# 7 Delayed Transfusion Reactions

**Definition**

Delayed transfusion reactions are defined in this report as those occurring more than 24 hours following a transfusion of blood or blood components. In practice, these are usually delayed haemolytic reactions due to the development of red cell alloantibodies. Simple serological reactions (antibody development without a positive DAT or evidence of haemolysis) are excluded.

This category accounted for 6.8% of non-infectious hazards reported and 6.7% of all hazards.

32 new initial reports were received and 2 were brought forward from the previous year. 2 additional reports were received which were not included in the analysis for this chapter. 9 reports received during the reporting period are still awaiting completion of a questionnaire and will be presented next year.

This chapter highlights the main findings from 25 completed questionnaires (23 from the current reporting year).

**Age and sex**

Age (25 reports)

| | |
|---|---|
| Age range | 31 -83 years |
| Median age | 70 years |

Sex (25 reports)

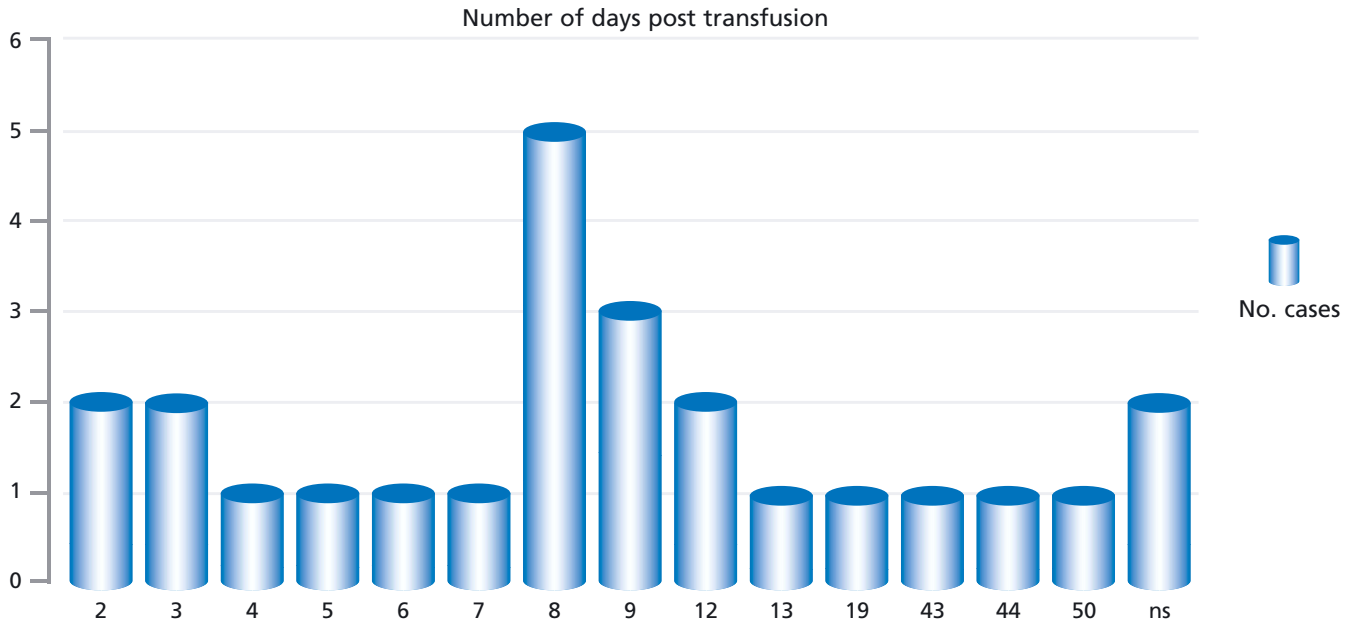
| | |
|---|---|
| Males | 8 |
| Females | 17 |

**Interval between transfusion and symptoms**

Figure 8 shows the interval in days between the implicated transfusion and signs or symptoms of a DHTR. The intervals given are necessarily those when the signs or symptoms were first noted. However, it is possible that some extravascular haemolysis was ongoing during or shortly after the transfusion in those cases where the causative antibody was detectable pre-transfusion, or when the reaction was clinically noted within 48 hours of the transfusion.

**Figure 8**

**Interval between transfusion and symptoms**




Median = 8 days

Range = 2 to 50 days

**Reactions reported**

There were 3 deaths in this group; none thought to be related to the transfusion. One patient required ICU admission, but did not suffer any long term morbidity. The remaining patients suffered minor or no morbidity.

