BCC with some studies finding BerEP4 expression in BCC having sensitivity and specificity close to 100% (Swanson *et al* 1998; Webb *et al* 2015; Sunjaya *et al* 2017). B-cell lymphoma 2 protein (Bcl2) is a regulator of apoptosis and has shown expression in BCC with high sensitivity and specificity (Swanson *et al* 1998; Zheng *et al* 2005; Gaballah and Ahmed 2015; Ramezani *et al* 2016). Carcinoembryonic antigen (CEA) is a group of related glycoproteins involved in cell adhesion. CEA immunohistochemistry is positive in a range of tumours including adenocarcinomas and SCC but is uncommon in BCC (Beer *et al* 2000; Hoang *et al* 2008; Ramezani *et al* 2016). Epithelial membrane antigen (EMA) is a mucin encoded by the MUC1 gene. EMA immunohistochemistry is positive in a range of tumours including nearly all mucosal SCC and the majority of cSCC. Less than half of BCC cases are positive. Therefore, it is most useful when negative, making metastatic mucosal SCC very unlikely (Ramezani *et al* 2016; Villada *et al* 2018). Alone, all antibodies are capable of both false positive and false negative errors but used in combination, they are more reliable. These antibodies are widely available and can be used in a panel approach to help identify aBCC (Figure 8).

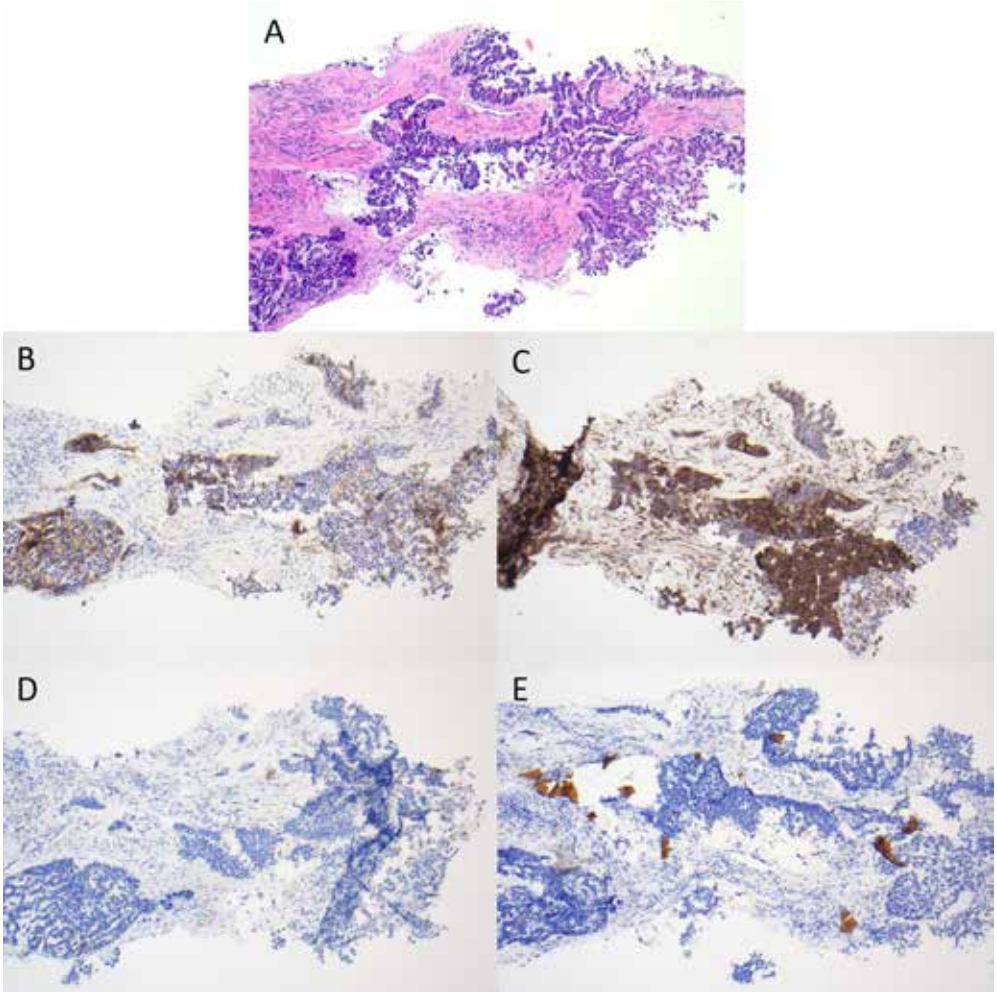

Figure 8. *Use of immunohistochemistry and a panel of common antibodies to diagnose mBCC. A. Metastatic BCC in bone; B. BerEP4; C. Bcl2; D. EMA; E. CEA (all original magnification x200).*

