Medicines & Healthcare products Regulatory Agency

MHRA Regulating Medicines and Medical Devices

Minutes for Blood Consultative Committee (BCC) Meeting
17th March 2016, 13:00-16:00
MHRA Buckingham Palace Road Offices, G1

Attendees:

Marie McQuade (SCTAC)
Chris Elliot (IBMS)
Alison Watt (SHOT)
Fidelma Murphy (NHSBT)
Jot Hyare (NTLMS)
Ann Benton (NHS Wales)
Brian Alexander (SNBTS)
Shirley Stag (HTA)
Shalinee Wickramasinghe (NHSBT)
Joan Jones (Wales NHS)
Stuart MacDonald (MOD)
Rashmi Rook (NHS)
Liz Carroll (Haemophilia Society)
Shubha Allard (NHSBT)
Jeremy Grindrod (MOD)
Jan Stewart (TDL Pathology)
Will Bowen (NHS)
Emma Lowe (NIHR)

MHRA:

Mark Birse (IE&S) – Chair
Beverley Malin-Smith (minutes)
Vivian Rowland (IE&S)
Chris Robbie (SABRE)
Rosalind Polley (Devices)
Michelle Rowson (IE&S)
David Churchward (IE&S)
Ian Rees (IE&S)
Kevin Page (IE&S)

1. Apologies Received

Sandra Gray (Retired)
Johnathan Wallis (NBTC)
Ian Bateman (NHSBT)
Paula Bolton-Maggs (SHOT)
Allan Morrison (NTLMS)
Cyril Taylor (TDL Pathology)
Tony Docherty (SNBTS)
Sheila MacLennan (JPAC)
Stephen Bassey (NTLM)
Angela Macaulay (NIBTS)
Caroline Lewis (Wales NHS)
Chris Phillips (NHSBT)

2. Introductions and Apologies for Absence

Mark Birse opened and chaired the meeting. He thanked everyone for attending, welcomed any new members, and noted the apologies. The minutes from the previous meeting held on 23rd September 2015 were reviewed. These were accepted and 3 ongoing actions were noted:
i) MHRA to create an online forum for Blood Stakeholders **– on agenda for today**
ii) Updated TOR **– any further comments requested by end of April 2016**
iii) BCC members to confirm the names of the primary representatives nominated to represent their respective organisation **– any further updates requested by end of April 2016**

3. SABRE Update

Chris Robbie provided an overview of reporting activity for 2015:

- The Deviation error category ‘other’ and Storage continue to be the highest reported error types but show very little change from reports received in 2014.
- Human Error is still the highest single SAE deviation.
- The increase in SAEs falling in the “other” category is largely a result of changes to SAE reporting arrangements where SHOT and MHRA see all reports. It is not thought to demonstrate a worsening of performance.

- For SAR reporting, Phase 1 of the Joint Haemovigilance Project was released on time with no major problems. Phase 2 is under construction which will incorporate SAE reporting.

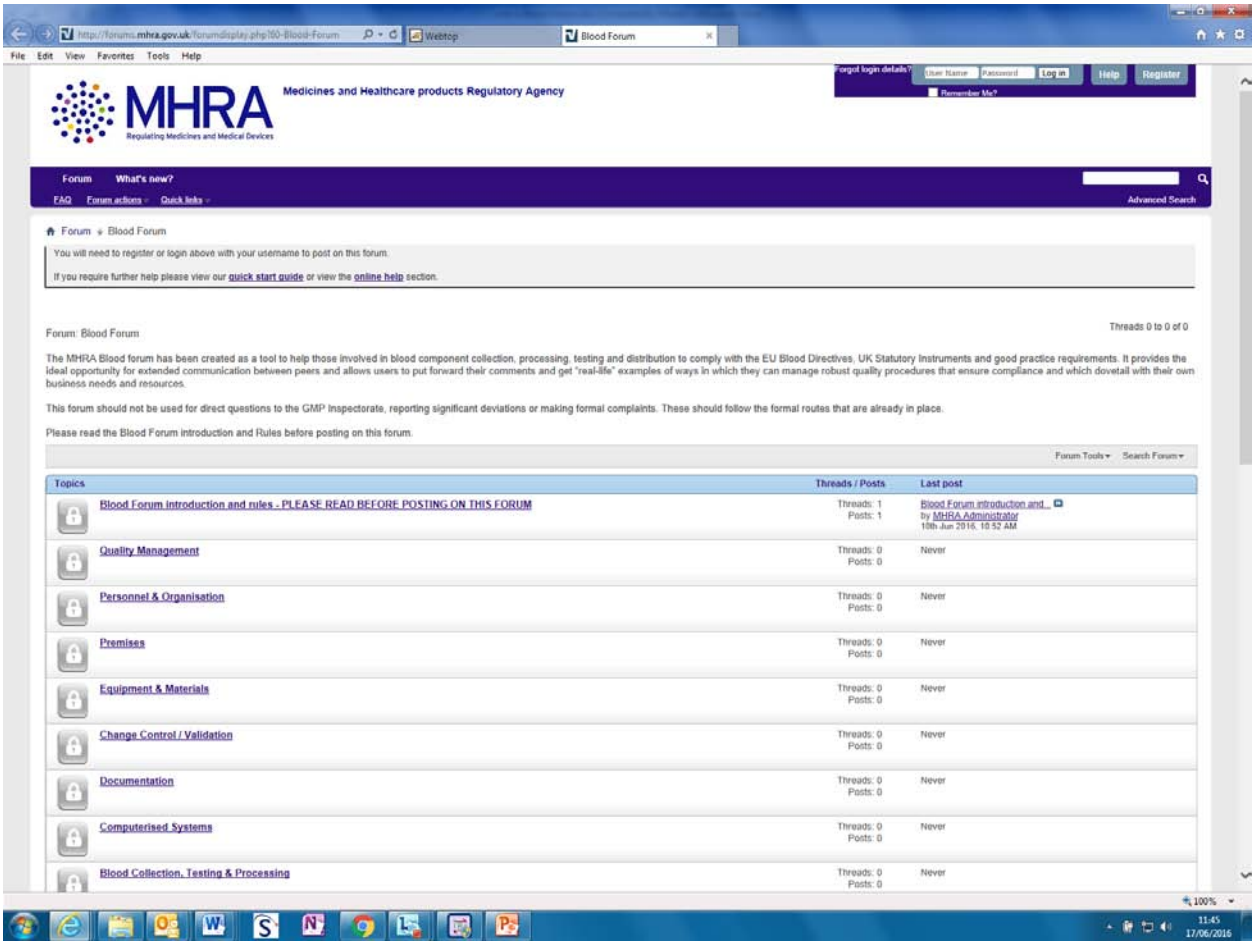
4. BCR Process Update

Vivian Rowland provided an overview of changes to the BCR report and assessment process for the April 2015 – March 2016 reporting year:

- The majority of questions requiring free-text responses have been removed to facilitate a fully automated assessment using the risk score programme.
- As a result of a reduction in the number of critical inspection deficiencies, compliance management and regulatory action cases, it is also proposed to reduce the number of inspections triggered 'for cause' as a direct result of the BCR assessment process.
- The inspectorate will develop the risk based inspection approach to react to risk factors identified throughout the year and maintain our commitment to proportionate regulation.
- Examples of non-BCR inspection triggers under consideration include notifications of significant site change and adverse SABRE reporting trends.
- Control inspections will also be performed to monitor the performance of the revised approach to BCR assessment and inspection scheduling.
- Blood Facilities will not be required to complete a compliance report for 2015. An alternative system of compliance declaration will be implemented. This will be aligned with elements of the system implemented by the Health Products Regulatory Authority in Ireland.
- Additional questions for inclusion in the BCR were proposed by BCC members. These have been considered and implemented where possible and justification provided where this was not possible, e.g. very site specific, considered too subjective, relevance to current compliance status unclear, or clinical decisions and number of staff required are not within MHRA's remit to advise.
- BCR template and guidance note added to GOV.UK on 07 March 2016. Reporting period 01 Apr 15 to 31 Mar 16.
- Jot Hyare suggests that another question for future consideration would be to ask: On how many occasions has the site dropped below the lowest threshold for qualified staff.
- It was suggested that BCC members consider benchmarking data for different Trusts versus the number of units. This is something that SHOT do and also the Welsh Blood Service do through regional transfusion committees.

5. Update on on-line forum for Blood Stakeholders

Michelle Rowson provided a demonstration of the on-line discussion forum on behalf of Stephen Grayson who is leading on the project following his return to the Inspectorate. The forum is currently on a test-site and so not available for public viewing.



- The titles for the headings have been created using the Chapters of the Good Practice Guide.
- The forum will be pre-populated with useful reference material and Q&A topics MHRA have previously developed and posted on the OIG website.
- It is hoped the forum will become a reference source, opportunity to post questions and share best practice ideas.
- All questions and comments need to be reviewed and moderated before they go live so background work has gone into ground rules and information on how the site will work.
- Anyone can view posts but need to register to add information and take part in discussions. Due to some concerns around confidentiality, users can register on a home e-mail address so that other users aren't aware of where they work or who they are.
- It is hoped that the forum will be ready to go live in the Summer 2016 and there will be an update on the Inspectorate Blog (https://mhrainspectorate.blog.gov.uk/) to make people aware of the launch.

6. Pilot of updated Compliance Report for Blood sites

Michelle Rowson reported that updated compliance reports had been designed for Blood sites. Michelle explained that prior to a routine inspection of BEA sites, there is a requirement to complete a pre-inspection compliance report and to provide an interim compliance report following key changes. Although the compliance report templates were updated about a year ago with improvements to the questions asked of blood establishments, the reports were more geared towards pharmaceutical sites and so a separate template for blood sites has been developed.

The updated templates are currently being piloted with a small number of sites.
A request was made to improve the formatting of free text boxes so that full answers can be given without it affecting the template. Once finalised, the templates will be made available on GOV.UK (https://www.gov.uk/guidance/blood-authorisations-and-safety-reporting).

7. **Regulatory Update**

i) IVD Directive changes – Rosalind Polley (Devices)

- Final text agreed in June however earliest publication date is expected to be October 2016. Commission guidance on classification will be published.
- Class D devices will not need to be CE marked, but will need to notify Competent Authority and are expected to have an appropriate quality management system in place. These 'in house' devices will only be permitted for use within the same legal entity. Any wider sale will require CE marking. It was confirmed that 'Health Institutions' can receive samples from other legal entities and perform testing under this exemption.
- Post marketing Surveillance is not currently part of the Directive but will be an expectation of MHRA.
- The Directive introduces rules around software for medical purpose. Laboratory information management systems (LIMs) must be CE marked if it is performing calculations or adding to data. BCC members reported that very few if any LIMs systems are CE marked and would welcome MHRA's help in contacting manufacturers.

Action – BCC members to provide list of suppliers to Beverley Malin-Smith for Devices to follow up.

ii) GMP blood good practice guidelines update – David Churchward

- A draft was released in December 2013 which has now been considered by the Commission and is likely to be accepted. Discussions are still required relating to how this document will be maintained in line with GMP developments (work on-going with Council of Europe).
- A revision to the UK Blood Safety and Quality regulations is imminent, to implement the EU Directive revision regarding West Nile virus NAT testing. When this requirement is transposed, a previous amendment to implement end of shelf-life platelet pH updates will also be incorporated.
- Hep E negative SABTO blood guidelines were updated on 14th March 2016. Discussions were held regarding the impact and effect this will have on ongoing services.

iii) Collaborative working – Ian Rees

- A UK Blood forum has been set up involving, MHRA, HTA, UKAS and CEOs of the UK Blood Services to look at ways of working, information sharing, reliance on others work, areas of overlap, to develop closer working.
- Potential for scope of BCC to be revised or incorporated into other meetings with a strategic focus, once the operational elements are covered by the on-line forum.

8. **Update from JPAC**

Shelia MacLennan presented the attached papers for information, as the changes agreed by JPAC will affect component handling in hospitals and not just the blood services. The topics relate to:

- Extension of post-thaw shelf life of FFP to 5 days to support management of massive haemorrhage
- Deviations from 4°C temperature storage for red cells:effect on viability and bacterial growth

The papers include a summary sheet and larger background paper for each topic. The aim is to reduce the amount of blood currently being wasted. Further background documents are also available on the JPAC website.

Dates for Next Meeting:
Tuesday 1st November 2016 14:00 – 16:00 at MHRA Offices, Buckingham Palace Road

Medicines and Healthcare Products Regulatory Agency

SABRE BCC report March 2016

Chris Robbie MHRA

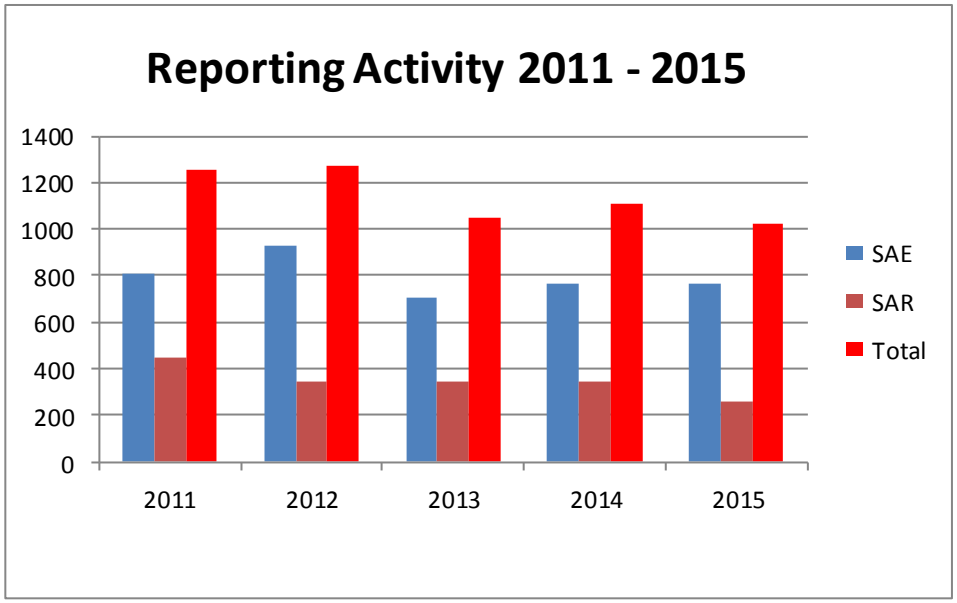
CPRD NIBSC MHRA

Reporting Activity

Medicines and Healthcare Products Regulatory Agency

Confirmed Reports 2011 – 2015

	2011	2012	2013	2014	2015
SAE	810	931	705	764	765
SAR	444	343	345	346	262
Total	1254	1274	1050	1110	1027



SAEs have remained the same as 2014

SARs have dropped by 24%

Changes to reporting arrangements with SHOT in Oct 2015 have affected reporting patterns and it is unwise to read anything into that at this moment

CPRD NIBSC MHRA

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SAEs by Deviation Jan 2015- 2015

SAE Deviation	Total No	Product Defect	Equipment Failure	Human Error	Other
Whole Blood Collection	**29**	0	0	28	1
Apheresis Collection	**3**	0	0	3	0
Testing of Donations	**6**	1	1	4	0
Processing	**10**	0	0	10	0
Storage	**198**	0	9	189	0
Distribution	**18**	0	0	17	1
Materials	**1**	0	0	1	0
Other	**500**	2	8	488	2
Overall Total	**765**	**3**	**18**	**740**	**4**

The Deviation error category ‘other’ and Storage continue to be the highest reported error types but show very little change from reports received in 2014.