All reported reactions were probably caused by the administration of allogeneic red cells. In 2 cases the causative antibody was detected pre-transfusion: in case 3, anti-M present in a mixture of antibodies was not initially detectable at 37°C, but contributed to a severe DHTR 9 days later; in case 21 an unidentified antibody present in a mixture caused a reaction within 2 days of the transfusion. In this latter case it is likely that there was ongoing extravascular haemolysis during or soon after the transfusion was started, and the same patient suffered an immediate haemolytic reaction following a subsequent transfusion (see vignette).

In 3 cases, the causative antibody may have been detectable pre-transfusion, but there is insufficient evidence to be certain. In case 13 (see vignette) one specificity was retrospectively found in the pre-transfusion sample but detectable only with an enzyme technique, and a second enzyme-only antibody was retrospectively detected in a further sample taken 8 days later, and 5 days prior to signs of a DHTR. In the other 2 cases (case 8 and 25), anti-Kidd antibodies were probably present in the pre-transfusion samples, but insufficient testing was undertaken to be certain (see vignettes).

Thirty-nine new antibodies were identified post-transfusion in 23 patients. In addition to the 5 cases already described, 7 had pre-existing antibodies: 6 received appropriately phenotyped blood and the 7$^{th}$ (with anti-Kp$^{a}$) received crossmatch compatible blood, but all developed further specificities as a result of the transfusion.

Six cases had no reported history of previous transfusion or pregnancy. In one of these (case 16), the reaction may have been due to primary sensitisation, the antibody being detected 44 days post transfusion (grade 2/3). In the other 5 cases (2 male, 3 female), the antibodies were found within 13 days of the transfusion, and it must therefore be assumed that these patients had received previous transfusions or had previously been pregnant.

Two had a negative DAT: both had clear-cut haemolytic reactions (grade 3) with identifiable red cell antibodies.

**Urgency of transfusion requirement**

The transfusion was said to be routine in 15 patients and an emergency in 10. However two of the routine transfusions took place between 8pm and midnight, classified as during a shift system, although testing took place during routine hours; in one case the pre-transfusion testing took place between midnight and 8am, classified as on-call, although the transfusion took place during routine hours, and in 1 case both the testing and the transfusion took place between midnight and 8am, classified as on-call.

**New post transfusion antibodies**

Table 12 shows the specificity of all new antibodies detected post-transfusion and table 13 antibodies in individual patients.

**Table 12 – New specificities by blood group system**

| Antibody specificity by blood group system | Number of cases | Sole *new* antibody |
|---|---|---|
| **Kidd** | | |
| $Jk^a$ | 10 | 7 |
| $Jk^b$ | 6 | 3 |
| **Rh** | | |
| $C^w$ | 1 | 0 |
| E | 7* | 2 (1 with anti-c) |
| c | 3 | 1 (with anti-E) |
| e | 1 | 0 |
| **Kell** | | |
| K | 2 | 0 |
| $Kp^a$ | 1 | 0 |
| **Duffy** | | |
| $Fy^a$ | 2 | 2 |
| **MNSs** | | |
| S | 2 | 0 |
| **Other** | | |
| $P^1$ | 1 | 0 |
| Ch | 1 | 0 |
| $Kn^a$ | 1 | 0 |
| $A_1$ | 1 | 0 |

* one retrospectively detected pre-transfusion but by enzyme only

**Table 13 New post-transfusion antibodies in individual patients**

| Case number | New antibody (ies) | Comments |
|---|---|---|
| **1** | $Jk^b$+S+$C^w$ | Pre-existing anti-$Fy^a$ |
| **2** | $Jk^b$ | Pre-existing anti-K |
| **3** | None | Pre-existing anti-$Fy^a$+ c+E+S+M+$Cs^a$. Unit not typed for anti-M as not detectable at 37°C until post transfusion |
| **4** | E+$Kp^a$+ non-spec enzyme antibody | |
| **5** | c+E | Known pre-existing anti-K but no identification panel performed pre-Tx |
| **6** | $Jk^a$ | Pre-existing anti-E+K |
| **7** | $Jk^a$ + non-spec enzyme antibody | |
| **8** | $Jk^b$+ $P_1$ | Known pre-existing anti-E but no identification panel pre-Tx |
| **9** | $Jk^a$ | |
| **10** | $Jk^a$ | |
| **11** | c+E+Ch+K | |
| **12** | $Jk^a$ | |
| **13** | $Jk^a$+K+E | Anti-$Jk^a$ by enzyme only, post tx. Anti-E and -$Jk^a$ both detected retrospectively by enz only. |
| **14** | $Jk^b$ | |
| **15** | $Fy^a$ | |
| **16** | E+$Jk^a$+?auto anti-c | |
| **17** | $Jk^b$ | |
| **18** | $Jk^a$ | Pre-existing anti-D+?C |
| **19** | e+$A_1$ | Pre-existing anti-K |
| **20** | $Fy^a$ | Pre-existing anti-$Kp^a$ |
| **21** | None | Anti-c+E+$Jk^b$+Cw+further unidentified antibody – eventually identified as anti-$Do^a$ |
| **22** | $Jk^b$+S+$Kn^a$ | Positive screen pre-transfusion but ID panel negative |
| **23** | E | |
| **24** | c+E+$Jk^a$+non-spec cold | |
| **25** | $Jk^a$ | Known pre-existing anti-K + cold auto, but no identification panel performed pre-Tx |