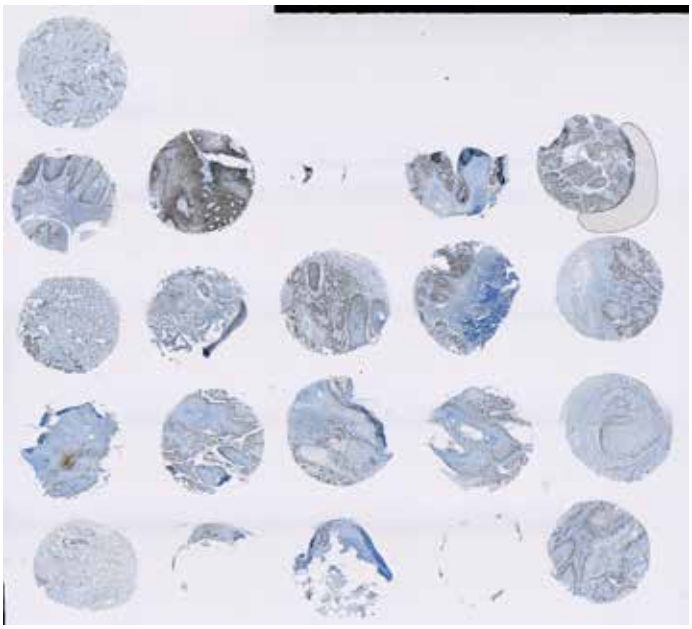

Figure 9. *Use of tissue microarray (TMA) to test an antibody on a number of tumours economically: TMA containing 20 x cSCCs stained with antibody P63 (original magnification x4).*

***Table 1**. The main differential diagnostic considerations for aBCC and useful antibodies (cSCC = cutaneous SCC; HNmucSCC = head and neck mucosal SCC).*

Antibody	aBCC	cSCC	HNmucSCC
BerEP4	+	-	+/-
Bcl2	+	-	-
EMA	-/+	+/-	+
CEA	-	-/+	-

There are many other antibodies that have been applied that show promise. These include CK20, CD34, androgen receptor, P63, CD10, bcl6, p16 (Zheng *et al* 2005), Uea1 (Webb *et al* 2015), sox2 (Villada *et al* 2018) and more specialised antibodies such as adipophilin, GLI1 (Kim *et al* 2019), and MCPyV (Reisinger *et al* 2010). The right antibody panel should be tailored to the entities in the differential diagnosis and should always contain "negative" BCC markers (Table 1).

Further research is needed to identify the optimal panel to use in day-to-day practice that is both highly reliable and affordable. Tissue microarrays (TMA) offer an excellent way to explore this question further (Tebcherani *et al* 2012) (Figure 9). TMAs offer an alternative method to 'full-face' sections which are more cost-effective and efficient than single-sample tissue sections. TMAs enable the assessment of the expression of multiple genes and proteins simultaneously on large numbers of clinical samples on one slide. Conventional histological and molecular pathology techniques can be performed on the same TMA, enabling DNA, RNA, and protein targets to be analysed from defined, almost identical regions of tissue, a facility important in determining molecular pathogeneses of disease at a cellular level (Kumar *et al* 2004; Jawhar 2009). Excellent reviews containing details of how to construct and use TMAs are available (Kumar *et al* 2004).

TMAs can be used for quality assurance purposes in a clinical setting, such as inter and intra-laboratory concordance, and are useful as initial tools to optimize and validate clinically relevant assays (Sabatino *et al* 2014). Today, instruments to construct TMAs are developed as either semi-automated or fully-automated, both have computer-controlled design graphical user interfaces for easy operation, automatic punch area selection for accuracy and precision.

Conclusion

We hypothesise that is it possible to design an immunohistochemistry panel that can help to distinguish aBCC from its mimics using common, inexpensive antibodies and thereby gain access to effective personalised treatment for this growing cohort of Australians. The panel of antibodies must be tailored to each case and depends on the differential diagnoses being considered. Further research is needed to identify the most effective combination of antibodies.