Human Error is still the highest single SAE deviation

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Other reports sub categories 2015

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Sub Category	2014	2015
Incorrect blood component selected and issued (IBCI)	135	137
Component labelling error (CLE)	85	86
Pre transfusion testing error (PTTE)	68	79
Sample processing error (SPE)	70	75
Data entry error (DEE)	56	48
Component collection error (CCE)	26	45
Failed recall (FR)	15	10
Incorrect blood component ordered (IBCO)	5	7
Component available for transfusion past de-reservation date (CATPD)	9	4
Unspecified (UNS)	1	4
Expired component available for transfusion (ECAT)	4	3
Handling Damage	0	1
Not Known (NKN)	0	1
Total	474	500

The increase in SAEs falling in the “other” category is largely a result of changes to SAE reporting arrangements where SHOT and MHRA see all reports. It is not thought to demonstrate a worsening of performance.

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SAR Reporting

- Phase 1 of the Joint Haemovigilance Project was released on time with no major problems
- Phase 2 is under construction which will incorporate SAE reporting
  - Improvements to information to fedback to reporters following analysis of reports by SHOT or MHRA
  - Provide a seamless link between SABRE and Dendrite systems
  - Allow SHOT to update SABRE confirmation reports directly from Dendrite
  - Reduce SAR reporting burden on reporters (MHRA/SHOT – Centralised reporting system)
- Regular consolidation of figures by SHOT and SABRE
- Phase 3 is under investigation

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2015 Data Summary Points

- Changes to reporting process have resulted in changes to the numbers of reports received in SABRE
- It will take at least another 12 months to assess the affect of these changes to analysis of the data
- Human error still remains the single largest cause of error
- SARs are being classified and categorised by clinical experts in SHOT where the reporting function, for SAR to the EU, will remain with the MHRA as the UK competent authority.
- Will give a better idea of the UK SAR type and numbers reported to the EU Annually.

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Blood Compliance Report (BCR) Process Update

Vivian Rowland

17 March 2016

Topics for discussion

Changes in BCR assessment 2015

2015 BCR Outcome

Proposed BCR questions

Additional BCR questions

2016 BCR Assessment

Changes in BCR assessment 2015

For Hospital Blood Banks

A removal of the majority of questions requiring free-text responses

Fully automated risk assessment

Reduced number of inspections as a direct result of the assessment

For Blood Facilities

No BCR for Blood Facilities

Implementation of compliance declaration

2015 BCR Outcome

HBB BCR received	308
Late submission	27
BAT referral required	45
Site compliant	287
Site required further assessment	10
Site required inspection	13 (including 2 control sites)

Proposed BCR questions (1)

1) Does the blood transfusion dept (or haematology/transfusion depts if combined) have any vacancies? if so, please state the number of posts and length of time vacant to date

a) Blood bank manager/ Technical Lead _____ length of time of vacancy___

b) Blood bank senior BMS_____ ___ length of time of vacancy___

c) BMS (Blood bank or shared rotational staff) _____ length of time of vacancy___

d) MLA (Blood bank or shared rotational)_____ length of time of vacancy___

e) Transfusion practitioner_____ length of time of vacancy___

Proposed BCR questions (2)

2) Was staffing levels raised as a major non- conformance at any previous MHRA inspection? Y/N or N/A

a) if 2 is Yes, has a Capacity Plan been produced and the total number of staff and skill mix to maintain all aspects of the service (technical and regulatory) identified ? Y/N

b) if 2 is Yes, has there been an increase in staff levels since the inspection? Y /N

Proposed BCR questions (3)

3) Does the technical lead participate in full shifts/oncall that would affect their core hours availability (09:00-17:00hrs)? Y/N

4) During Oct 2015 how many **week days** was the Blood bank adequately staffed to enable all technical work, training and regulatory activities to be performed? _____ days (0 -20)

5) Has your laboratory performed a GAP analysis against the UK Transfusion laboratory Collaborative 2014 standards? Y/ N

Proposed BCR questions (4)

6) Does the laboratory record the following as quality incidents / non-conformances:

a) lab errors, clinical errors related to blood ,

b) Audit non-conformances

c) customer/ staff and supplier complaints?

d) Equipment failures

Proposed BCR questions (5)

7) How many Blood bank quality incidents were recorded from 1/4/15 to 31/3/16 :

0-50

50-100

100-200

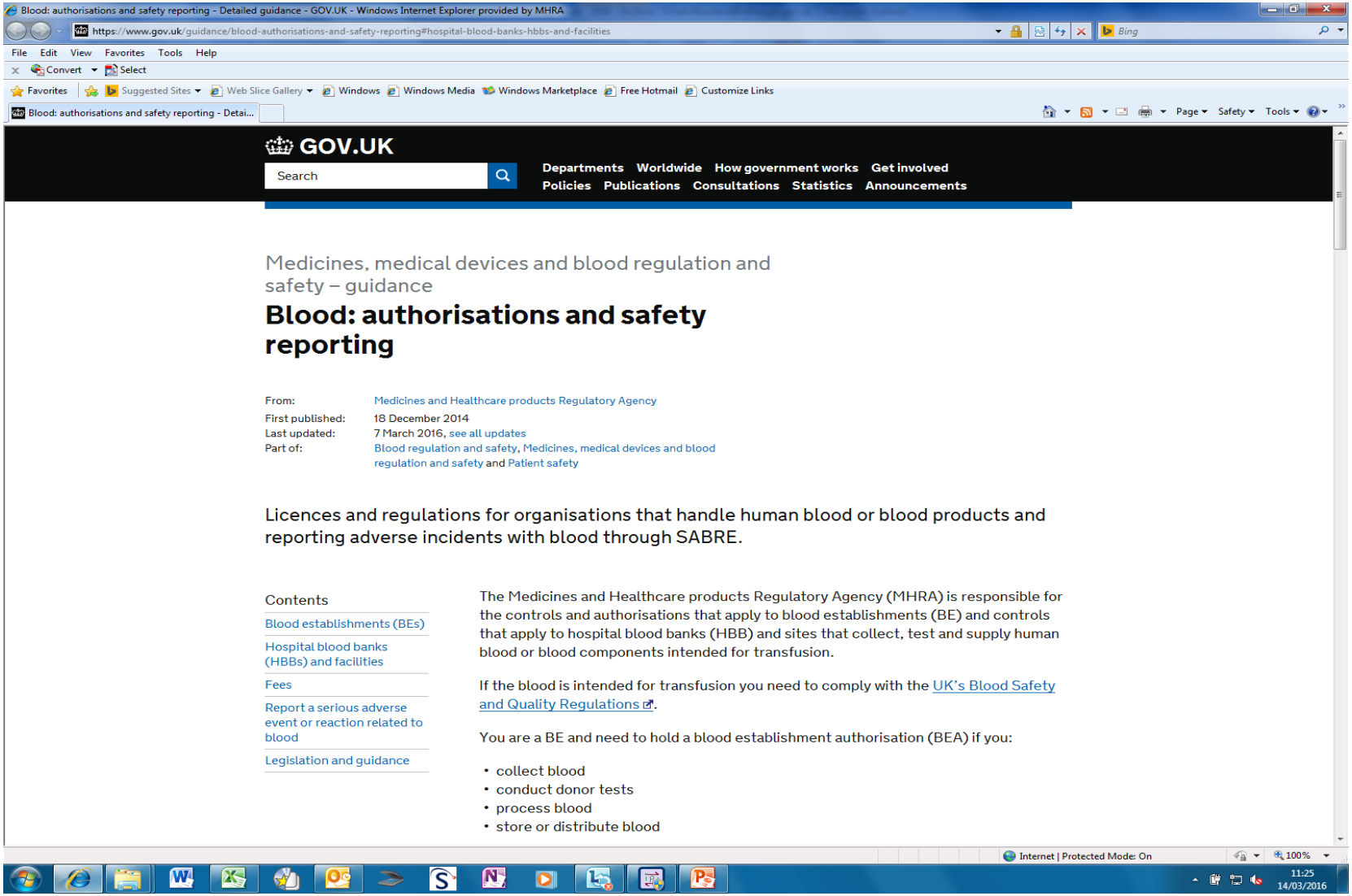
200-300

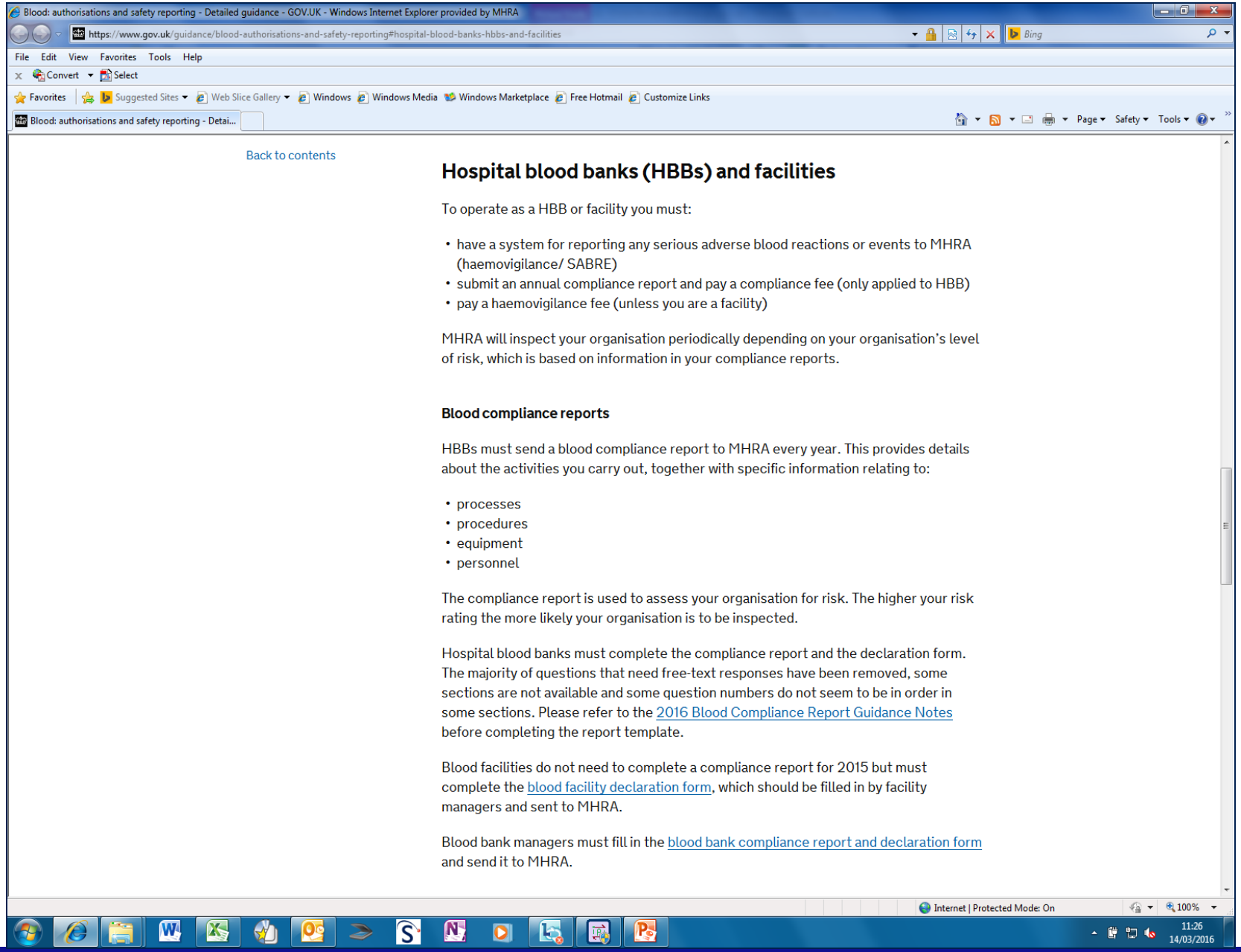
300+

Additional BCR questions

B1.10	Are pre transfusion testing activities routinely performed by an off-site (remote) laboratory? If yes please provide details in Section S below
P3.4.2	Are “quality control” (QC) tests performed on the analyser in accordance with the manufacturers specification?
P3.4.3	Are patient samples permitted to be run concurrent with the QC test?

2016 BCR Assessment




Blood: authorisations and safety reporting - Detailed guidance - GOV.UK - Windows Internet Explorer provided by MHRA
https://www.gov.uk/guidance/blood-authorisations-and-safety-reporting#hospital-blood-banks-hbbs-and-facilities
File Edit View Favorites Tools Help
Convert Select
Favorites Suggested Sites Web Slice Gallery Windows Windows Media Windows Marketplace Free Hotmail Customize Links
Blood: authorisations and safety reporting - Detai...
Page Safety Tools
Back to contents
Hospital blood banks (HBBs) and facilities
To operate as a HBB or facility you must:
• have a system for reporting any serious adverse blood reactions or events to MHRA (haemovigilance/ SABRE)
• submit an annual compliance report and pay a compliance fee (only applied to HBB)
• pay a haemovigilance fee (unless you are a facility)
MHRA will inspect your organisation periodically depending on your organisation's level of risk, which is based on information in your compliance reports.
Blood compliance reports
HBBs must send a blood compliance report to MHRA every year. This provides details about the activities you carry out, together with specific information relating to:
• processes
• procedures
• equipment
• personnel
The compliance report is used to assess your organisation for risk. The higher your risk rating the more likely your organisation is to be inspected.
Hospital blood banks must complete the compliance report and the declaration form. The majority of questions that need free-text responses have been removed, some sections are not available and some question numbers do not seem to be in order in some sections. Please refer to the 2016 Blood Compliance Report Guidance Notes before completing the report template.
Blood facilities do not need to complete a compliance report for 2015 but must complete the blood facility declaration form, which should be filled in by facility managers and sent to MHRA.
Blood bank managers must fill in the blood bank compliance report and declaration form and send it to MHRA.
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2016 BCR Assessment

2016 HBB blood compliance report guidance note

2016 HBB compliance report

2016 HBB declaration form

2016 Blood facility declaration form

Last submission date: 30th April 2016

Assessment: May to June 2016

BAT assessment: July 2016

BAT Assessment Team

Graham Carroll

David Churchward

Stephen Grayson

Andrew Hopkins

Graeme McKilligan

Kevin Page

Vivian Rowland

Michelle Rowson

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Thank you

Any questions?