**Severity of reaction/ clinical sequelae**

Symptoms and signs could be divided into 4 categories as follows:

- Group 1 Asymptomatic (with positive DAT only)
- Group 2 Falling haemoglobin (⬇Hb)/positive DAT/spherocytes (2 of these parameters)
- Group 3 ⬇Hb + jaundice±positive DAT±spherocytes
- Group 4 As group 3 + renal impairment

**Group 1**

There were 7 patients in this group. One was already on ICU but all 7 survived with no sequelae. One was jaundiced and had a falling Hb, but it is likely that these signs could be attributed to the underlying clinical condition. One had a fever reported during transfusion, but there is no evidence that this was related to the antibody.

**Group 2**

There were 4 patients in this group. Two were already on ICU, but all 4 in this category survived with no sequelae. Although one of these patients was jaundiced and was reported to have dark urine (case 19), these findings could also be explained by the underlying clinical condition.

**Group 3**

There were 11 patients in this group, of whom 7 survived with only short term symptoms and no risk to life, although 2 did require admission to the ward (cases 1 and 16). One of these (case 16) who gave no previous history of transfusion was re-admitted after 44 days, with an Hb of 44g/L, a raised bilirubin and no evidence of any remaining transfused cells; the picture was complicated by his history of bleeds due to alcoholism.

Two patients died, with the cause unrelated to the transfusion.

One patient with sickle cell disease (case 3 – see vignette), suffered a life-threatening drop in Hb to 25g/L, but survived with no long term ill effects. One patient required ICU admission (case 22) but also survived with no long term ill effects.

**Group 4**

There were 3 patients in this group. One was already on ICU and died as the result of a ruptured abdominal aortic aneurysm. One (case 11) had to be re-admitted with haemoglobinuria. The third survived with no long term ill effects.

**Table 14**

**Individual new antibodies grouped by severity of signs and symptoms**

| Group 1 | | Group 2 | | Group 3 | | | | Group 4 | |
|---|---|---|---|---|---|---|---|---|---|
| Case No. | Ab specificity | Case No. | Ab specificity | Case No. | Ab specificity | Case No. | Ab specificity | Case No. | Ab specificity |
| 4 | E+Kp$^a$ | 15 | Fy$^a$ | 1 | Jk$^b$+S+C$^w$ | 18 | Jk$^a$ | 11 | c+E+K+Ch |
| 7 | Jk$^a$ | 17 | Jk$^b$ | 2 | Jk$^b$ | 21 | None | 14 | Jk$^b$ |
| 8 | Non specific +E+Jk$^b$+P$_1$ | 6 | Jk$^a$ | 3 | None | 22 | Jk$^b$+S+Kn$^a$ | 20 | Fy$^a$ |
| 9 | Jk$^a$ | 19 | e+A$_1$ | 5 | c+E | 24 | c+E+Jk$^a$+non-spec cold | | |
| 12 | Jk$^a$ | | | 10 | Jk$^a$ | | | | |
| 23 | E | | | 13 | Jk$^a$+K+E | | | | |
| 25 | Jk$^a$ | | | 16 | E+Jk$^a$+ ?auto anti-c | | | | |

### Analysis of serological information

Table 15 gives information on the techniques used for antibody screening in the 25 reported cases. An IAT crossmatch was performed in 18 cases (including all those where the antibody screen was positive), an immediate spin crossmatch in 1 case, and electronic issue in 6 cases.