Acknowledgements

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References

Beer TW, Shepherd P, Theaker JM 2000. Ber EP4 and epithelial membrane antigen aid distinction of basal cell, squamous cell and basosquamous carcinomas of the skin. *Histopathology* 37(3):218–223.

Bisceglia M, Panniello G, Galliani CA, Centola M, D'Errico MM, Minenna E, Tucci FA, Ben-Dor DJ 2020. Metastatic Basal Cell Carcinoma of the Skin: A Comprehensive Literature Review, Including Advances in Molecular Therapeutics. *Adv Anat Pathol* 27(5):331–353.

Bonilla X, Parmentier L, King B, Bezrukov F, Kaya G, Zoete V, Seplyarskiy VB, Sharpe HJ, McKee T, Letourneau A, Ribaux PG, Popadin K, Basset-Seguin N, Ben Chaabene R, Santoni FA, Andrianova MA, Guipponi M, Garieri M, Verdan C, Grosdemange K, Sumara O, Eilers M, Aifantis I, Michielin O, de Sauvage FJ, Antonarakis SE, Nikolaev SI. 2016 Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. *Nat Genet* 48(4):398–406.

Cameron MC, Lee E, Hibler BP, Barker CA, Mori S, Cordova M, Nehal KS, Rossi AM 2019. Basal cell carcinoma: Epidemiology; pathophysiology; clinical and histological subtypes; and disease associations. *J Am Acad Dermatol* 80(2):303–317.

Ciążyńska M, Kamińska-Winciorek G, Lange D, Lewandowski B, Reich A, Sławińska M, Pabianek M, Szczepaniak K, Hankiewicz A, Ułańska M, Morawiec J, Błasińska-Morawiec M, Morawiec Z, Piekarski J, Nejc D, Brodowski R, Zaryczańska A, Sobjanek M, Nowicki RJ, Owczarek W, Słowińska M, Wróbel K, Bieniek A, Woźniacka A, Skibińska M, Narbutt J, Niemczyk W, Ciążyński K, Lesiak A 2021. The incidence and clinical analysis of non-melanoma skin cancer. *Sci Rep* 11(1):4337.

Clements S, Khachemoune A 2021. Upstaging of basal cell and squamous cell carcinomas during definitive surgery: a review of predictive preoperative clinical and histologic features. *Arch Dermatol Res* 313(5):319–325.

Gaballah MA, Ahmed RA 2015. Diagnostic value of CD10 and Bcl2 expression in distinguishing cutaneous basal cell carcinoma from squamous cell carcinoma and seborrheic keratosis. *Pathol Res Pract* 211(12):931–938.

Goldenberg G, Karagiannis T, Palmer JB, Lotya J, O'Neill C, Kisa R, Herrera V, Siegel DM 2016. Incidence and prevalence of basal cell carcinoma (BCC) and locally advanced BCC (LABCC) in a large commercially insured population in the United States: A retrospective cohort study. *J Am Acad Dermatol* 75(5):957-966.e2.

Hoang MP, Dresser KA, Kapur P, High WA, Mahalingam M 2008. Microcystic adnexal carcinoma: an immunohistochemical reappraisal. *Mod Pathol* 21(2):178–185.

Hoashi T, Kanda N, Saeki H 2022. Molecular mechanisms and targeted therapies of advanced basal cell carcinoma. *Int J Mol Sci* 23(19):11968.

Jacobsen AA, Aldahan AS, Hughes OB, Shah VV, Strasswimmer J 2016. Hedgehog Pathway Inhibitor Therapy for Locally Advanced and Metastatic Basal Cell Carcinoma: A Systematic Review and Pooled Analysis of Interventional Studies. *JAMA Dermatol* 152(7):816–824.

Jawhar NMT 2009. Tissue Microarray: A rapidly evolving diagnostic and research tool. *Ann Saudi Med* 29(2):123–127.

Jeng KS, Chang CF, Lin SS 2020. Sonic Hedgehog Signaling in Organogenesis, Tumors, and Tumor Microenvironments. *Int J Mol Sci* 21(3):758.

Katona TM, Perkins SM, Billings SD 2008. Does the panel of cytokeratin 20 and androgen receptor antibodies differentiate desmoplastic trichoepithelioma from morpheaform/infiltrative basal cell carcinoma? *J Cutan Pathol* 35(2):174–179.

Kim HS, Kim YS, Lee C, Shin MS, Kim JW, Jang BG 2019. Expression profile of sonic hedgehog signaling-related molecules in basal cell carcinoma. *PloS one* 14(11):e0225511.