JPAC 15-54

Joint UKBTS Professional Advisory Committee (1)
Summary Sheet

1. Paper for the JPAC meeting on:	18th June 2015
2. Date submitted:	10th June 2015
3. Title (including version no.):	Deviations from 4 °C temperature storage for red cells: effect on viability and bacterial growth
4. Author(s):	Dr Rebecca Cardigan, Dr Stephen Thomas and Dr Ty Pitt for the SAC on Blood Components
5. Brief summary:	There are various points in the blood supply chain where red cells may be temporarily stored out with their designated storage temperature (2-6°C). This could include transport of red cells between the blood centre and hospital, but also from a hospital transfusion department to a satelilite fridge or another external site, or when red cells are issued and collected for transfusion to a patient. Guidance on this issue is currently found in three sources: 1) specifications for red cells in the Red Book contain a statement regarding equipment breakdown and transport of red cells –detailed in full on page 3 and 14: 2) BCSH guidelines and 3) the Handbook of Transfusion Medicine state that red cells out of controlled temperature for more than 30 minutes should not be re-issued, and that the transfusion should be completed within 4 hours of removal from controlled temperature (except for neonatal red cells which is 4.5 hours, to allow for a maximal 4 hour transfusion time). Previously data relating to component quality to justify 1) was reviewed and accepted by JPAC in 2010 (JPAC10-76). However, data on bacterial risk has not been reviewed, nor data that have been generated recently in the UK and elsewhere that provide evidence that the 30 minute rule might safely be extended. The paper attached summarises data on component quality and bacterial risk when red cells are stored at ambient temperature for multiple short periods and makes recommendations for changes to the current guidance.
6. Action required by JPAC: (What do you want JPAC to do in response to this paper?) e.g. • endorse a specific recommendation • advise where there is a choice of possible actions • advise on priorities within the	Endorse the following recommendations 1) That the guidance in the Red Book relating to transport of red cells be changed to indicate that the 12 hour maximal transit time outwith 2-6°C, where the surface temperature can reach 10°C, is on one occasion only.

work plan • provide a steer on policy	2) That this paper and data are provided to the BCSH group reviewing the guidelines on the administration of blood to consider amending the 30 minute rule in accordance with the statement on page 14 of this paper. In addition, the next revision of the Handbook of Transfusion Medicine should also consider amending in line with any changes in BCSH guidance. 3) That the bacteriology studies conducted by NHSBT are submitted for publication, and JPAC be made aware of any significant modifications to the conclusions in light of reviewers comments. 4) That a requirement to assess the effect of deviations in the normal storage temperature of red cells be added to the exisiting requirements to validate a novel red cell product given in chapter 8 of the Red Book, e.g new red cell additive solutions etc.
7. Any other relevant information:	Completed JPAC decision making framework attached

(1) **Joint U**nited **K**ingdom **B**lood **T**ransfusion **S**ervices **Professional Advisory Committee**

Joint UKBTS Professional Advisory Committee (*)

UKBTS General Information 03

Deviations from 4 °C temperature storage for red cells: effect on viability and bacterial growth

May 2015

Prepared by: Standing Advisory Committee on Blood Components

This document will be reviewed whenever further information becomes available. Please continue to refer to the website for in-date versions.

1. Introduction

There are various points in the blood supply chain where red cells may be temporarily stored out with their designated storage temperature (2-6°C). This could include transport of red cells between the blood centre and hospital, but also from a hospital transfusion department to a satelilite fridge or another external site, or when red cells are issued and collected for transfusion to a patient. This document summarises data on component quality and bacterial risk when red cells are stored at ambient temperature for multiple short periods and makes recommendations for changes to the current guidance base on the available evidence.

a) Current regulatory requirements for storage and transport temperature of red cells

The current requirement in the UK and Europe is that red cell components must be stored with their core temperature in the range 2 to 6 °C, whereas AABB Standards state 1-6°C[1-4].

Exceptionally, it is allowed that the core temperature may extend from 1 to 10 °C, providing that this deviation has happened on one occasion only, and that the duration is no longer than five hours[2]. In addition, the UK Guidelines allow surface temperatures up to 10 °C for up to 12 hours during transport, although currently it is not stated on how many occasions. The Council of Europe Guidelines [3] allow up to 10 °C for 24 hours during transit. The AABB Standards [4] and AABB Technical Manual state that blood storage and transit temperature should not exceed 10 °C but no time limit is stated. None of the published guidelines on transport of red cells state on how many occasions during the shelf-life of a red cell this may occur, and are unclear about whether these recommendations relate to blood centres or hospitals or both.

The EU Directive and Blood and Safety Quality Regulations state that ‘transport and distribution of blood and blood components at all stages of the transfusion chain must be under conditions that maintain the integrity of the product’.

These storage and transportation regulatory requirements are in place to a) inhibit the growth of any bacteria introduced into the bag at the point of collection, processing, or storage; and b) to preserve red cell quality.

b) The ’30 minute’ rule

The other occasion when red cells are removed from their normal refrigerated storage is prior to transfusion. Although the UK Guidelines do not give any guidance on how long blood can be out of controlled temperature before transfusion is commenced, the British Committee for Standardisation in Haematology (BCSH) guidelines [1]state that ‘If red cell units are out of temperature controlled storage for more than 30 minutes they should not be put back into storage for re-issue. If an IT tracking system is being used it should be able to immediately highlight to laboratory staff the presence of any returned units that need withdrawal from stock.’ The BCSH guidelines also recommend that transfusions are completed within 4 hours of removal from a controlled temperature, apart from neonatal transfusions which can be upto 4.5 hours to allow for 4 hours for the transfusion itself in order to allow for transfusions up to 20mls/kg at a rate of 5 ml/kg/hr.

It is likely that the 30 minute rule originated as a result of the 1971 publication of Pick and Fabijanic [5] who investigated the time taken for a unit of cooled blood to reach 10 °C when removed from the refrigerator. They found that, whether the unit was handled or not, the surface temperature reached 10 °C between 15 and 30 minutes after removal into ambient conditions, whereas the core temperature took 45 to 60 minutes to reach 10 °C . Thirty minutes thus would appear to be a reasonable cut-off to ensure that the core temperature did not rise above 10 °C. Since this original work, there have been a number of studies that have confirmed the rate of warming, in increasingly sophisticated ways[6-9]. It is not clear why Pick and Fabijanic chose 10 °C as the upper limit, it may have been on the basis of data published by Hughes-Jones [10] that showed reduced, but acceptable, recovery of red cells following transfusion when stored at 10 °C for 34 days. In addition, 10^{o}C may have been chosen as a practical limit based on the wet ice type of transit containers that were available at that time. The relevance of short-term exposures to 10 °C, and thus the relevance of the 30 minute rule, is therefore worthy of review.

The 30 minute rule can result in wastage of red cells in two respects:

a) If a patient is not ready to receive a planned transfusion, and red cells are out of controlled storage for more than 30 minutes they cannot be returned to stock for issue to that or another patient.
b) Red cells sent to a location remote from a blood refigerator or off-site in case a transfusion is needed, cannot be returned to stock if not transfused within 30 minutes of removal from controlled storage.

There is general concern among blood services and hospitals that a considerable number of RCC are lost unnecessarily as a result of the 30-minute rule. Data from the UK Blood Stocks Management Scheme (BSMS) repeatedly shows approximately 10, 000 RCC are discarded every year due to out of temperature control excursions outside of the laboratory, and this represents almost one quarter of all red cell wastage[11]. In a recent survey of hospitals by the BSMS, over 96% of respondents indicated that extending the 30 minute rule to 60 minutes would enable most of their out of temperature control units to be re-issued.
A systematic review published by Brunskill et al in 2011[12], concluded that “It is possible that the 30-minute rule could be extended to a “60-minute rule,” but the main concern remains bacterial growth in any contaminated units that are returned to clinical stock… and further studies are required before that question is answered.” The papers reviewed by Brunskill et al are summarised below, along with those subsequently published and recent reports from NHSBT in response to the call for “robust, modern studies using

multiple combinations of blood, current anticoagulant and additive solutions, and with defined temperatures and times of exposure that are relevant to current clinical practice."

2. Impact of changes in the temperature of red cells on their quality

a) In-vitro studies

Storage of red cells at 4 °C decreases the metabolic rate of the cell and enables blood to be stored for longer periods. At higher temperatures, the rate at which glucose is consumed and lactate produced is increased, leading to a lowering of pH. This is turn stimulates 2,3 DPG phosphatase, resulting in a rapid reduction of 2,3 DPG, a molecule that competes with oxygen for the same site on the haemoglobin molecule, reducing the oxygen affinity of haemoglobin and increasing oxygen delivery to the tissues. At 30 °C it has been estimated that within four hours 2,3 DPG will have fallen to 35% of the initial concentration, and it will be totally depleted within 18 hours[13].

Several workers have assessed the effect of intermittent and repeated warming on red cell metabolism. Strauss et al [14]stored ACD anticoagulated blood at different temperatures, and on the basis of changes to 2,3 DPG, ATP, pH, extracellular K^+ and Hb concentration, concluded that acceptable shelf-life was nine days at 10 °C, six days at 15 °C and three days at 20 – 25 °C. If adenine and guanosine were added, the storage times were increased to 20, 10 and five/four days respectively.

Shields stored units of plasma reduced-whole blood in ACD-A at 4 °C or at 10 °C for 28 days, and saw no difference between the two groups for plasma haemoglobin (Hb), supernatant potassium (K^+), haematocrit (Hct) or osmotic fragility[15]. This provides some evidence to support the upper limit of 10 °C being acceptable with respect to red cell metabolism, but the extensive range of laboratory assays that would have been employed currently were not available at that time. Most notably, the authors are not aware of any study that has assessed ATP levels in red cells exposed at 10°C for multiple or prolonged periods.

Shields [15] also exposed plasma-reduced WB units (otherwise stored at 4 °C) repeatedly to either 10 °C or 22 °C for varying periods of time (between 1 – 24 hours). Those exposed to 10 °C at weekly intervals showed no difference from the controls for parameters tested (plasma Hb, K^+, Hct). However, repeated exposure to 22 °C showed elevation of plasma Hb at day 21, though not earlier in the storage period. Unfortunately, data is only provided for 16 and 24-hour periods of exposure at this temperature and not shorter periods.

Ruddell et al compared CPDA-1 anticoagulated packed red cell units warmed to 25 °C for 24 hours at either day 6 or day 20 with control units[16]. The warmed units had lower concentrations of glucose, higher lactate, and lower pH than controls. The rates of decrease in adenosine triphosphate (ATP) were greater in the warmed units during the week after warming compared with controls. There was no statistically significant increase in plasma Hb, and haemolysis did not exceed 1% in any of the units. The authors concluded that one day of storage at 25 °C accelerates essential metabolic breakdown equivalent to 10 days of storage at 1 – 6 °C, and extrapolated this observation to predict that a single two hour exposure to ambient temperature might be expected to reduce the storage life of a unit by one day.

Reid et al studied red cells in additive solution (AS-5). Units were warmed to 25 °C for 24 hours on day 14 or day 28[17]. Glucose, ATP and pH declined more in the warmed units,

and haemolysis was less than 1% in all units. Mean cell ATP concentrations in the warmed cells at day 30 of storage were approximately equivalent to those in cold-stored cells at 42 days, suggesting an enhanced aging of the cells.

Ecker & Hitzler performed a similar study, but exposed units to ambient temperature for a shorter length of time[18]. CPDA-1 red cells were exposed to 20 °C for 6 hours on day 5, day 15 or day 30 of shelf life and compared to continuously refrigerated controls. The warmed units had a lower ATP content than controls, but this was greater than 50% of the initial concentration and all values were above the level considered necessary for adequate post-transfusion survival of >2 μmol/g Hb. There was no significant difference between the groups for lactate, glucose, sodium (Na^+) or K^+, and haemolysis was < 0.5%.

Hancock and colleagues at NHSBT's Component Development Laboratory (CDL) studied the effect of the storage and transport deviations permitted by the UK Guidelines (10 °C for 5 hours and 12 hours, as described in the introduction) on red cells stored in SAGM and found no significant effect on any *in vitro* red cell parameters tested (including haemolysis, ATP, K^+)[19].

In a second study Thomas and colleagues studied red cells in SAGM subjected to repeated short term exposures to 21°C, and showed that three exposures of three hours on days 3,8 and 15 of storage plus one exposure of five hours at 21°C (to model the transfusion itself) did not have a negative effect on *in vitro* markers of red cell quality (including haemolysis, ATP, K^+)[20].

The concluding study from Thomas and colleagues at CDL investigated the effect of multiple 30 minute or 60 minute exposures to warm (30 °C) temperatures (up to three exposures on each of three separate days of storage, days 15, 17 and 21)[21]. The study design also included the 5 and 12 hour exposures to 10 °C and a five hour transfusion period (also at 30°C), and used both adult-sized and paediatric-sized components in SAGM. No significant differences were seen in ATP or K^+, but increased haemolysis was seen in the units that had been subjected to multiple exposures (adult units subjected to at least two 60 minute exposures on each of three days and in paediatric units exposed for 60 minutes once on each of three days). The authors concluded that, on the basis of *in vitro* quality markers, an extension of the 30 minute rule to 60 minutes could be considered but only if the total number of exposures was limited to no more than three, and that adequate time was allowed to cool the units properly in between exposures.

Ramirez-Arcos and colleagues from the Canadian Blood Service reported on two studies using red cells in SAGM. In the first study, a single five hour exposure to room temperature showed no immediately significant effects on the *in vitro* quality of the red cells, although six days after the exposure ATP and K^+ levels were significantly lower than in unexposed controls[22]. In the second study, units were exposed to room temperature for 30 minutes on each of five separate days, and no significant effects on *in vitro* red cell quality markers were reported[23].

de Grandmont and colleagues from Hema-Quebec studied red cells in SAGM and in AS-3, which were exposed to room temperature for 30 or 60 minutes at weekly intervals for five weeks. No significant differences were seen in the *in vitro* markers tested (including haemolysis, ATP, K^+)[24].

Wagner and colleagues in Austria studied red cells in SAGM that were stored constantly at one of three temperatures (1 - 6, 13 or 22 °C) for 42 days or at 2-6°C in conjunction with five weekly exposures at 13 or 22 °C for 24 h each time[25]. The data show that long term storage at 13 or 22 °C results in increased haemolysis. Repeated exposure to 22 °C

for 24 hours also increased haemolysis which was not obsereved in units subjected to repeated exposure to 13 °C. Due to lack of control data on ATP the effects on this variable are difficult to assess. Nonetheless, 24 hour exposures to 22^{o}C appeared to have the expected effect of reducing ATP levels significanlty.

Gulliksson and colleagues in Sweden have also assessed red cells in SAGM after transient warming to ambient temperature for 6h on multiple occasions or a single exposure to ambient temperature on day 5 or 21 for 6,12,18 or 24 hours[26]. Exposure to ambient for multiple periods of 6 hours appeared to have little effect on in vitro measures of red cell quality. Warming during the second half of shelf life led to more haemolysis than when exposed at day 5, but not to an excessive degree. As expected, exposure for longer periods, such as 18-24 hours, had a more pronounced effect on haemoylsis and reduction in ATP.

b) In-vivo studies

Strauss and Raderecht [27]tested the in-vivo recovery of WB collected into either ACD or ACD with added adenine and guanosine (ACD-AG) and stored at temperatures ranging from 4 °C to 25 °C. 24-hour recovery of the cold-stored ACD-AG blood was 83%. This declined to “unacceptably low values” between 20 – 27 days at 10 °C, 10 – 14 days at 15 °C, and 4 - 5 days at 25 °C. Time of acceptable storage was lower for warmed ACD blood.

In two different studies, exposure to ambient temperatures for 24 hours has been shown to reduce red cell recovery following transfusion to normal subjects. Shields warmed ACD packed red cells stored for 7, 21 or 28 days to 22 °C for 24 hours immediately prior to transfusion[15]. They found that 24-hour recovery was reduced in 21 day old blood, and the difference was statistically significant with 28 day old blood (75% compared to 62%). This was considered to be an equivalent loss of viability to that seen with an additional week of storage at 4^{o}C.

In the study of Reid *et al* [17] red cells in additive solution (AS-5) were warmed to 25 °C for 24 hours on day 14 or day 28 and then stored to day 35, with control units kept at 4^{o}C for 42 days. The 24 hour recovery of red cells at day 35 for units exposed to ambient temperature for 24 hours was similar to controls at day 42. The reduction in recovery was paralleled by a reduction in ATP, and therefore this appears to be a useful laboratory marker of the reduction in red cell recovery that may occur to due warming of red cells. The conclusion reached was that one day of storage at 25 °C reduces the storage time by 12 days, but shorter exposures such as two hours would produce differences in viability and recovery that are too small to measure, and is consistent with laboratory studies showing little effect of multiple short-term exposures on ATP levels.

Hogman [28]warmed 42-day old SAG-M red cells for one hour at 37 °C, immediately prior to transfusion. There were no observed differences in haemolysis, K^{+}, glucose, lactate, or 24-hour recovery. The ATP concentration decreased slightly, but there was no difference in adenylate energy charge. Warming was noted to improve the cellular shape significantly.