**Table 15**

**Techniques used for antibody screening and crossmatching**

| IAT screening technology | Number of cases |
|---|---|
| CAT | 23 |
| NISS tube | 1 |
| Unknown to reporter* | 1 |

* Performed by the Blood Centre

### IAT Technology

Excluding the case where pre-transfusion testing was performed at the reference centre, 96% of cases were performed using Column Agglutination Technology (CAT). This reflects current practice in the UK where approximately 82% of antibody screens in non-reference laboratories are performed using CAT (data from UK National External Quality Assessment Scheme (UK NEQAS) questionnaire, February 2003).

### Automation

At least 83% of the CAT users utilised automation (no data was available in 2 cases). In 2002, only 43% of UK laboratories utilised automation for antibody screening (data from UK NEQAS questionnaire, July 2002). The significance of this finding is as yet unknown, given the small number of cases.

### Plasma/serum

Plasma was used in 22 cases (92%) and serum in 2 cases (1 not stated). This probably reflects current practice with data obtained from a UK NEQAS questionnaire (Feb 2003) showing that approximately 86% of antibody screens undertaken in UK hospitals are performed using plasma rather than serum.

### Screening cells

In eighteen cases the screening cells bore apparent homozygous expression of the relevant antigen. In 6 cases, although not stated, it is probable that they did, and in the last case no information was available as the pre-transfusion testing took place at a different site.

The majority of reporting laboratories utilising a CAT technique, used screening cells that were validated for use by the supplier. Of the 5 that used alternative screening cells, only 2 stated that they had validated the cell suspension in-house prior to use. There was no information available from 4 reporting laboratories.

### Incubation time and details of techniques

Incubation times for antibody screening/identification and crossmatching by CAT varied from 10 to 30 minutes, but all were within recommended limits. All cell:serum ratios were correct for the column technology used and manufacturer's instructions were followed in all cases where information is available (21 of 23 cases). The serum:cell ratio used in the NISS tube case was 2:1; this is lower than the recommended 4:1[18] (case 25 – see vignette).

**Retrospective testing**

In only 6 (25%) cases was the pre-transfusion sample retested, and the same result was obtained in 5 (83%) of them. In all 6 cases a different individual repeated the testing, but in the 5 cases where the same result was obtained, the same techniques were used as for pre-transfusion testing. In the sixth case an enzyme technique was included in the retrospective testing and enzyme only antibodies were detected, one of which became reactive by IAT post transfusion (see vignette). In these 6 cases the DTR was not recognised until 6-12 days post transfusion. In 4 cases where retrospective testing was not performed, the DTR was reported to have occurred 2-4 days post transfusion and samples for crossmatching were taken within 48 hours of the transfusion.

**Direct Antiglobulin Test**

The post transfusion DAT was reported to be negative in 2 cases. In 8 cases the coating was IgG only, in 2 cases C3d only and in 9 cases both (in addition, one had IgM coating).

**Interval between drawing the crossmatch sample and transfusion**

**Table 16**
**Interval between drawing the crossmatch sample and transfusion**

| Interval between sampling and transfusion | No. cases |
|---|---|
| <48 hours | 22 |
| 5 days | 1 |
| 6 days | 1 |

As far as it is possible to tell from the questionnaires, all of the samples were taken within the time limits recommended in the BCSH guidelines. [19]

**Reporting to Blood Centres and Hospital Transfusion Committees**

A total of 15/24 (63%) incidents were reported to the local Blood Centre and 21/24 (88%) to the HTC. No data was available for the remaining case. There seems to be a gradual upward trend in reporting cases of DTR to the local Blood Centre (46%, 49% and 58% for the previous 3 years), whereas reporting to the HTC is more frequent but more erratic (79%, 85% and 73% for the previous 3 years).

**Case histories of some of the more informative or unusual cases are given below:**

***Case 3***

*A 37 year old female patient with sickle cell disease received an 8-unit emergency exchange transfusion following a stroke. The patient was known to have anti-Fy$^a$+c+E+S+M+Cs$^a$ and crossmatched antigen negative blood was provided for all except the anti-M, which at the time was not detectable at 37°C and was therefore thought to be of no clinical significance. Six days later she received a 3 unit top-up transfusion, crossmatched against a fresh sample, and again antigen negative for all except the anti-M. Two days later she had dropped her Hb to 25gL, the DAT was positive and anti-M was identified both in the plasma at 37°C and in an eluate made from the patient's red cells (tested by IAT). A further 3 units of M- blood were transfused and produced a short-lived increase in Hb. The short-lived increase in Hb combined with an increase in the level of HbS% and no reticulocytopenia, suggests destruction of both transfused cells and patient's own cells. The haemolysis was therefore likely to be the result of a combination of DHTR due to anti-M, and hyperhaemolysis (of patient's own and transfused cells).*[20]