Kumar B, De Silva M, Venter DJ, Armes JE 2004. Tissue microarrays: a practical guide. *Pathology* 36(4):295–300.

Lomas A, Leonardi-Bee J, Bath-Hextall F 2012. A systematic review of worldwide incidence of nonmelanoma skin cancer. *Br J Dermatol* 166(5):1069–1080.

Messina J, Epstein EH Jr, Kossard S 2018. Basal cell carcinoma. In: Elder DE, Massi D, Scolyer RA, Willemze R eds. *WHO Classification of Skin Tumors*. IARC Press pp26–34.

Niculet E, Craescu M, Rebegea L, Bobeica C, Nastase F, Lupasteanu G, Stan DJ, Chioncel V, Anghel L, Lungu M, Tatu AL 2022. Basal cell carcinoma: Comprehensive clinical and histopathological aspects, novel imaging tools and therapeutic approaches (Review). *Exp Ther Med* 23(1):1-9.

Perera E, Gnaneswaran N, Staines C, Win AK, Sinclair R 2015. Incidence and prevalence of non-melanoma skin cancer in Australia: A systematic review. *Australas J Dermatol* 56(4):258–267.

Pollack A, McGrath M, Henderson J, Britt H 2014. Skin cancer by state and territory. *Aust Fam Physician* 43(8):507.

Ramezani M, Mohamadzaheri E, Khazaei S, Najafi F, Vaisi-Raygani A, Rahbar M, Sadeghi M 2016. Comparison of EMA, CEA, CD10 and Bcl-2 Biomarkers by Immunohistochemistry in Squamous Cell Carcinoma and Basal Cell Carcinoma of the Skin. *Asian Pac J Cancer Prev* 17(3):1379–1383.

Reisinger DM, Shiffer JD, Cognetta AB, Chang Y, Moore PS 2010. Lack of evidence for basal or squamous cell carcinoma infection with Merkel cell polyomavirus in immunocompetent patients with Merkel cell carcinoma. *J Am Acad Dermatol* 63(3):400–403.

Richmond-Sinclair NM, Pandeya N, Ware RS, Neale RE, Williams GM, van der Pols JC, Green AC 2009. Incidence of basal cell carcinoma multiplicity and detailed anatomic distribution: longitudinal study of an Australian population. *J Invest Dermatol* 129(2):323–328.

Rubin AI, Chen EH, Ratner D 2005. Basal-cell carcinoma. *N Engl J Med* 353(21):2262–2269.

Sabatino R, Santonastaso C, Franco R, Scognamiglio G 2014. Use of tissue microarrays in translational research. *WCRJ* 1:e356.

Seidl-Philipp M, Frischhut N, Höllweger N, Schmuth M, Nguyen VA 2021. Known and new facts on basal cell carcinoma. *J Dtsch Dermatol Ges* 19(7):1021–1041.

Sekulic A, Von Hoff D 2016. Hedgehog Pathway Inhibition. *Cell* 164(5):831.

Snow SN, Sahl W, Lo JS, Mohs FE, Warner T, Dekkinga JA, Feyzi J 1994. Metastatic basal cell carcinoma. Report of five cases. *Cancer* 73(2):328–335.

Stratigos AJ, Sekulic A, Peris K, Bechter O, Prey S, Kaatz M, Lewis KD, Basset-Seguin N, Chang ALS, Dalle S, Orland AF, Licitra L, Robert C, Ulrich C, Hauschild A, Migden MR, Dummer R, Li S, Yoo SY, Mohan K, Coates E, Jankovic V, Fiaschi N, Okoye E, Bassukas ID, Loquai C, De Giorgi V, Eroglu Z, Gutzmer R, Ulrich J, Puig S, Seebach F, Thurston G, Weinreich DM, Yancopoulos GD, Lowy I, Bowler T, Fury MG 2021. Cemiplimab in locally advanced basal cell carcinoma after hedgehog inhibitor therapy: an open-label, multi-centre, single-arm, phase 2 trial. *Lancet Oncol* 22(6):848–857.

Sunjaya AP, Sunjaya AF, Tan ST 2017. The Use of BEREP4 Immunohistochemistry Staining for Detection of Basal Cell Carcinoma. *J Skin Cancer* 2017:2692604.

Swanson PE, Fitzpatrick MM, Ritter JH, Glusac EJ, Wick MR 1998. Immunohistologic differential diagnosis of basal cell carcinoma, squamous cell carcinoma, and trichoepithelioma in small cutaneous biopsy specimens. *J Cutan Pathol* 25(3):153–159.