A limitation of the data to date is that studies have assessed basic measures of red cell quality. More recent concepts such as oxidative changes during red cell storage have not been assessed.

Although studies by NHSBT have included paediatric-sized units that will warm up more quickly when removed from controlled storage, components that have undergone secondary processing such as irradiation, washing, or red cells for exchange transfusion have not been assessed. In terms of bacterial risk, exposure of irradiated or washed red cells to ambient temperature is unlikely to result in a higher risk than standard red cells, since they are stored in the same medium and for shorter duration. The shelf-life of both washed and irradiated red cells is restricted to 14 days due to the effects of both processes on red cell quality. It is not known whether there would be more of a detrimental effect on red cell quality of exposing cells that have already been subjected to additional stress such as washing and irradiation to periods at ambient temperature since these studies have not been performed. This highlights the importance of trying to restrict the amount of time that red cells are out of controlled temperature to a minimum.

c) Conclusions on red cell quality

The current recommended red cell component storage temperature of 2 – 6 °C is correct. Storage of red cells out with their recommended temperature should be kept to a minimum.

The current upper limit of surface temperature of 10 °C for a single period no longer than 12 hours for transportation between blood centres and hospitals and to cover refrigerator breakdown (a single occurrence no longer than 5 hours) is acceptable.

Periods of 24 hours or more of warming to ambient temperature have been shown to accelerate metabolism and ageing of red cells, resulting in reduced red cell recovery in vivo and therefore to potentially shorten the shelf life of the component by 10 – 12 days per 24 hours of exposure. Shorter periods of warming are less likely to cause the same effect.

In terms of component quality, the 30 minute rule could be extended to 60 minutes, but a limitation is required on the total number of exposures, as increased haemolysis has been seen in adult and paediatric units exposed more than once on each of three occasions, and sufficient time must be allowed in between multiple exposures for the red cells to return to 2-6 ^{o}C.

3. Impact of changes in temperature of red cells on bacterial contamination

a) Incidents of bacterial contamination in red cells

The most significant concern is that the rate of growth of any bacteria that have entered the unit, either from the donor skin or blood-borne, may increase when red cells are removed from the cold environment. Contamination of red cells with bacteria is a very rare occurrence and usually involves Gram-negative species which are able to survive and/or multiply in cold storage. Data compiled by NBL on organisms implicated in bacterial transmissions from RCC between 1995 and 2014 identified six incidents reported to NHSBT from approximately 40 million units, giving an incidence of contamination of 1 in 6 million (see Table 1).

	Organism	Frequency	Potential Source	Patient Outcome

Gram negative	*Pseudomonas putida*	2	Environment	Death (1) Morbidity (1)
	*Yersinia enterocolitica**	1	Gut	Death
	Enterobacter cloacae	1	Gut	Morbidity
	Serratia liquefaciens	1	Gut	Morbidity
Gram positive	*Staphylococcus* sp.	1	Skin	Morbidity

* prior to introduction of universal LD in the UK. Since this organism is leucocyte-associated LD is expected to reduce this risk and no cases have been reported since the introduction of universal LD in1999

Table 1 – Bacterial infections transmitted by transfusion of red cells (1995 – 2014)

b) In vitro studies

In 1990, Hamill and colleagues reviewed the literature and performed experimental studies on a panel of various organisms, showing that exposure of spiked units of non-leucodepleted red cells in AS-1 additive solution to 26 °C for two hours on two occasions had no significant effect on the rates of bacterial multiplication[29, 30]. The authors therefore called for extension of the 30 minute rule to two hours. However, Brunskill's systematic review concluded that whilst overall there was no evident increased risk of bacterial contamination outside of the rule, further studies were called for, using a more representative range of microbes, and more current anticoagulants and additive solutions, to resolve the issue[12].

NBL investigated the currently permitted storage and transport temperature conditions, with spiking experiments used to determine bacterial growth. Four groups of RCCs were spiked separately with six species and stored to reflect deviations in refrigeration due to a) equipment breakdown (10 ^{o}C for 8h on day 2-equating to a core temperature of 10 ^{o}C for 5 h), b) transport (surface temperature 10 ^{o}C for 12 h on day 3), c) a combination of both a and b and were compared to units stored at 4^{o}C throughout. Samples were taken over the shelf life of the unit. The species tested included *Pseudomonas putida, Bacillus cereus, Enterobacter cloacae, Serratia liquefaciens, Yersinia enterocolitica* and *Staphylococcus epidermidis*. Of these, only the cold tolerant *P. putida* exhibited a statistically significant increase in numbers (>1 log) compared with controls over all temperature deviations, reaching counts of 10^7 to 10^8 cfu/ml by day 21.

There are no comparable studies in the literature exploring the growth response of *P. putida* in similar storage and temperature deviations. Ramirez-Arcos and colleagues [23] investigated the growth of bacteria in RCC held out of storage temperature for more than four hours but utilised single strains of *S. liquefaciens* and *S. marcescens*. *S. liquefaciens* grew at a constant rate in refrigerated units and reached *c.* 10^6 cfu/ml within one week of storage. By contrast *S. marcescens* grew markedly slower and required 21 days to reach a comparable count. *S. liquefaciens* was further tested in a single five hour exposure to room temperature, three days post spiking of the unit, and exhibited a significant increase in viable count at three hours of exposure. At five hours the mean count was 3.4 x 10^3 cfu/ml.

The level of risk due to the current storage and transport conditions must therefore be considered to be very low given the rarity of contamination events. None of the six bacterial transmissions since 1995 are known to have been associated with deviations in storage temperatures of the implicated units.

The debate over the 30 minute rule was further advanced by Ramirez-Arcos *et al* of the Canadian Blood Service, whose experiments with the cold tolerant species *Serratia marcescens*, showed a statistically significant difference in bacterial numbers in spiked RBC units exposed to room temperature on five occasions for 30 minutes compared with non exposed controls[23]. The range of species was extended to include *Yersinina enterocolitica*, *Escherichia coli* and *Staphylococcus epidermidis* in a follow up study [22]. This showed that *E. coli* and *S. epidermidis* failed to grow in control or ambient temperature exposed RCC. *Y. enterocolitica* grew to similar levels in tests and controls but *S. marcescens* grew to numbers of 1-log higher (10^4 cfu) in units exposed for 30 or 60 minutes on multiple occasions (once each on day 7, 14, 21, 23, 28 and 35 post collection). However, there was no significant difference in counts between units exposed for 30 or 60 minutes and the authors concluded that an extension of the 30 minute rule to 60 minutes was therefore 'reasonable'.

More recent data from a separate but similar study from Hema-Quebec using RCC spiked with 1-5 cfu/unit each of reference strains of *S. epidermidis*, *S. marcescens* and *S. liquefaciens*, in two additive solutions, also showed that 30 and 60 minute exposures to room temperature did not result in significantly increased bacterial growth with the exception of S.marcesens[24]. However, the latter was not significantly different between red cell units exposed for 30 or 60 minutes. An interesting observation was that *S. marcescens* failed to grow in additive AS-3 while *S. liquefaciens* grew to similar levels in this and SAGM solutions. This finding is difficult to explain as the significant differences between AS-3 and SAGM with regard to growth promotion is the presence of citrate in the former and mannitol in the latter; both species are able to utilize these compounds for growth.

NBL performed a study that modelled similar multiple warm exposures of RCC in SAGM (adult and paediatric packs) as used by Thomas et al at CDL[21], with the units spiked with six clinically relevant isolates (*Pseudomonas putida, Bacillus cereus, Enterobacter cloacae, Serratia liquefaciens, Yersinia enterocolitica* and *Staphylococcus epidermidis)*. The units were exposed to 30 ^{o}C either once on three separate days, or on three occassions on the same day, for either 30 or 60 minutes. Units were sampled before and after each deviation and viable counts compared with control units maintained in refrigeration. An increase in bacterial numbers of ≥ 1-log in test over controls was considered evidence of significant multiplication of the test organism in view of the variability in measuring these endpoints. The key finding was that none of the exposure conditions tested had a significant impact on bacterial counts of five of the six species tested. The exception was *P. putida* which showed a > 1-log difference in count from the negative control in an adult pack following three exposures for 60 minutes at 30^{o}C on the same day (day 15). Other exposures of adult or paediatric packs to three 30- and 60-minute temperature deviation cycles showed no significant difference in counts of *P. putida*. Since the difference between control and test units in the adult unit study was only observed when tested on day 15 (the same day as exposure to 30^{o}C), and not in the same units later during storage.On repeat experiment, this difference was not confirmed and counts did not differ significantly from the control units suggesting that the earlier finding was an artifact. Based on these results up to three exposures of 30 minutes at 30 ^{o}C on the same or separate days and three exposures of 60 minutes on separate days do not appear to constitute an increase in risk for the component. It is noteworthy that with the exception of *S. epidermidis* in control adult units, which declined in count below the limit of detection, all of the species tested were culturable from both control pack types at day 35, underlining their ability to survive, albeit in low numbers, for the duration of cold storage.

A limitation of the studies conducted by NBL is that for S.liquafaciens and Y.enterocolitica, growth of the bacteria had reached stationary phase by day 15 when the units were first exposed to deviations in temperature. Further work was undertaken using lower spiking inocula to ensure that growth on day 15 was in exponential phase. For S. liquefaciens, counts of test and control units were closely similar for all units and confirmed that the stated deviations had no effect on the growth response of this organism. Repeating of these experiments for Y. enterocolitica with a lower initial inoculum resulted in exponential growth as desired and no differences were observed between tests and controls for paediatric units. Considerable fluctuation in test and control counts was evident in adult units with pre temperature deviation counts on day 15 exceeding the 1 log difference threshold. However, by the end of the deviations no further increase in counts between tests and controls was observed. These findings indicate that repeated excursions of adult units out of temperature control for greater than 30 minutes did not result in a significant acceleration of growth of this organism.

A notable outcome from the second Ramirez-Arcos study was the demonstration of elevated and clinically significant levels of endotoxin in both *Y. enterocolitica* and *S. marcescens* spiked RCC. Concentrations of endotoxin (> 1000 EU/ml) for *Y. enterocolitica* in tests and controls were evident by day 20 post collection and correspondingly by day 35 for *S. marcescens[22]*, but were not significantly different in units exposed to 30 or 60 minutes at ambient temperature compared to controls stored at 4^{o}C throughout. However, the accurate measurement of endotoxin in whole blood is difficult due to non specific protein binding and technical factors such as the high haematocrit.

c) Discussion

The in depth review by Brunskill et al in 2012 concluded that RCC temperature excursions did not result in significant bacterial growth, but insufficient studies had been published[12]. However, this view has been underlined by studies published since then and the current investigations by NBL.

Few bacterial species are able to multiply at 4 ^{o}C and thus be classified as obligate psychrophiles although several species (mesophiles) are tolerant of cold and may survive storage due to selective shut down of metabolic systems. The problematic species for RCC contamination are mainly those groups able to survive and multiply, albeit slowly, in cold storage such as the Gram-negative organisms *Pseudomonas putida/fluorescens* and *Serratia marcescens/liquefaciens*. Of the organisms identified by NBL from incidents of contamination, only *P. putida*, *Y. enterocolitica*, and *S. liquefaciens* are associated with growth at refrigerated temperatures but this property is likely to vary with the origin of the strain from environmental and human sources[31]. *E. cloacae* is an environmental saprophyte and could be expected to survive in the cold, while the recovery of *Staphylococcus* sp. most likely represents initial gross contamination from the skin at venepuncture.

Serratia spp., are environmental bacteria which have long been associated with contamination of blood components. Few Gram-negative species are able to withstand the bactericidal effect of plasma and killing by phagocytes in the reduced oxygen concentration in a blood bag. The *Serratia* group are facultative anaerobes, which means that they are able to survive and grow in an atmosphere severely limited in oxygen. Some work has shown that a strain of *S. marcescens* isolated from a contaminated blood unit was able to grow in deionised water supplemented with materials derived from the plastic bag, and in deionised water alone, and growth was greatest under anaerobic conditions [32]. This apparent intolerance of oxygen in nutrient limited conditions was considered to be a consequence of growth promoting compounds leaching out of the

blood bag materials [33]. Both *S. marcescens* and *S. liquefaciens* produce potent haemolysins, and proteolytic enzymes that degrade complement, and other defence related plasma proteins thus enhancing their survival in blood. Indeed, it has been demonstrated that *S. liquefaciens* after inoculation into plasma from standard blood donations multiplied 22 million times greater than *E. coli* and 11,000 times more so than *P. fluorescens* [34]. Skin carriage of *Serratia* is believed to be rare in the healthy but screening data are limited and mostly anecdotal. If derived from the decontaminated skin of a donor, initial counts are most likely to be low ($< 10^2$) but by the end of the 35 day shelf life of a unit of RCC counts could reach $\geq 10^8$ /ml and thus constitute a significant risk to a recipient. However, both the Canadian studies and data from NBL show that when compared to refrigerated control units, counts of *S. liquifaciens* were not significantly greater in units exposed to warm temperatures for up to 60 minutes. By contrast in the Canadian study, the cold tolerant species *S. marcescens*, showed an increased in count of 1 Log over controls in units exposed multiple times to room temperature for 30 or 60 minutes but the increase was not considered to be of clinical significance for this species as counts fell below the threshold of 10^5 cfu/ml advocated by Jacobs et al in platelet component transfusions and was not different between those units exposed for 30 or 60 minutes.

Pseudomonas putida, and its near relative *P. fluorescens*, are primarily pathogenic for plants, being ubiquitous in the natural environment. As a consequence of a general ability to thrive in refrigerated temperatures and high proteolytic activity, they are commonly isolated as food and dairy spoilage agents. *Pseudomonas* spp. were found colonizing the arms of approximately 1% of blood donors, with *P. fluorescens*, specifically, being present in 0.3% of donors [35]. These species are rarely found in clinical specimens and are often of doubtful significance outside of immunosuppressed individuals (see Appendix).

Nevertheless, initially low numbers of either *Pseudomonas* or *Serratia* spp., in a red cell unit, even if rapidly refrigerated post collection, could proliferate over time to constitute a significant microbial hazard to a susceptible recipient and this could be exacerbated by repeated and/or prolonged excursions from refrigeration in excess of those validated.

d) Conclusions on bacterial risk

- Sepsis following transfusion of RCC is exceedingly rare (*c.* 1 in 6 million).
- The level of risk due to the current storage and transport conditions is considered to be very low given the rarity of contamination events. None of the six bacterial transmissions since 1995 are known to have been associated with deviations in storage temperatures of the implicated units.
- Although based in antiquity and on little evidence, the 30-minute rule has served to reduce the risk of bacterial proliferation in units contaminated with the cold-tolerant species *Pseudomonas* and *Serratia* spp., which could constitute a significant clinical hazard to recipients.
- Published research and studies by NBL suggest that a reasonable case can be made to extend the out of temperature control rule to 60 minutes for no more than three exposures to ambient temperature when red cells have been returned to 2-6^{o}C between exposures. This is based on the finding that the increase in bacterial counts following each exposure did not exceed the threshold limit of 1log difference.

4. Current practice in UK hospitals

In response to data demonstrating a continued significant wastage of red cells in hospitals being 'out of temperature control', the Blood Stocks Management Scheme (BSMS) undertook a survey of at the end of 2014 to better understand the issue and how it could be improved. A total of 130 hospitals responded to questions relating to temperature control of areas of the supply chain from Blood Centre to patient.