Tang S, Thompson S, Smee R 2017. Metastatic basal cell carcinoma: case series and review of the literature. *Australas J Dermatol* 58(2):e40–e43.

Tebcherani AJ, de Andrade HF, Sotto MN 2012. Diagnostic utility of immunohistochemistry in distinguishing trichoepithelioma and basal cell carcinoma: evaluation using tissue microarray samples. *Mod Pathol* 25(10):1345–1353.

Villada G, Kryvenko ON, Campuzano-Zuluaga G, Kovacs C, Chapman J, Gomez-Fernandez C 2018. A Limited Immunohistochemical Panel to Distinguish Basal Cell Carcinoma of Cutaneous Origin From Basaloid Squamous Cell Carcinoma of the Head and Neck. *Appl Immunohistochem Mol Morphol* 26(2):126–131.

Wadhera A, Fazio M, Bricca G, Stanton O 2006. Metastatic basal cell carcinoma: a case report and literature review. How accurate is our incidence data? *Dermatol Online J* 12(5):7.

Webb DV, Mentrikoski MJ, Verduin L, Brill LB, Wick MR 2015. Basal cell carcinoma vs basaloid squamous cell carcinoma of the skin: an immunohistochemical reappraisal. *Ann Diagn Pathol* 19(2):70–75.

Zheng ZL, Kim AR, Kye YC, Kim IS, Zhang XL 2005. Expression of p63, bcl-2, bcl-6 and p16 in basal cell carcinoma and squamous cell carcinoma of the skin. *Korean J Pathol* 39(2):91–98.

Implementation of risk control measures to manage flammable liquid storage in the Australian Standard AS ISO 15189:2023 accredited medical laboratory

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Introduction

Flammable liquid is a routinely used hazardous chemical in the medical laboratory. The medical laboratory must establish and maintain relevant risk control measures to protect laboratory personnel when flammable liquid is stored and used. The main concern is fire caused by the ignition of flammable vapours that could burn fiercely and spread rapidly. The medical laboratory must implement relevant control measures based on the design and structure of facility according to AS ISO 22367:2021 to support the risk management specified in Clause 5.6 of AS ISO 15189:2023. Implementation of additional requirements other than those stated in Table 1 may be required depending upon the measures required to reduce the fire risks. The main objective of this paper is to enhance the medical laboratory's awareness of requirements relating to provision of relevant risk control measures for indoor storage of minor quantities of flammable liquid.

Location of storage

The medical laboratory must ensure the location can provide good physical security with sound environmental factors and does not hinder safety measures of the surrounding areas. The requirements may be mutually conflicting, such as the provisions of good security with adequate airflow ventilation. Specific air change rates for different areas are recommended by ANSI/ASHRAE/ASHE Standard 170-2021.

Fire hazard management

The medical laboratory must observe relevant fire safety precautions to reduce the likelihood of fire risk. This must be supported by the implementation of classification of hazardous areas of operations that leads to the modification of laboratory activities, management of holding volume as it relates to fire load density, provision of fire extinguishing capability, maintenance of electrical wiring to prevent dangerous outlets from sparking, and hazard warning display.

Method of storage

The medical laboratory must ensure the flammable liquids are stored inside purposely designed chemical storage cabinets and 'explosive-safe' non-sparking refrigerators. The chemical storage cabinet door must have a warning sign describing the dangerous goods that are stored. The flammable liquids can also be stored on shelves and racks equipped with edge guards at an appropriate height.

Further considerations

Guidance from Safe Work Australia (SWA) and the International Labour Organization (ILO) should also be considered by the medical laboratory. The SWA provides relevant recommendations relating to storage of chemicals (SWA 2020a), including flammable liquids (SWA 2020b), and the ILO provides recommendations for control measures for the storage of hazardous chemicals (ILO 1993).

The medical laboratory must provide what is reasonably practicable to ensure the relevant risk control measures for flammable liquid storage are identified, implemented, and displayed unambiguously for hazard communication to laboratory personnel.

References

Safe Work Australia 2020a. *Managing risks of storing chemicals in the workplace*. Canberra: Safe Work Australia.

Safe Work Australia 2020b. *Storage of flammable liquids*. Canberra: Safe Work Australia.

International Labour Organization 1993. *Safety in the use of chemicals at work*. Geneva: International Labour Office. Section 6.7, Control measures for the storage of hazardous chemicals; 28-29.