- The movement of red cells from blood supplier to hospital is a controlled process, both in terms of temperature and also inability for units to be tampered with, and there was no evidence that this area causes significant problems.
- Transfer of red cells from receipt to stock refrigerator in the hospital transfusion laboratory and from the transfusion laboratory to the issue refrigerator is also very well controlled. For transit times of >15 minutes from the transfusion laboratory within the same hospital location, 98% of respondents use boxes validated to keep red cells between 2-6^{o}C, this figure reduces to 72% for 5-15 minutes and 48% for <5 minutes.
- For transfer from the transfusion laboratory to off-site locations such as hospices, all respondents indicated that where this is applicable validated transport boxes are used with (13%)/without (87%) temperature monitoring.
- Temporary storage of red cells in validated, storage portable containers for periods between 2 and 24 hours (mainly less than 4) was more common than expected for transfusions at home/hospices and use on haematology wards, trauma packs and air ambulances. The portable containers appear to be validated appropriately but few (13%), had temperature monitoring devices and 27% of respondents allowed access to the container to check the contents.
- The greatest wastage of red cells out of a controlled environment occurs in the transfer from an issues refigerator to the location where the patient is to be transfused; 59% of respondents do not use validated transport containers when collecting blood for a patient. 32% use validated transport boxes, of which 3% are temperature monitored.
- The majority of respondents (94%) follow the 30 minute rule and discard units that either exceed this, or where there is uncertainty that the time out of controlled storage was <30 minutes. A minority (3%) reduce this to 15 minutes, and 3% extend this to 60 minutes if they check the temperature of the units.

The storage of red cells for significant durations in portable containers, and also when issued to patients appears to be areas where improvements in practice could be achieved.

5. Conclusions and recommendations

- The current recommended red cell component storage temperature of 2 – 6 °C is appropriate to ensure red cell quality is maintained and the risk of bacterial proliferation is minimised.

- The current upper limit of 10 °C surface temperature for <12 hours of transportation between blood centres and hospitals is acceptable, but it should be clarified that this is on a single occasion since bacterial studies where red cells have undergone prolonged or multiple shorter deviations in storage temperature due to transportation in combination with deviations that may occur later in shelf-life (i.e representing the 30 minute rule) have not been conducted.

- The current upper limit of a core temperature of 10 °C in the case of refrigerator breakdown for a maximum of <5 hours on a single occasion is acceptable. If this occurs in the hospital setting, then hospitals must ensure this is the only excursion due to equipment failure that has occurred through discussion with their Blood Supplier.

- We advocate minimising the time that red cells are stored out with 2 – 6 °C in order to optimise red cell quality and reduce bacterial risk as far as possible, by using blood transport containers that are validated to keep red cells at 2 – 6 °C, especially for transit times that will exceed 15 minutes.

- For occassions when removing red cells from 2-6^{o}C controlled storage is unavoidable then:

    - It is best to restrict time out of a controlled temperature environment to <30 minutes
    - If 30 minutes is exceeded the unit should not be returned to the issue location in the refridgerator, but returned to the transfusion department or quarantined remotely using electronic blood tracking.
    - Up to 60 minutes out of controlled temperature is acceptable, provided the unit is quarantined, by placing in a secure refrigerator for at least 6 hours, to allow the unit to return to 2-6^{o}C, prior to reissue.
    - Hospitals will need to identify these units so that they are not subject to out of controlled temperature storage for >30 to <60 minutes on more than three occasions.

- At this time, there is insufficient evidence to extend the 4 hour rule covering the maximum time that may be taken for transfusion.

6. Proposed changes to specifcations for red cells in the Red Book

(Please note that in light of the discussion at JPAC in March, we will now include guidance around deviations in storage temperature in the Red Book, as yet this is not included here)

The proposed changes to text to incorperate the above recommendations and to improve clarity are highlighted in red below.

a) storage - equipment breakdown

‘Exceptionally, i.e. due to equipment failure at a Blood Centre or hospital, red cell components where the core temperature has not exceeded 10 °C or less than 1 °C may be released for transfusion provided:

o that the component has been exposed to such a temperature change on one occasion only
o that the duration of the temperature excursion has not exceeded five hours
o that a documented system is available in each Blood Centre or hospital to cover such eventualities
o that adequate records of the incident are compiled and retained.

b) transport

For red cell components, transit containers, packing materials and procedures should have been validated to ensure the component surface temperature can be maintained between 2°C and 6°C for transportation periods exceeding 15 minutes. Additionally:

- the validation exercise should be repeated periodically
- if melting ice is used, it should not come into direct contact with the components
- dead air space in packaging containers should be minimized
- as far as is practicable, transit containers should be equilibrated to their storage temperature prior to filling with components
- for transportation between blood supplier and hospital an upper limit of 10°C surface temperature is acceptable on a single occasion provided that the transport time does not exceed 12 hours
- in some instances it is necessary to issue red cell components that have not been cooled to their storage temperature prior to placing in the transit container. The transport temperature specified above is not applicable for such consignments.

Appendix 1. Hazard profiles of cold-tolerant bacteria from contaminated RCC

P. putida/*P. fluorescens*

Both species are primarily plant pathogens and common in soil, water. They are cold tolerant, metabolically versatile, and readily isolated from the general environment as well as transient colonists of human skin. They are among the most common species recovered from contaminated blood products and infusion solutions. They have an intrinsic ability to form biofilms on inanimate surfaces including plastics and this may contribute to survival in blood collection bags. Although low grade pathogens for man they produce high affinity iron-chelating compounds to acquire iron from haemoglobin to facilitate growth in blood. Transmission is through direct or indirect contact with contaminated inanimate surfaces or transfer from the skin to an adjacent body site, e.g. at venesection. The likelihood of infection is low except in patients with long term indwelling venous access devices and onset of infection is often delayed several days following exposure to a contaminated source. Some strains exhibit increased resistance to disinfectants and antiseptics and are often recovered from non sterile blood collection tubes where they contribute to false positive blood cultures or 'pseudobacteraemia.'

Their infectious dose is unknown but likely to be in excess of 10^4 organisms/ml if introduced directly into the blood stream. Similarly, there are scant data on the activity of the endotoxins produced by these species. In keeping with most pseudomonads, they exhibit relatively high levels of natural resistance to antibiotics but most strains should be susceptible to third and fourth generation cephalosporins, carbapenems and the newer fluoroquinolones.

Y. enterocolitica

This species is a common cause of infection in animals and less so in humans. It is widespread in nature owing to asymptomatic shedding from the animal gut. Relatively few strains are enteropathogenic in man and infection is usually a self-limiting diarrhoea, on occasion with fever. *Y. enterocolitica* is one of the most frequent contaminants of blood owing to its ability to thrive at refrigerated temperatures. Most strains are susceptible to a wide variety of antimicrobials with the exception of ampicillin and cephalothin.

In immunosuppressed individuals, the organisms can spread from the gut to liver and spleen and form abscesses. It is speculated that subclinical or mild episodes of gastroenteritis may result in extension of the bacteria from the gut to the blood stream leading to a transient bacteraemia in a donor. They are potent producers of siderophores which sequester iron from haemoglobin and individuals with haemochromatosis are particularly susceptible to infection. Infections are sometimes associated with inflammatory sequelae such as arthritis and Reiter's syndrome.

S. marcescens/*S. liquefaciens*

Both species are relatively widespread in nature and are transient but rare colonists of human skin. *S. marcescens* is the most clinically relevant species particularly as an opportunist agent in neonates and the severely immunocompromised. The intrinsic pathogenicity of *S. liquefaciens* for man is debatable but it has been repeatedly cited as a cause of serious sepsis and death in recipients of contaminated RBC units. Both species survive well in refrigeration but *S. liquefaciens* is held to be more rapid growing in the cold than *S. marcescens* but evidence for this is scant. Strains are often susceptible to a variety of antimicrobials

Transmission of the organism most likely occurs at venesection and clinically significant numbers and possibly endotoxin accumulate steadily over storage time.

7. References

Reference List

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Joint UKBTS / NIBSC Professional Advisory Committee ([1])
Summary Sheet

1. Paper for the Blood Consultative Committee meeting on:	17 March 2016
2. Date submitted:	14 March 2016
3. Title (including version no.):	Extension of post-thaw shelf life of FFP to 5 days to support management of massive haemorrhage
4. Author(s):	Sheila MacLennan, Laura Green and Rebecca Cardigan
5. Brief summary:	See attached draft Change Notification and paper
6. Action required by the Blood Consultative Committee: (What do you want BCC to do in response to this paper?) e.g. • endorse a specific recommendation • advise where there is a choice of possible actions • advise on priorities within the work plan • provide a steer on policy	For information
7. Any other relevant information:	

([1]) **Joint U**nited **K**ingdom **B**lood **T**ransfusion **S**ervices and **N**ational **I**nstitute for **B**iological **S**tandards and **C**ontrol **Professional Advisory Committee**

Date of publication: ###########	**Implementation:** To be determined by each Service

Change Notification UK National Blood Services No. ? - 2016

Fresh Frozen Plasma, Leucocyte Depleted

Applies to the Guidelines for the Blood Transfusion Services in the United Kingdom – 8th Edition 2013

Background

The recently published BCSH guideline "A practical guideline for the haematological management of major haemorrhage" (Hunt *et al*, 2015), recommends that transfusion laboratories seeing major haemorrhage due to trauma should consider having pre-thawed plasma on standby to allow FFP to be immediately available for the management of major bleeding. Some centres are already doing this; however this practice is leading to practical difficulties including FFP wastage due to the current shelf-life of thawed FFP being only 24 hours.

JPAC have therefore reviewed the available data on FFP with a view to possible extension of post-thaw shelf-life to enable rapid clinical provision without excessive wastage . JPAC agreed that the shelf-life of Fresh Frozen Plasma, Leucocyte Depleted following thawing should be changed from 24 hours to a maximum of 120 hours to permit the use of extended-thawed plasma according to revised BCSH guidelines. An addendum to BCSH Guidelines for the use of fresh frozen plasma, cryoprecipitate and cryosupernatant is expected to be published soon. Further details on the rationale for this change can be found in the JPAC supporting paper posted on http://www.transfusionguidelines.org.uk/document-library/supporting-papers.

These changes **DO NOT** apply to specifications for plasma components other than 7.15 Fresh Frozen Plasma, Leucocyte Depleted i.e. they **DO NOT** apply to 7.16, 7.17, 7.18, 7.19, 7.20, 7.27 and 7.28.

The following changes are necessary to specification 7.15: Fresh Frozen Plasma, Leucocyte Depleted.

7.15.2: Labelling

Change the penultimate bullet point as follows:

- a warning that the component must be used within 4 hours of thawing if maintained at 22 ±2°C, or up to a maximum of 120 hours of thawing if stored at 4 ±2°C, depending on indication.

7.15.3: Storage

Change the fourth and fifth bullet points, and two additional points added as follows:

- Protocols must be in place to ensure that the equipment is cleaned daily and maintained to minimise the risk of bacterial contamination. After thawing, and at the time of administration, the content

should be inspected to ensure that no insoluble cryoprecipitate is visible and that the container is intact. If to be stored thawed for an extended period (>24 hours from thawing), thawing methods that do not directly expose units to water must be used to minimise bacterial contamination.

- Once thawed, the component must not be refrozen and should be transfused as soon as possible. If delay is unavoidable, the component may be stored and should be used within 4 hours if maintained at 22 ±2°C or up to a maximum of 120 hours if stored at 4 ±2°C, but it should be borne in mind that extended post-thaw storage will result in a decline in the content of labile coagulation factors.

- Pre-thawed FFP that is out of a controlled temperature environment (4 ±2°C), can be accepted back into temperature controlled storage if this occurs on one occasion only of less than 30 minutes. Transfusion of FFP should be completed within 4 hours of issue out of a controlled temperature environment.

- For indications other than unexpected major haemorrhage, the component should be used within 24 hours of thawing.

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Joint UKBTS / NIBSC Professional Advisory Committee [1]
Summary Sheet

Paper for the Blood Consultative Committee meeting on:	17 March 2016
Date submitted:	14 March 2016
Title (including version no.):	Deviations from 4^0C temperature storage of red cells: effect on viability and bacterial growth
Author(s):	Dr Rebecca Cardigan, Dr Stephen Thomas and Dr Ty Pitt
Brief summary:	JPAC have considered the effect on viability and bacterial growth when red cells are removed from cold storage and concluded that: 1. The guidance in the Red Book relating to transport of red cells be changed to indicate that the 12 hour maximal transit time outwith 2-6^{o}C, where the surface temperature can reach 10^{o}C, is on one occasion only. 2. For occasions when removing red cells from 2-6^{o}C controlled storage is unavoidable then: • It is best to restrict time out of a controlled temperature environment to <30 minutes • If 30 minutes is exceeded the unit should not be returned to the issue location in the refrigerator, but returned to the transfusion department or quarantined remotely using electronic blood tracking. • Up to 60 minutes out of controlled temperature is acceptable, provided the unit is quarantined, by placing in a secure refrigerator for at least 6 hours, to allow the unit to return to 2-6^{o}C, prior to reissue. • Hospitals will need to identify these units so that they are not subject to out of controlled temperature storage for >30 to <60 minutes on more than three occasions. Change notifications will be issued to include both of these changes in component specifications and this paper published on the JPAC website. In addition the change to the '30 minute rule' will be included in a forthcoming revision to the BCSH blood Administration Guidelines.
Action required by the Blood Consultative Committee: (What do you want BCC to do in response to this paper?) e.g. • endorse a specific recommendation	For information

• advise where there is a choice of possible actions • advise on priorities within the work plan • provide a steer on policy	
Any other relevant information:	

([1]) **Joint U**nited **K**ingdom **B**lood **T**ransfusion **S**ervices and **N**ational **I**nstitute for **B**iological **S**tandards and **C**ontrol **Professional Advisory Committee**

JPAC 15-89 amended

Joint UKBTS Professional Advisory Committee ([1])
Summary Sheet

1. Paper for the JPAC meeting on:	12 November 2015
2. Date submitted:	09 November 2015
3. Title (including version no.):	Review of the shelf life of fresh frozen plasma components following thawing
4. Author(s):	Dr Laura Green and Dr Rebecca Cardigan for the SAC on Blood Components
5. Brief summary:	The new BCSH guideline on the management of major bleeding, recommends that blood banks seeing major haemorrhage due to trauma should consider having pre-thawed plasma on standby to allow FFP to be immediately available for the management of major bleeding. Some centres are already doing this by operating a pre-thawed FFP policy, but this results in wastage of FFP due to the limited (24 hour) shelf-life of thawed FFP. We have therefore reviewed the available data on FFP with a view to possible extension of post-thaw shelf-life to enable rapid clinical provision without excessive wastage, and have made specific recommendations for consideration by JPAC.
6. Action required by the Joint Professional Advisory Committee: (What do you want JPAC to do in response to this paper?) e.g. • endorse a specific recommendation • advise where there is a choice of possible actions • advise on priorities within the work plan • provide a steer on policy	Endorse the recommendation that: 1) the shelf life of thawed MB FFP should remain the same (i.e. 24 hours) and not be extended 2) the shelf life of thawed FFP be extended to 5 days to permit use of extended thawed plasma according to BCSH guidelines. Possible options for clinical indications of extended-thawed FFP include management of major bleeding associated with trauma only, or management of any unexpected major bleeding. 3) clinical indications for extended-thawed FFP and re-issue when out of controlled storage (30 minute rule) need to be included in BCSH guidelines. 4) further data on FFP wastage, and the effect of the changes recommended in this paper on both wastage and speed at which plasma can be provided are gained Data on the component and the importance of risk mitigation for bacterial contamination will need to be communicated to hospitals if these recommendations are accepted by JPAC, and SHOT will need to be notified of this change.
7. Any other relevant information:	This paper should be reviewed together with the previous JPAC papers: • JPAC 11.59: November 2011 • JPAC 13.48: July 2013 • JPAC 15-37 Position statement

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Review of the shelf life of fresh frozen plasma components following thawing

Laura Green and Rebecca Cardigan on behalf of SACBC

1. Background

The British Committee for Standards in Haematology (BCSH) guidelines (O'Shaughnessy *et al*, 2004), and the Guidelines for the UK Blood Transfusion Services (2013) recommend that fresh frozen plasma (FFP) be used as soon as possible after thawing, or within 24 hours of thawing if stored at 4°C. However, the thawing process and the transit time needed to deliver FFP may lead to significant delays in its availability, particularly detrimental during emergencies such as major haemorrhage. JPAC were previously asked to review whether the shelf-life of FFP following thawing should be extended beyond 24 hours (extended-thawed FFP here after), a summary of which is given below.