***Table 1**. An action list for the medical laboratory to ensure the relevant risk control measures for flammable liquid minor storage are identified, implemented, and displayed for hazard communication.*

Areas	Action list	Sources
Location of storage	The locality of storage does not pose negative impacts relating to: • Safety of any areas on lower levels of the building; • Conduct of firefighting operations; • Containment of flammable vapours; and • Ventilation.	Clause 2.3.1 of AS 1940:2017
	The locality of storage is a secure location.	Subclause 8.5.1 a) of ISO 15190:2020
	The locality of storage has adequate ventilation.	Subclause 8.5.1 d) of ISO 15190:2020
	The locality of storage is away from direct heat, sunlight or highly variable temperatures.	Subclause 8.5.1 e) of ISO 15190:2020
Control of ignition sources	The relevant areas of operations are classified according to AS/NZS 60079.10.1.2022, if the volume of flammable liquids is: • > 100 L in closed containers; • > 25 L for decanting purposes; • > 5 L in open containers for occasional use; or • > 1 L in open containers for continuous use.	Clause 2.3.3 of AS 1940:2017
	No uncontrolled sources of ignition in any space where flammable vapours is present.	Clause 2.3.3 of AS 1940:2017
Fire protection	The relevant areas of operations used for decanting flammable liquids have: • ≥ 1 portable, readily accessible, fire extinguisher(s) with a suitable rating for use; • ≥ 1 portable fire extinguisher(s) with suitable rating for use must be located adjacent to the storage area; and • A sign on display bearing the following words: 'DANGER–FLAMMABLE LIQUIDS–NO SMOKING–KEEP FIRE AWAY' Note. The design of the sign must meet the requirements of AS 1319—1994.	Clause 2.3.5 of AS 1940:2017
Quantities of minor storage	Appropriate packing densities are observed for: • Packing Group I (≤ 50 L per 50 m^2 of floor space or ≤ 50 L in a room of ≤ 50 m^2 of floor space); • Packing Group II (≤ 50 L per 50 m^2 of floor space or ≤ 50 L in a room of ≤ 50 m^2 of floor space); and • Packing Group III (≤ 100 L per 50 m^2 of floor space).	Clause 2.2.1 of AS 1940:2017 Clause 7.3.2 of AS 2243.2:2021

Method of storage	Flammable liquids stored outside chemical storage cabinets are stored on shelves or racks.	Clause 8.5.2.1 of AS/NZS 2982:2010
	Flammable liquids are stored inside a closable cabinet or on a sturdy shelf.	Subclause 8.5.1 c) of ISO 15190:2020
	Flammable liquids are stored in approved chemical storage cabinets.	Subclause 8.5.2 a) of ISO 15190:2020 Subclause 11.1.2 of ISO 15190:2020
	Flammable liquids are not stored on tops of chemical storage cabinet, on the floor, or on bench tops and in chemical fume hoods.	Subclause 8.5.2 e) of ISO 15190:2020
	Packing Group I of flammable liquid is stored at an appropriate volume (≤ 2.5 L); unless they are required for daily operations and handled by trained laboratory personnel.	Clause 2.2.2 of AS 1940:2017
	Refrigerated flammable liquids are stored in 'explosive safe' non sparking refrigerators.	Subclause 11.1.2 of ISO 15190:2020
Chemical storage cabinet design	The construction of chemical storage cabinet is designed to specifications of Clause 4.4 of AS 1940:2017.	Clause 6.3.4 of AS 2243.2:2021 Clause 7.3 of AS/NZS 2982:2010
Chemical storage cabinet capacity (cabinets under benches)	Each chemical storage cabinet has an appropriate capacity for flammable liquids (≤ 30 L).	Clause 7.2.3 of AS/NZS 2982:2010
Chemical storage cabinet siting considerations	The locations of chemical storage cabinets are not situated one above the other, under stairs, or in corridors, and do not obstruct emergency escape.	Clause 6.3.6 of AS 2243.2:2021
	The siting of chemical storage cabinets has an appropriate separation distance if located next to each other (≥ 300 mm of air-space).	Clause 8.5.1 of AS 2243.2:2021
Chemical storage cabinet (segregation in storage)	The chemical storage cabinet used for storage of flammable liquids is not to be used for the storing of dangerous goods of any other class.	Clause 7.6.2 of AS 2243.2:2021
Shelves and racks (open storage)	The flammable liquids are stored at an appropriate height from the floor (≤ 1.5 m).	Clause 6.8 of AS 2243.2:2021
	The shelves used for storing flammable liquids are equipped with edge guards, sturdy with specified capacity, and properly assembled.	Subclause 8.5.2 g) of ISO 15190:2020
Display of hazard identification information	The entrance to a dedicated room where flammable liquids are stored has appropriate signage regarding dangerous goods that are stored. The chemical storage cabinet door has displayed an appropriate signage regarding dangerous goods that are stored.	Clause 6.5 of AS 2243.2:2021
Electrical wiring precaution	The certification of electrical wiring of the storage facility is valid according to Clause 8.4 of AS/NZS 3000:2018.	Clause 4.4.3 of AS 2243.2:2021

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Journal-based CPD No. 90

Questions relating to the article *'Effectiveness of oral iron supplementation for treatment of iron deficiency anaemia'* at page 62 of this issue.

1.	Iron deficiency anaemia (IDA) disproportionately affects children and adult females.	True/False
2.	The bioavailability of oral iron is affected by factors including diet, iron compound, dosage and dosing frequency.	True/False
3.	In adults 50% of iron replenishment is required from the diet.	True/False
4.	Serum ferritin concentration is reflective of the total iron stores and is considered the most accurate measure of iron status.	True/False
5.	The normal level of iron stored in the body is confirmed by the concentration of serum ferritin of >15 µg/L.	True/False
6.	Ferrous sulfate contains the highest amount of elemental iron with 530 mg.	True/False
7.	FeNa-EDTA allows a 2-3 x increase in absorption compared with other iron compounds and is regarded as the most favourable iron compound.	True/False
8.	The bioavailability of oral iron supplementation is not affected by whether it is taken with or without a meal.	True/False
9.	Coffee impairs iron absorption in the stomach.	True/False
10.	The current Australian recommended dose for adults with IDA is 100-200 mg of elemental iron administered daily in divided doses.	True/False

Name: _____

Email: _____

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Australian Professional Acknowledgement of Continuing Education (APACE)

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Journal-based CPD No. 91
Page 1 of 1

Questions relating to the article '*Pre-analytical errors and their prevention in an emergency department setting*' at page 45 of this issue.

1.	Approximately 70% of all clinical decisions are based on laboratory results.	True/False
2.	In this study the pre-analytical phase refers to specimen collection.	True/False
3.	Statistical analysis of pre and post intervention error rates were analysed using Graph Pad Prism.	True/False
4.	Request-based error type accounted for the highest number of pre-analytical errors.	True/False
5.	The highest phlebotomy/collection error was sample haemolysis.	True/False
6.	A delay in specimen transport greater than 30mins from the ED to the laboratory was the highest transport-based error.	True/False
7.	In this study, 2.34% pathology samples were haemolysed which is lower than the Karcher & Lehman error rate.	True/False
8.	Switching from an IV catheter Jelco system to a system that is bi-directional may reduce the number of haemolysed samples.	True/False
9.	A pneumatic tube system may reduce TAT.	True/False
10.	This study showed that there was a need for ongoing commitment to pre-analytical training to reduce error rates.	True/False

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Australian Institute of
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An APACE Certificate is usually awarded on attaining 100 CEU credits within a two year period.

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Therefore, the extended time frame due to Covid-19 will no longer apply.

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AUSTRALIAN JOURNAL OF MEDICAL SCIENCE

Instructions to authors

The following instructions are based on the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals", also known as the Declaration of Vancouver, and on the *Australian Government Style manual: for authors, editors and printers*, 6th edition, 2002. URLs were correct on September 29th, 2008.

Manuscripts that do not fully comply with the following 'Instructions to Authors' may be returned for revision before they are considered for publication.

The *Australian Journal of Medical Science (AJMS)* will consider for publication any paper relevant to the field of Medical Science. Disciplines include blood banking, clinical biochemistry, haematology, histopathology, immunology, microbiology and molecular biology. Areas of general interest to medical laboratory scientists, including toxicology, epidemiology, public and community health, and professional and management issues will also be considered.

Papers published in the *AJMS* are in the form of:

- Review Articles
- Original Articles
- Brief Communications
- Technical Notes
- Case Studies
- Letters to the Editor
- Book Reviews

Articles submitted for publication are understood to be offered only to the *AJMS* and those accepted become the property of the *AJMS*.