1.1 Summary of previous recommendations from the UK Joint Professional Advisory Committee (JPAC)

1.1.1. Considerations for extending the shelf life of FFP to 5 days (JPAC 11.59)

In 2011 the UK Standing Advisory Committee on Blood Components (SACBC) was asked by JPAC to review the current evidence on whether the shelf life of standard FFP following thawing can be extended from 24 hours to 5 days in order to:

1) reduce the wastage of plasma
2) improve timely availability of FFP for urgent use, e.g. trauma
3) pilot the remote supply of plasma to hospitals by NHSBT

To this end the literature was reviewed with 2 main issues in mind: (1) the efficacy of the components; and (2) the risk of bacterial contamination and other side effects.

Recommendations

JPAC recommended that the shelf life of FFP following thawing should **not be changed** because:

- the laboratory data showed that coagulation factors declined during storage and therefore there is a possibility that their efficacy would also decline
- there are no clinical studies evaluating the use of FFP which have been thawed and stored for 5 days
- it would not be possible for hospitals to label plasma beyond 24 hours of thawing as a separate component, since this would require them to hold a manufacturing license from the Medicines and Healthcare Regulatory Authority (MHRA) under the Blood and Safety Quality Regulations.
- there may be a new recommendation from the Advisory Committee on the Safety of Blood Tissues and Organs (SaBTO) in 2012 for the type of FFP to be used in the UK
- it was felt that there wasn't a strong clinical drive for extending the shelf life of FFP

1.1.2. Considerations for extending the shelf life of FFP to 48 or 72 hours (JPAC 13.48)

In March 2012, the National Blood Transfusion Committee requested that JPAC examine the evidence on efficacy and safety of extending the shelf life of thawed FFP to 48-72 hours. The following were reviewed:

a. coagulation factor content of FFP at 48-72 hrs;
b. clinical demand and potential reduction in FFP wastage through a national questionnaire: should thawed FFP be extended to 48-72hrs?

Recommendations

JPAC recommended that the shelf life of FFP following thawing should **not be changed** because:

- There was an unknown effect of extended storage of thawed plasma on the clinical efficacy of the component since clinical studies on thawed plasma are lacking
- Several laboratory studies showed that FVIII levels reduce significantly at Day-2 or Day-3; this reduction does not meet the European/UK requirements for FFP.
- The questionnaire results showed that:
    - the majority of participants were satisfied with FFP delivery times but there is still room for improvement.
    - Half of the respondents had concerns about the possible loss of efficacy with extended-thaw FFP, and just over a third would definitely use it for treating major haemorrhage.
    - Only a minority predicted significant wastage reduction.
- The use of extended thawed plasma could not easily be restricted to selected patient groups as the component is not labelled as a separate product.

1.2 Reasons for revisiting the shelf life of thawed FFP

1.2.1. Increase availability of FFP for management of major bleeding of trauma

Recently, a randomised controlled study (The PROPPR - Pragmatic, Randomized Optimal Platelet and Plasma Ratios) compared outcomes in patients with severe trauma and major bleeding. For selected post-hoc analyses, the early administration of plasma, platelets, and RBC in a 1:1:1 compared with a 1:1:2 ratio reduces death due to exsanguination at 24 hours, albeit with no significant differences in mortality at 24 hours and 28 days (Holcomb *et al*, 2015). The results of this study informed the new BCSH guideline on the management of major bleeding, which recommends that FFP be given empirically and early in the initial resuscitation process in a high dose ratio of 1:1 with red cells, before coagulation test results are available (Hunt *et al*, 2015a). For other major haemorrhage however, the guideline recommends that FFP be administered in a ratio of 1:2 with the red cells (Hunt *et al*, 2015b). Currently the National Institute for Health and Care Excellence (NICE) has distributed a draft clinical guideline for consultation on the assessment and management of major trauma: the draft document recommends that in adults and children a ratio of 1 unit of plasma to 1 unit of red cell should be used to replace fluid volume of patients who develop major bleeding (NICE 2015 draft guideline). In order to fulfil these guideline recommendations, major trauma centres will need to make FFP readily available for the management of major bleeding in trauma.

However, findings from a prospective observational study at 22 hospitals in the UK, including both major trauma centres and smaller trauma units, indicated that for patients who received plasma, the median time to first FFP transfusion was 87 min after arrival; for patients with more severe bleeding (massive haemorrhage) the timing was slightly faster at 68 min. Therefore, it is clearly important to look at the pathways of timely transfusion support and consider all options to support rapid administration of blood component.

1.2.2. Reduce FFP wastage

Some hospitals are pre-emptively thawing FFP and storing it for 24 hours (the current maximal shelf-life): this approach, however, is leading to significant FFP wastage. Since SACBC and JPAC last reviewed the shelf life of thawed FFP in 2012, it is believed that FFP wastage has increased significantly.

Until recently, data on FFP wastage has been scant as this was not collected by the Blood Stocks Management Scheme. From August 2015, BSMS hospitals have been submitting data on all types of component wastage, but data on FFP is not yet available for many hospitals. Recent FFP wastage

rates (Aug-Oct 2015) reported to NHSBT is variable and ranges from 1% - 86% of adult FFP issued, with an average wastage rate of 13%. For Adult FFP, in those hospitals providing pre-thawed plasma, (mainly trauma centres) the wastage range is 1% - 45%, with an average wastage rate of 14%. During Aug-Oct this represents over 1500 units - approximately a third of all FFP wastage. Intelligence and feedback from hospitals indicates that there is an upward trend for FFP wastage, especially for those centres providing pre-thawed plasma for trauma which anecdotally may be as high as 50%.

Wastage data for FFP overall for SNBTS for 2014/15 is 18%. The wastage rate in 2014/15 for WBS for thawed & returned unused FFP was 12% which has increased from 7% in 2010/11. In discussions with hospitals supplied by WBS their most challenging issue is the requirement for maintaining the cold chain – as if this is out of Blood Bank control for > 30 minutes and returned it is discarded.

1.3 Aims of current paper

In light of new clinical evidence, NHSBT has asked JPAC to re-evaluate the shelf life of thawed FFP and methylene blue treated FFP (MB FFP) beyond 24 hours (and up to 5 days). The literature review on the *in vitro* characteristics of the coagulation factors within thawed plasma during storage have been described in a previous paper (Cardigan & Green, 2015). For the current paper we will summarise the most recent NHSBT laboratory data and use it as the starting point for considering extending the shelf life of thawed FFP. In this paper we will not discuss solvent detergent FFP (Octaplas) as this is a licensed medicinal product and therefore its shelf-life following thawing is governed by the manufacturer (Octapharma) – currently 8 hours at 20-25^{o}C and 24 hours at 2-8^{o}C.

Data relating to MB FFP following thawing were reviewed by JPAC in 2006 in order to extend the shelf-life from 4 to 24 hours (JPAC 06-55). The data presented from NHSBT showed almost a doubling of thrombin generation between 24 and 48 hours. The reason for the increase in could be due to cold activation of plasma occurring beyond 24 hours post-thaw and is consistent with data from Germany showing an increase in activation of FVII and FX after 7 days storage of thawed MB FFP. It is unclear why this occurs with MB but not standard FFP. Currently we are not aware of any countries using thawed MB FFP, or indeed any PI FFP, beyond 24 hours of thawing. Due to the increased thrombin generation profile on extended post-thaw storage, the known reduction in clotting factors due to the initial PI treatment, and a desire to take a precautionary approach to minimising exposure of neonates to DEHP, we do not recommend that the shelf-life of thawed MB FFP be extended beyond 24 hours. MB FFP is thus not considered further here, and all subsequent discussion relates to untreated single donor FFP.

Liquid (never frozen) plasma is not considered within. A separate paper will be presented to JPAC in March 2016 with data and a specification for this component, dependent upon the outcome from phase 1 studies ongoing in NHSBT.

2 Data on coagulation factor content of standard FFP once thawed

The most recent data from FFP produced by NHSBT using current production processes are given in Tables 1, 2 and 3. These studies focused on the loss of factors V, VII and VIII and protein S as previous studies have identified these as being those most affected by storage following thawing.

SACBC also considered data in between day 1 and day 5, but due to the conclusions reached regarding the acceptability of data at day 5, these data are not presented to JPAC to simplify the paper.

Table 1. Coagulation factors in FFP at days 1 and 5 after thawing – NHSBT data

Clotting factor tests	Pre freeze	**post thaw** (T0)	**24 hours** post thaw	% change T0 vs. D1	**D5 (120 hours)** post thaw	% change T0 vs. D5	p-value (ANOVA)
PT (ratio)	1.02 ±0.05	1.03 ±0.05	1.06 ±0.06	2.4 ±1.4	1.13*** ±0.05	9.5 ±1.5	<0.0001
APTT (ratio)	1.14 ±0.04	1.17 ±0.05	1.23 ±0.05	5.0 ±2.0	1.26*** ±0.05	8.2 ±2.4	<0.0001
Fg (g/L)	2.55 ±0.35	2.57 ±0.40	2.60 ±0.46	0.7 ±5.7	2.61 ±0.39	1.80 ±5.9	0.4
FII (IU/mL)	0.91 ±0.08	0.91 ±0.09	0.88 ±0.07	-3.5 ±3.4	0.84*** ±0.06	-7.7 ±4.2	<0.0001
FV (IU/mL)	0.87 ±0.13	0.85 ±0.13	0.81 ±0.12	-5.1 ±4.0	0.74*** ±0.11	-12.9 ±4.6	<0.0001
FVII (IU/mL)	0.94 ±0.14	0.97 ±0.17	0.95 ±0.16	-2.5 ±4.5	0.79*** ±0.13	-18.3 ±4.8	<0.0001
FVIII (IU/mL)	0.85 ±0.19	0.82 ±0.20	0.62 ±0.16	-23.6 ±3.5	0.57*** ±0.14	-30.7 ±3.7	<0.0001
FXI (IU/mL)	0.90 ±0.16	0.94 ±0.17	0.89 ±0.15	-5.1 ±2.9	0.87* ±0.16	-7.2 ±3.9	<0.0001
FXII (IU/mL)	1.04 ±0.18	1.06 ±0.19	1.05 ±0.19	-1.2 ±4.3	1.03* ±0.17	-2.5 ±4.2	0.0014
Free PS (%)	99.0 ±14.3	98.2 ±12.9	96.3 ±14.0	-2.0 ±3.9	94.0** ±13.9	-4.4 ±3.5	<0.0001
PS activity (%)	89.4 ±15.2	84.6 ±13.9	82.3 ±13.6	-2.5 ±5.7	72.5*** ±11.7	-14.0 ±6.8	<0.0001
PC (%)	94.7 ±9.64	95.6 ±9.56	94.5 ±9.54	-1.1 ±2.4	93.8 ±9.90	-1.9 ±2.7	0.01

Results are given as Mean ±Standard deviation n=x, from a pool of n=2 units pooled and split
*One-way ANOVA performed with post test comparing 1 day post thaw versus 5 days post thaw ONLY, significant differences shown as * P<0.05 ** P<0.01 *** P<0.001.*
P value shown is from overall ANOVA, not post test.

Data in Table 1 from NHSBT show that for all factors studied, except protein C, there is a statistically significant decrease between 24 and 120 hours (5 days) after thawing. Previous studies have shown that most of the loss of FVIII occurs within the first 24 hours following thawing, and then the rate of loss decreases. For other factors the loss of activity is more linear during storage once thawed. However, with the exception of FVIII, mean levels remain above 70% of normal at day 5. Levels of FVIII and vWF in FFP are highly dependent upon ABO blood group, with the lowest values in group O donors. Therefore levels of FVIII at day 5 will vary by ABO group: in group A plasma mean levels are approximately 68% of normal. This is relevant as the current BCSH guidelines recommend the use of group A plasma as the universal group for the treatment of major haemorrhage due to trauma (Hunt *et al*, 2015b).

Data on thrombin generation from NHSBT (Table 2) suggests that extending the storage time from 24 hours to 5 days results in a small increase in the time to initiate thrombin generation, but no effect on the overall capacity of plasma to generate thrombin (ETP).

Data on markers of contact activation (cleavage of the amidolytic substrate S2302 and FXIIa:C1-inhibitor complexes) as well as levels of C1-inhibitor do not suggest that there is measurable

contact activation occurring during storage for up to 7 days, although we have only assessed a relatively small number of units and cold activation of plasma is known to be highly variable between donations. NHSBT is currently assessing a larger number of units of liquid plasma to confirm that this finding is reproducible in a larger data set.

Table 2. Thrombin generation, ROTEM and activation markers following thawing and storage of FFP for up to 7 days

	Time following thawing				
	Pre-freeze	0	1	5	7
Lag (min)	2.32 ±0.41	2.51 ±0.34	2.71 ±0.41	2.87* ±0.41	2.93 ±0.44
Peak (nM/L)	367 ±65.7	370 ±36.4	335 ±37.3	314 ±61.0	301 ±59.6
ETP (nM/min)	1813 ±412.1	1878 ±263.6	1851 ±259.5	1825 ±432.7	1757 ±440.6
ttPeak (min)	4.58 ±0.61	4.93 ±0.55	5.42 ±0.68	5.92*** ±0.85	6.05 ±0.95
CT (sec)	41.9 ±4.12	43.5 ±4.20	45.2 ±3.82	48.4* ±6.06	51.5 ±6.54
MCF (mm)	21.2 ±3.45	21.8 ±3.63	19.1 ±3.25	21.9*** ±3.41	20.5 ±4.53
AA (deg)	79.4 ±2.15	79.5 ±2.38	77.8 ±2.36	78.7* ±2.54	78.7 ±2.96
C1INH (%)	100 ±11.9	101 ±14.9	101 ±15.4	101 ±10.7	99 ±15.9
C1INH-FXIIa (IU/mL)	2.92 ±1.86	2.19 ±2.08	2.13 ±2.17	2.21 ±2.26	2.14 ±2.29
S-2032 (max V)	1.6 ±0.5	1.5 ±0.5	1.5 ±0.4	1.4 ±0.5	1.4 ±0.5

Thrombin generation, ROTEM and activation markers of FFP at various time points: pre-freeze, immediately post thaw, 1 day (current shelf life), 5 day and 7 days post thaw. Mean ±SD. Right: Percentage change of immediately post thaw versus 1, 5 and 7 days post thaw. One-way ANOVA performed with post test comparing 1 day post thaw versus 5 days post thaw ONLY, significant differences shown as * P<0.05 ** P<0.01 *** P<0.001.

In 2014, Newcastle upon Tyne Hospital tested 40 units of group A pre-thawed FFP for fibrinogen, FVII, FVIII, and signs of leakage at 24, 72 and 120 hours. Similar to the NHSBT data, their results show that the % reduction in fibrinogen was insignificant up to 120 hours, whilst FVII and FVIII levels reduced significantly (Table 3, data courtesy of Dr Jonathan Wallis). They also noted a decrease in FV by 72 hours, which is consistent with the most recent data from NHSBT.