All individuals listed as authors must have made a substantial contribution to the conception and design of the study, the acquisition of data or the analysis and interpretation of data; the drafting of the article or revising it critically for important intellectual content; and final approval of the version to be published. The corresponding author must take responsibility for obtaining permission from all the authors for the submission of any version of the manuscript and for any changes in authorship.

When the manuscript is submitted the authors must disclose any potential conflict of interest and/or commercial support.

Requirements & preparation of manuscripts

General

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

Number pages consecutively commencing with the title page.

Arrange the article in the following sequence:

- Title page
- Abstract and key words
- Main Text
- Acknowledgements
- References
- Tables - each table, complete with title and footnotes, on a separate page
- Legends for illustrations.

Authors should ensure that their manuscript communicates their ideas and concepts simply and clearly so that the article is easily read and understood. Authors are strongly recommended to refer to the recommendations on reporting standards as outlined in the statements and checklists of the CONSORT group (see: http://www.consort-statement.org/) and similar groups such as STARD (see: http://www.stard-statement.org/). The principles outlined in these standards may be used as general guidelines and not just as applied to clinical trials and diagnostic studies.

Title page

The title of the article should not exceed three lines (40 characters per line), including punctuation and spacing. All authors must be identified on the title page (e.g. William Smith, Susan Yeo, ..."). Where applicable, the title page should also include the name of the institution with which each author is affiliated and to which the work should be attributed. In the case of multiple authors, the name, postal address, email address, telephone and facsimile number of the author responsible for correspondence relating to the manuscript should be indicated.

Abstract & keywords

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

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

Text

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

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

- Introduction
- Materials and methods
- Results
- Discussion
- References

For other types of articles such as commentaries, reports and reviews, use an appropriate format or consult the Editors for guidance. Do not include a separate section for conclusions, these should be given in the discussion.

Introduction

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

Materials & methods

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

Results

Present the results in the same sequence as given in the Materials and methods; use tables and illustrations where these will help the reader understand the work being presented. Do not repeat in the text all the data in the tables or illustrations.

Discussion

Indicate the new and important aspects of the study and emphasise the conclusions that follow. Do not repeat in detail data given in the Results section and do not add new data. Include in the Discussion the implications of the findings and their limitations and compare the observations to other relevant studies. Recommendations may be included if appropriate. Link the conclusions with the goals of the study and answer the experimental question stated in the Introduction. However, avoid unqualified statements and conclusions not completely supported by your data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

Acknowledgements

Acknowledge individuals who have made substantial contributions to the study including technical work and financial support. Authors are responsible for obtaining consent from all the individuals acknowledged by name as inclusion may be interpreted as an endorsement of the article's contents.

References

The AJMS uses a modified Harvard System (author-date system).

Throughout the body of the manuscript cite the author/s name and the publication year in parentheses as in the following examples:

(i) Research in this area (Jones 1999) …

(ii) It has been successfully demonstrated that (Smith and Brown 1981; Auteur 1995; Scienziato *et al* 2007).

(iii) Following further investigation, Wetenschapper (2002 highlighted the difficulties inherent in…

Where there are three or more authors, acknowledge only the first author, e.g. (Smith *et al* 2007). For two authors the following style should be used: (Smith and Brown 2007).

The reference list should be in the format described below. Journal titles should be abbreviated in Index Medicus format (see: ftp://nlmpubs.nlm.nih.gov/online/journals/ljiweb.pdf) using standard abbreviations from the ISSN List of Title Word Abbreviations (see: http://www.issn.org/en/node/344) All authors should be given in the reference list.

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

Examples of the correct form for references are given below:

Journal Reference:

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

Personal Author(s) of a book:

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

Editor, Compiler, Chairman as Author:

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

Chapter in Book:

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

Online documents:

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

Tables

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

For footnotes, use the following symbols in this sequence:

* † ‡ § ¶ ** ††

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

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

Illustrations

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

When plotting points, the following symbols are preferred:

○ ● ⏄ ▲ □ ■

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

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

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

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

Abbreviations

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

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

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

Commonly used abbreviations

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

Additional information

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

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

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

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

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

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

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

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Australian Institute of Medical and Clinical Scientists

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Changes to Certification arrangements for the Medical Laboratory Science Profession

From April 2023, the Australian Council for the Certification of the Medical Laboratory Scientific Workforce (CMLS) Board are no longer accepting applications for certifications directly. Instead, professional bodies operating CMLS approved CPD schemes will be able to issue certification on behalf of the Council for their members who meet the requirements for certification as detailed on the CMLS website.

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