Table 3. Post Thaw FFP Coagulation Factor levels over time

	24hrs post thawing					72 hours post thawing					120 hours post thawing				
	FI	FV	FVII	FVIII	Leakage/ integrity check	FI	FV	FVII	FVIII	Leakage/ integrity check	F1	FV	FVII	FVIII	Leakage/ integrity check
Average	2.288	94.173	110.39	75.308	No Leakage or floculation observed.	2.333	88.373	101.3	68.08	No Leakage or floculation observed.	2.2775	82.568	94.148	66.78	No Leakage or floculation observed.
Max	3.44	128.5	180.7	125.4		3.4	122.5	180.7	102		3.11	128.5	164.9	93.7	
Min	1.63	63.4	66.3	37.6		1.65	50.7	63.6	11.3		1.52	50.7	58.6	35.7	
SDEV	0.9162	32.566	57.699	44.045		0.882	35.915	59.78	45.83		0.79529	39.111	54.113	29.02	
MEDIAN	2.26	95	109.74	73.5		2.23	90.6	101.5	68.74		2.28	82.084	90.75	65.4	

Data from Newcastle upon Tyne Hospital (provided by Dr Jonathan Wallis). Data are based on 40 units of group A FFP from NHSBT as expressed as a % of pooled normal plasma.

To put the data on thawed plasma into context we have included data on pathogen inactivated plasma components for comparison (Table 4). PI-treatment of plasma, by any of the available methods, results in a reduction in clotting factors, the magnitude of which is dependent upon the PI method and clotting factor in question. For some factors there is a 30-40% loss of activity following PI. However, these plasmas are used in Europe for the same indications as FFP with the exception of the treatment of TTP (MB is thought to have reduced efficacy).

The large majority of FFP units transfused are given to support patients with major haemorrhage, either obstetric, surgical or traumatic. FFP is less commonly used to treat coagulopathy in sick patients with liver disease prior to invasive procedures, DIC and rarely to treat single factor deficiencies or thrombotic thrombocytopenic purpura (TTP). FFP usage in adults is always given as multiples of units, generally a minimum of 4 (1 litre). As such any variation in factor content between individual donor units, bar that associated with blood group type, is averaged out to give final levels similar to those in the table above. In small children a clinically effective dose may be as little as a single whole unit (220-290 mLs). For such patients' variation in factor content in individual donor units may therefore be clinically significant. These patients are also mandated to receive plasma from a source with a low risk of vCJD. Currently hospitals use either SD FFP or MB FFP for this indication. FFP is also used to correct certain single factor deficiencies where concentrates are not available (Factor V, factor XI). For these uses it is recommended that patients should receive pathogen inactivated plasma. In most centres this is interpreted as SD-treated pooled plasma. A minority of patients may receive MB treated single donor plasma, and in the future, possibly single donor PI plasma. These usages can be excluded from the current analysis in that they are generally less acute demands, will usually use a non standard FFP and are overall very small volume use.

FFP may be used to treat TTP. SD pooled plasma is recommended and MB plasma is not recommended. Untreated standard FFP may be used and in the absence of testing of ADMANTS13 levels post thaw should be used within 24 hours of thaw as at present.

The evidence for use of FFP to support coagulation in non bleeding patients is weak. It is therefore difficult to make any firm recommendations with regard to type of plasma or length of storage. However, if either SD or MB treated plasma is considered suitable, then prolonged storage thawed standard FFP may be considered equivalent given the equivalent factor levels.

Table 4. Data on thawed FFP in comparison to PI-treated plasma

* Internal data from NHSBT Component Development Laboratory, ** data reproduced in part from Backholer et al Vox Sanquinis 2016 Jan 12. doi: 10.1111/vox.12368. [Epub ahead of print], *** data from product insert

	Thawed FFP*				**MB****	**Intercept****	**Octaplas LG*****
	post thaw (T0)	**24 hours** post thaw	**120 hours** post thaw	**168 hours** post thaw	**post thaw** (T0)	**post thaw** (T0)	**post thaw** (T0)
PT (ratio)	1.03 ±0.05	1.06 ±0.06	1.13 ±0.05	1.15 ±0.06	1.06 (0.04)	1.08 (0.04)	
APTT (ratio)	1.17 ±0.05	1.23 ±0.05	1.26 ±0.05	1.26 ±0.05	1.40 (0.04)	1.34 (0.05)	
FibC (g/L)	2.57 ±0.40	2.60 ±0.46	2.61 ±0.39	2.63 ±0.43	1.70 (0.15)	2.05 (0.17)	2.6 ± 0.1
FII (IU/mL)	0.91 ±0.09	0.88 ±0.07	0.84 ±0.06	0.82 ±0.07	0.90 (0.07)	0.90 (0.07)	1.01± 0.07
FV (IU/mL)	0.85 ±0.13	0.81 ±0.12	0.74 ±0.11	0.74 ±0.10	0.73 (0.05)	0.77 (0.05)	0.76 ± 0.05
FVII (IU/mL)	0.97 ±0.17	0.95 ±0.16	0.79 ±0.13	0.72 ±0.11	1.08 (0.12)	1.05 (0.14)	1.09 ±0.05
FVIII (IU/mL)	0.82 ±0.20	0.62 ±0.16	0.57 ±0.14	0.54 ±0.14	0.61 (0.08)	0.58 (0.08)	0.80 ± 0.07
FXI (IU/mL)	0.94 ±0.17	0.89 ±0.15	0.87 ±0.16	0.90 ±0.17	0.42 (0.05)	0.58 (0.06)	0.88 ± 0.04
FXII (IU/mL)	1.06 ±0.19	1.05 ±0.19	1.03 ±0.17	1.03 ±0.18	0.81 (0.07)	0.80 (0.09)	1.04 ± 0.08
Free ProS (%)	98.2 ±12.9	96.3 ±14.0	94.0** ±13.9	92.7 ±13.6	74 (6)	75 (6)	
Pro S (activity %)	84.6 ±13.9	82.3 ±13.6	72.5*** ±11.7	68.8 ±12.7	NA	NA	0.63 ± 0.08
ProC (%)	95.6 ±9.56	94.5 ±9.54	93.8 ±9.90	94.4 ±9.94	95 (8)	95 (7)	86 ± 8
C1-INH	101 ±14.9	101 ±15.4	101 ±10.7	99 ±15.9			

3. Discussion

Determining how long to extend the post-thaw shelf life of FFP consists in getting the right balance of:

a. Efficacy of the component
b. Safety of component
c. Practical consideration/challenges

a. Efficacy of the component – standard FFP

Clinical indications for FFP transfusion include: single coagulation factor deficiency (i.e. FV deficiency); reversal of warfarin effect in the presence of life-threatening bleeding (together with prothrombin complex concentrate); disseminated intravascular coagulation in the presence of bleeding; massive transfusion; coagulopathy prior to invasive procedures; and plasma exchange for thrombotic thrombocytopenic purpura (O'Shaughnessy *et al*, 2004). With the exception of the last, the recommendations on FFP for the other clinical indications are based on expert opinion rather than randomised control studies.

Efficacy of FFP can be estimated by its haemostatic properties and the clinical impact of these properties on patients' outcome (i.e. correction of haemostasis and improved morbidity and mortality).

Haemostatic properties

Currently we do not know the levels of individual clotting factors (or inhibitors) in FFP which are necessary for its efficacy or safety in different clinical indications. Our data demonstrate a decrease in clotting factors in thawed FFP over time, meaning that insofar as haemostatic properties are concerned, extended-thawed FFP (both at days 3 and 5) is inferior to thawed FFP stored for 24 hours. However, despite this decrease, the average of all clotting factors (excepting FVIII) remain >70% of normal at Day 5, although no clinical studies have compared the efficacy and safety of extended-thawed FFP with that stored for 24 hours. There is no evidence from usage in other countries that a reduction to these levels reduces clinical efficacy of FFP in helping to correct haemostasis in the setting of bleeding patients.

Any reduction in haemostatic efficacy of extended-thawed FFP might be balanced by its usage enabling transfusion of FFP earlier in the course of major bleeding. Benefit of this was demonstrated in the PROPPR study, whereby the early use of FFP reduces death from exsanguinations (although the average shelf life of thawed FFP in this study remains unknown). Further, in the case of bleeding trauma patients, FVIII levels do not drop significantly in the first 24 hrs (Frith & Brohi, 2012); thus, it could be argued that the use of extended-thawed FFP would be satisfactory (despite containing lower levels of FVIII), since the replacement of FVIII at the onset of bleeding may be relatively less critical. Furthermore, dependent on the volume of thawed plasma individual hospitals hold, the volume of plasma stored >24hours given to any individual is limited.

Having optimum levels of clotting/inhibitor factors in plasma determines not only the efficacy but also its safety. For example, in the past low levels of protein S and alpha2 antiplasmin (anticoagulants) in solvent-detergent treated (SD) plasma has resulted in increased risk of thrombotic complications (Yarranton *et al*, 2003;Magner *et al*, 2007). Further, in 2010 an increase in thromboembolic events was also reported following Octagam (intravenous immunoglobulin) administration in both the US and the EU (2010) due to increased levels of FXIa. Currently, the finished product for Octaplas LG is tested for coagulation factors V, VIII, and XI, and the inhibitors

protein C, protein S, and plasmin inhibitor: a minimum of 0.5 IU/mL is obtained for each of the three coagulation factors, whereas the inhibitor levels are guaranteed equal or higher than 0.7, 0.3, and 0.2 IU/mL respectively (*Octapharma: product insert for Octaplas LG*). Our results show that the mean level for Protein S/C and FXI remain within the normal range at day 5. This is not surprising when we consider that FFP in the UK is produced from male donors (protein S/C levels are higher than women), and that our plasma is not pathogen inactivated (Protein C and S are labile towards solvent detergent treatment).

Other properties

Some authors have also suggested that the efficacy of FFP may in part be due to its effect in promoting vascular stability. The *in vitro* impact of FFP on vascular endothelium have been discussed in a recent review (Cardigan & Green, 2015), and the overall conclusion is that the effect of FFP on endothelium is lessened when FFP is thawed and stored for 5 days, although clinical data are needed to confirm these findings. FFP also contains adiponectin which is known to have vascular protective function. In one study –using a haemorrhagic shock mouse model - FFP resuscitation reversed the lung vascular damage-induced by haemorrhagic shock and this was shown to be partly attributable to adiponectin (Deng *et al*, 2015). Currently, we do not know what happens to adiponectin levels in FFP after prolonged storage once thawed.

International experience

Extended thawed FFP is used in several countries, with the shelf life varying between 5 and 14 days. Examples are:

- some centres in the USA (shelf life 5 days, labelled as a separate component to FFP)
- the Dutch Military Blood Bank in Leiden, Netherlands (for up to 7 days)
- the Academic Hospital Leiden, Netherlands (stored for up to 14 days; the average time at usage is 3 days post-thaw)
- some centres in Germany (stored for 7 days following thawing)
- Canada (5 days post-thaw shelf life)
- Australia (5 days following thawing if used to treat coagulopathies other than FVIII deficiencies)
- Some centres in Sweden (7 or 14 day shelf-life)

An EU survey was carried out in 2015 as part of the recent EU Symposium on plasma. Of the 13 countries that responded, only 2 are using thawed FFP beyond 24 hours of storage (i.e. 7-14 days) (data provided by Dr Sheila MacLennan). The current Council of Europe Guide states that FFP should be used immediately on thawing. Although there was no immediate agreement to change this, following the symposium a group is being set up to consider this issue with a view to recommending what should be in the next edition.

In the PROPPR study (in USA), out of the 12 trial sites that participated in the study, 11 used extended thawed FFP (shelf life 5 days), and only one site used liquid plasma with the shelf life of 26 days (Novak *et al*, 2015).

b. Safety

Two main safety considerations are:

a. Bacterial contamination
b. Levels of Phthalates

Bacterial contamination

The risk of bacterial transmission of FFP is very small, and to date there has been no SHOT report of infections arising from plasma. The two main junctures where bacterial contamination could be introduced for FFP are during blood donor phlebotomy or the FFP thawing process in hospitals. The most significant concern is bacterial growth when plasma is removed from the cold environment.

The risk of bacterial contamination arising from donors and proliferating during subsequent extended post thaw storage at 4°C is very small, and in the worst case scenario we can assume that this is equal to that of red cells. In the case of red cells, between 1995 and 2014, data on organisms implicated in bacterial transmissions identified six incidents reported to NHSBT from approximately 40 million units, giving an estimated incidence of transmission of 1 in 6 million (*JPAC paper 15-54: Deviations from 4°C temperature storage for red cells: effect on viability and bacterial growth, May 2015*).

The risk of bacterial contamination arising during the process of thawing and subsequent extended storage remains unknown. Some of the potential risks that could increase the likelihood of contamination during thawing include:

- The presence of pinholes or cracks in the packs: this could be visible or invisible
- Leaking or burst bag
- Dirty thawing equipment
- Prolonged thawing process
- Fluctuations of temperatures during thawing

In 2011 SACBC and JPAC reviewed the variations in practice during thawing of plasma and concluded that the thawing temperature of all frozen plasma components be changed from 37°C to 33-37°C (*JPAC 11-58*). The risk of bacterial contamination could be reduced by ensuring there is no direct contact of FFP with water, that thawing devices are kept clean and regulatory decontaminated, and that there is careful visual inspection of units following thawing and prior to administration. All UK Blood Services provide FFP in a vacuumed packed outer container, and thus FFP should not come into direct contact with thawing devices.

Canada, Australia and the USA have not reported any cases of bacterial sepsis associated with extended thawed plasma (personal communication Dana Devine, Joanne Pink, Louis Katz). In excess of 750,000 units of FFP have been issued in Canada since introducing a 5 day thawed product (2011) and over 700,000 units of FFP in Australia since 2010. The latest published data available from the USA (AABB Blood Survey Report 2013) suggests that 53% of all plasma transfused is thawed plasma, an increase from 24.5% in 2011. However, it is not known at what time post thaw (up to the maximal 5 day shelf-life) that most extended-thawed FFP is actually transfused in these countries. Within Australia, only a small number of laboratories currently use pre-thawed plasma. These are predominantly large metropolitan and regional laboratories that support hospitals with major surgical, intensive care, emergency and trauma services; and normally hold between 2 and 8 units of pre-thawed group A and/or AB FFP.

Phthalates

Plasticisers such as di(2-ethylhexyl)phthalate (DEHP) added to blood bags can leach out of the bags into the blood component and therefore be transfused to patients. Since DEHP is lipophyllic, there is a potential for increased levels due to leaching into plasma during storage at 4°C. Concerns about DEHP have been discussed in the recent review (Cardigan & Green, 2015), and it is acknowledged that levels of DEHP in plasma thawed and stored for 5 days are two-fold higher than that in red cells and platelets at the end of their shelf-life. This issue is of most concern for patients receiving

massive transfusion, chronic transfusion and neonates.

c. Practical consideration/challenges

The main practical considerations for extended thawed FFP are:

- Labelling of the components
- Clinical indication (the best way to control this)
- Management of FFP wastage
- Timing of 'out of controlled temperature'
- Quality monitoring

Labelling of the components

In the USA and Australia, once thawed, FFP is relabelled as a different component after 24 hours ('thawed plasma' or 'extended thawed plasma'). Some centres use standard 24 hour thawed FFP and extended-thawed FFP interchangeably, others do not.

Given the data on component quality, extended-thawed FFP should be labelled as a different component to standard thawed 24 hour FFP. However, the vast majority of hospitals in the UK do not hold a blood establishment licence so if extended-thawed FFP is to be introduced, they cannot introduce new labels to the blood component once thawed. Thus the only possible option for how to manage an extension to storage following thawing is to change the post-thaw shelf life in the Red Book for FFP from 24 hours to a new agreed extended-thawed shelf-life. An example of how the wording could be changed is given below, the exact wording would be notified through a change notification.

From

'Once thawed, the component must not be refrozen and should be transfused as soon as possible. If delay is unavoidable, the component may be stored and should be used within 4 hours if maintained at 22 ± 2 °C or 24 hours if stored at 4 ± 2 °C, but it should be borne in mind that extended post-thaw storage will result in a decline in the content of labile coagulation factors'

To

- *'Once thawed, the component must not be refrozen and should be transfused as soon as possible. If delay is unavoidable, the component may be stored and should be used within 4 hours if maintained at 22 ± 2 °C or 24 hours if stored at 4 ± 2 °C. For management of major bleeding, thawed FFP that has been stored at 4 ± 2 °C can be used for up to 5 days, but it should be borne in mind that extended post-thaw storage will result in a decline in the content of labile coagulation factors'*

There is a precedent for having a shorter shelf-life of a component for some clinical indications than is given on the label. This currently applies for Red Cells in Additive Solution, Leucocyte depleted for Large Volume Transfusion of Neonates/Infants, where the component should be used in < 5 days for neonates and infants, but for other indications it has a shelf life of 35 days.

When thawing FFP, hospitals would write on the expiry date/time of thawed units as they do now, and put this in their LIMS system. The extended thaw option would require that hospitals are extra vigilant in identifying any safety issue relating to the component, as well as, ensuring its appropriate use.

The main disadvantage of this option is that extended-thawed FFP will not be labelled as a separate component and as such there are risks that:

- any safety concerns may not be identified
- the manual entry of new expiry date and time could lead to component being used beyond its recommended shelf life - because of mismanagement of stocks or because of human error when entering information. Some hospitals have indicated that regardless of shelf-life having the final expiry of the component on the label when issued from UKBTS (as is the case for red cells and platelets) is attractive as it ensures (through LIMS) units are not used after expiry once thawed.

3.2 Clinical indications for extended-thawed FFP

If JPAC approves the use of extended-thawed FFP, there will have to be clear clinical indications for its use. These should be recommended by the BCSH FFP guideline, currently being revised.

Possible clinical indications for extended thawed FFP are: a) management of major bleeding associated with trauma only; or b) management of any major bleeding; or 3) all current indications for standard FFP. Extended-thawed FFP would not be appropriate for situations where pathogen inactivated FFP is recommended, e.g. for patients who have a single factor deficiency, thrombotic thrombocytopenic purpura.

SACBC have discussed these options. SACBC members were in agreement that as well as excluding single factor deficiency or therapeutic plasma exchange, extended thawed FFP should not be used for children/neonates. The latter is a precautionary approach to reduce exposure to DEHP and because there is little clinical experience with its use in these patients - most other centres that use thawed plasma exclude its use in neonates. There was less consensus on which FFP recipients extended-thawed FFP would be suitable for. SACBC concluded that extended-thawed FFP could be used in trauma based on evidence from the PROPPR study. They also felt that it might be reasonable to extrapolate from usage in trauma to other situations of major haemorrhage, although the pathophysiology is different. SACBC were less confident that extended-thawed FFP should be used for other less urgent indications. Therefore in the first instance SACBC suggest that the use of extended thawed FFP be restricted to clinical indications where immediate availability will improve timely supply to the bedside with documented clinical benefits, and the extended post thaw shelf life will help to reduce wastage.

3.3 Other aspects

Management of FFP wastage

Reducing FFP wastage is one of the main drivers for extending the shelf life of thawed FFP, particularly for those hospitals who are pre-thawing FFP in anticipation of demand. Although it is difficult to predict by how much extending the shelf life of thawed FFP will reduce wastage, it seems uncontroversial that broadly speaking, the shelf life of thawed FFP is inversely related to the level of wastage experienced. It is also likely to be the case that by extending the use of thawed plasma from trauma to all major bleeding that the likelihood of plasma pre-thawed for use in trauma being re-issued to other patients would increase.

Currently the wastage rate in NHS hospitals varies widely (data provided by Blood Stocks Management Scheme, see Appendix). The quantity of attributable wastage will depend on:

- hospitals' ability to manage effectively the usage and wastage of plasma, with some being better than others.
- the size of the hospitals - the bigger the hospital the higher the chances of recycling the pre-thawed FFP to other non-trauma patients

- clinical indications - if BSCH guidelines restrict the clinical indication of extended thawed FFP, then it is unlikely that FFP wastage will be diminished.

From the PROPPR study, which used a shelf life of 5 days for thawed FFP - the average FFP wastage rate was between 1- 7% (Novak *et al*, 2015).

It should be acknowledged that it is not clear from the data that is currently available what effect introducing extended thawed plasma either for trauma or trauma plus other causes of major bleeding will have on wastage rates.

Maximum timing of extended thawed FFP out of controlled temperature

Another important practical consideration is the maximum timing that extended thawed FFP could be outside controlled temperature (i.e. 2-6^{o}C). In the case of red cells (also stored at 2-6^{o}C) the 30 minute rule applies, primarily to reduce risk of bacterial growth and to preserve the quality of red cells. For extended thawed FFP, in the absence of any data we recommend the same rule be applied and this would be mainly to prevent bacterial growth. Currently Canada and Australia also operate a 30 min rule for thawed plasma, while in the United States the guidance from the AABB is not clear. Canada is currently performing laboratory studies to ascertain whether this could be extended.

Some laboratory or blood tracking IT systems may limit time out of controlled temperature to a single programmable time for all components.

In order to reduce bacterial risk it will be important (via BCSH) to provide guidance to hospitals on time out of controlled temperature. The application of the 30 minute rule, whilst essential, may limit the amount of plasma that can be re-issued to other patients.

Quality monitoring

Currently for FFP, quality monitoring (QM) testing includes the measurement of FVIII levels in the component at the point of production. Apart from thawing, no further manufacturing process will be applied to extended thawed FFP, and thus no additional QM is required, assuming that the initial QM testing is adequate. However, better understanding of the impact of different thawing methods/processes used by hospitals, on the quality of extended-thawed FFP, would be advisable during any implementation of extended thawed FFP, since this is a variable that could influence final product quality that has not been assessed.

Summary

Following the results of the PROPPR study the BCSH guideline on the management of major bleeding, has recommended that for trauma patients who are bleeding, FFP should be given in the initial resuscitation process in a dose ratio of 1:1 with red cells, before coagulation test results are available.

Currently some hospitals are pre-emptively thawing FFP and storing it for 24 hours: this approach however is leading to significant FFP wastage.

In vitro data from NHSBT has shown that the longer the standard FFP is left after thawing, the lower the clotting factors are. However, the average level of clotting factors measured (except FVIII) remain >70% of normal at Day 5.

Currently, we do not know what the optimum level of individual clotting factors (or inhibitors) in FFP required for its efficacy or safety. In the PROPPR study, 11 of the 12 trial sites used extended

thawed FFP (shelf life of 5 days), although we do not know the average shelf life of FFP transfused in the study.

Recent data in animal models suggest that FFP may also be important in exerting a protective effect on the endothelium, and this is lessened in thawed plasma. The effect appears to be partly attributable to adiponectin, and it is not known what effect storage of thawed plasma has on this.

Extended thawed FFP is used in several countries (USA, some European countries, Australia and New Zealand), with the shelf life varying between 5 and 14 days.

The extension of shelf life of thawed FFP could theoretically increase the risk of bacterial proliferation, particularly if a water method is used for thawing, but steps could be taken to mitigate this risk.

Plasticisers such as di(2-ethylhexyl)phthalate (DEHP) added to blood bags can leach out of the bags into the blood component and therefore be transfused to patients. This is particularly of concern for children/neonates, massive transfusion and chronic transfusion. However for major haemorrhage, the benefit of early administration of plasma appear to outweigh theoretical toxicology concerns.

Recommendations from SACBC

MB FFP

- The shelf life of thawed MB FFP should remain the same (i.e. 24 hours) and not be extended

Standard FFP

- In light of new clinical evidence of benefit for giving FFP early in bleeding in trauma, we support the extension of the shelf life of thawed FFP to 5 days for enabling hospitals to reduce wastage while increasing availability.

If extension of shelf life of thawed FFP is approved the following would need to be considered:

- *Clinical indications* would need to be determined by the BCSH guideline, and this is currently being updated. Possible options for clinical indications of *extended thawed FFP include*: a) management of major bleeding associated with trauma only; or b) management of any major bleeding.

- *Labelling:* change the post-thaw shelf life in the Red Book for FFP from 24 hours to an agreed shelf-life: the wording in the specification could be changed in line with BCSH recommendations:

  From
  'Once thawed, the component must not be refrozen and should be transfused as soon as possible. If delay is unavoidable, the component may be stored and should be used within 4 hours if maintained at 22 ± 2 °C or 24 hours if stored at 4 ± 2 °C, but it should be borne in mind that extended post-thaw storage will result in a decline in the content of labile coagulation factors'

  To

- *'Once thawed, the component must not be refrozen and should be transfused as soon as possible. If delay is unavoidable, the component may be stored and should be used within 4 hours if maintained at 22 ± 2 °C or 24 hours if stored at 4 ± 2 °C. For management of major*

bleeding, thawed FFP that has been stored at 4 ± 2 °C can be used for up to 5 days, but it should be borne in mind that extended post-thaw storage will result in a decline in the content of labile coagulation factors'

- *Quality monitoring: we do not recommend any extra quality monitoring*

- *Thawing process*: In order to reduce the risk of bacterial contamination/growth the following mitigating factors must be followed
  - Use thawing methods that do not directly expose FFP units to water
  - If water baths methods are used, hospitals need to ensure that direct contact of units with water is avoided (i.e. vacuum packs)
  - Ensure that plasma thawers are decontaminated on regular basis
  - There has to be visual inspection at the time of thawing and at the time of administration of FFP

- *30 minute rule*: If extended thawed FFP is taken out of the controlled temperature environment (i.e. 2-6^{o}C), then it should be transfused within 4 hours and only be re-issued if returned to controlled storage within 30 minutes.

Future work:

It would be desirable to gain a better understanding of:

- FFP wastage management for hospitals, as the current data are limited but variable and suggest that there may be room to improve practice. Further, it is not known whether the change to extended thawed plasma would improve either the rapidity of provision of plasma or wastage rates and this could be examined as part of any implementation.
- The difference that different thawing methods/process have on quality of extended thawed FFP

NHSBT is concurrently assessing a never frozen plasma (or liquid plasma) component. SACBC is planning to submit a paper to JPAC in March 2016 containing phase 0/1 data on for approval of a specification for this component. Currently work is being undertaken to ensure that levels of RBC and WBC are sufficiently low to obviate the need to irradiate or RhD match this component. If in the future, both liquid plasma and extended thawed FFP are used by hospitals, further work is needed to assess their cost (both from NHSBT and hospital ends) and safety.

Appendix Table 1. Studies assessing levels of coagulation factors in thawed untreated plasma

No of units and ABO group	Length of time as WB prior to freezing and temp	LD?	Stored for up to	Key findings
4 (2A & 2O)	<3 hrs	no	28d	At d28 loss of 8% fibrinogen, 40% FV, 64% FVIII (39% by d3), 20% of FIX and FXI.
15 (5A, 5B, 5O)	Not stated	Not stated	5d	No loss of FII, V, VII, X or fibrinogen. 40% loss of FVIII.
20 (5 of each ABO)	24hr 4oC (FP24)	Not stated	5d	No increase in FVIIa
5 all O	Not stated but apheresis so probably short	Not stated	14d	At d14: 35% loss of FV, 45% loss FVIII, 8% loss fibrinogen.
18 apheresis, 19 from WB	For WB<8 hrs, plasma not frozen but 4oC	Yes	28d	At d28: 60-65% loss FVIII, 25% loss FV, no loss C1-INH, no increase in d-dimers
10 FFP (5A, 5O) 10 FP24 (5A, 5O)	<8 hrs <24 hrs at 4oC	Not stated	5d	No decrease in ADAMTS13 activity after 5d for either product
30 WB FFP (all A) 39 apheresis (Ab or A)	<8hrs <2hrs	Yes	42d	Change in prekallikrein, C1-INH and PC dependent upon donor. Kallikrein generation more predominant in females. No change in ATIII or d-dimers, increase in TATover 42d.
18 (5 of each A,B, O, + 3AB)	<24hr 4oC	Not stated	5d	At d5: minimal change in fibrinogen, FII, FVIII, ATIII, PC ro ADAMTS13. Loss of FV (34%), FVII (18%), FIX (11%), PS (31%). Increase in FXI (52%) and FXII (21%).
20 (5 of each ABO)	<1 hr (apheresis)	Yes	6d	Miminal changes in fibrinogen, FXII, FXIII, PS, PC, ATIII, vWF ag. Loss of FII (10%), FV (14%), FVII (36%), FVIII (38%), FIX (11%), FXI (16%).
15 (5A, 5B, 5O)	<8 hr <24hr 4oC	Mix	5d	For FP24: minimal changes in fibrinogen, FII, FIX, FX, vWFag, ATIII, PC. Loss of FV (31%), FVII (14%), FVIII (28%), vWF activity (17%), PS (15%).
15 (8A, 4O, 2B, 1AB)	<24 hr 'room temp'	Yes	7d	Minimal loss of fibrinogen, FX, ATIII, FXI, free PS, FV. At day 5 21% loss FVII and 25% loss FVIII
14 (O:non O 1:1.8) 16 (O:non O 1:1)	<8 hour <24 hour 4oC	Not stated	5d 5d	Reduced thrombin generation day 5 v day 1 (Increased lag time, reduced ETP and peak thrombin). ROTEG: alpha angle, maximum amplitude similar Day 0 v day 5, but increase in reaction time.
30 (13 O: 17 non-O)	< 8 hour	Not stated	5d	Thrombin generation/ROTEG results as above. 50% decrease in platelet microparticles day 5 v day 0 by flow cytometry. Filtration to remove MP reduces thrombin generation.
28 O & 26 non-O	<24 hr ambient temp	Yes	5d	
10 O thawed 37oC 10 O thawed 45oC	Not stated	Not stated	20d	Loss of FV and FVIII similar whether thawed at 37 or 45oC, but thawing times faster at the higher temperature. Loss of 25% FV and 39% FVIII at day 5, with further decline to day 20. A 20% increase in FVII by day 5.

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Table 2. Residual coagulation factor/inhibitor levels in untreated plasma 5 days following thawing (% of baseline immediately post-thaw)

Type of plasma	FFP	FFP?	FFP	FFP	FFP	FFP	FFP	FP24	FP24	FP24	FP24	RT-FP24	RT-FP24	NS	NS
Fibrinogen	97	95			95	99	99	92		100	102	96	100	100	
FII					90	97	94			97	99			80	
FV	74	80			86	79	70	75		66	69	97	80	66	75
FVII					64	67	91	81		82	86	78	91	72	119
FVIII	75	60			62	53	75	65		97	72	75	65	41-67	61
FIX					89	98	94	100		89	96				
FX					89	93	98				97	100		80	
FXI					84		97	100		152		100			
FXII					102		99			121					
FXIII					100		97								
VWF antigen					100	99	92				96				
VWF activity						92	84				83				
ATIII				100	100	97	100			95	104	96			
PC					100	100	100			94	102				
PS					100	93	87	36		69	85	100			
ADAMTS13		94	100						100	93					
Antiplasmin								100							

Reproduced from (Cardigan & Green, 2015)

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