***Case 13***

*An 83 male patient with MDS received a 2-unit top up transfusion on day 1; the antibody screen was negative. On day 9, a new sample was taken; again, the screen was negative and a further 2 units were crossmatched and transfused. On day 12, a new sample showed a weakly positive antibody screen by IAT, but the specificity was not clear; however anti-E and anti-Jk$^a$ were revealed by a 2-stage enzyme technique and the DAT was weakly positive. On day 13, a further sample was taken, and anti-E and anti-K were identifiable by IAT; anti-Jk$^a$ was still only detectable by 2-stage enzyme. The patient's Hb fell from 96gL to 80gL between day 10 and day 16, the bilirubin rose from normal levels to 53µmol/L by day 16 and spherocytes were noted on the blood film. A new sample, taken on day 16 and plasma aliquots from previous samples were referred to the NBS*

*reference laboratory. They confirmed anti-E+K by IAT and enzyme-only anti-Jk$^a$ in the day 16 sample, enzyme-only anti-E and anti-Jk$^a$ in the day 9 sample, and enzyme-only anti-E in the day 1 sample. One of the units transfused on day 1 was Jk(a+) and the other K+; one of the units transfused on day 9 was E+. It is not clear which of these antibodies contributed to the DHTR.*

***Case 21 (and case 3 from the ATR section)***

*A 68 year old female patient with myelodysplastic syndrome required a 2-unit top-up transfusion. The patient was known to have anti-c+E+Jk$^b$+C$^w$, but further reactions by IAT could not be identified, even by the reference centre. It was suspected that the unidentified antibody was HLA related, and $R_1R_1$ Jk(b-) red cells, weakly incompatible by IAT, were issued as suitable for transfusion. Two days later the patient had chills, fever, jaundice and a falling Hb, and the DAT (not performed pre-transfusion) was positive with anti-IgG. The patient had a further red cell transfusion three weeks later with weakly incompatible $R_1R_1$ Jk(b-) cells, which were selected and crossmatched by the Reference Laboratory, using an IAT and one stage papain technique; at this stage the antibody was suspected to be a 'high-titre, low-avidity' (HTLA) specificity (within the Knops blood group system). However, she became restless, and developed a fever, chills and hypotension during the transfusion, which was continued at a slower rate. The International Blood Group Reference Laboratory confirmed the presence of an anti-Do$^a$ in the post-transfusion sample four weeks later.*

The frequency of Do$^a$ is 66% in northern European donors; therefore Do(a-) blood is not difficult to find. However, Dombrock antibodies are difficult to identify and usually occur in mixtures of antibodies. For this reason, their presence should be considered when unidentifiable reactions occur in the presence of other red cell alloantibodies.

***Case 5***

*A 31 year old female patient with anaemia due to Crohn's disease was routinely transfused as an outpatient with 3 units of red cells. She was known to have anti-K, last investigated 6 weeks previously and as there was no history of transfusion, an identification panel was not performed (in line with laboratory policy). The patient reported verbally that she had anti-E. The blood was K-, E- and crossmatch compatible. Eight days later the patient presented with dark urine and jaundice; the DAT was positive and anti-c+E+K was identified in her plasma. A retrospective panel on the pre-transfusion sample confirmed that only anti-K was detectable (by both IAT and enzyme techniques). A subsequent enquiry from a neighbouring hospital revealed detection of enzyme only anti-c+E in 1994.*

Two similar cases are described where pretransfusion testing did not include antibody identification, despite known existing antibodies.

***Case 8***

*A 35 year old male patient with alcoholic liver disease and bleeding varices required an emergency transfusion of several different blood components. The patient had a known anti-E and E negative units were crossmatched by a manual DiaMed technique and issued as compatible without an identification panel. Two days after transfusion, a further sample revealed non-specific reactions by IAT in addition to the anti-E; the DAT was positive with IgG, IgM and C3d reagents (not tested pre-transfusion). No retrospective testing was undertaken on the pre-transfusion sample as it was no longer available. The Blood Service reference laboratory identified anti- E+Jk$^b$+$P_1$. The patient had raised levels of plasma bilirubin and alanine aminotransferase (ALT), and the Hb fell, all of which were consistent with his clinical condition. It is therefore not clear whether or not the patient suffered from a haemolytic transfusion reaction, and the case has been classified as a category 1.*

***Case 25***

*Two units of blood were requested for a 71 year old female patient on-call for a non-urgent top-up transfusion, the reason for the anaemia not being recorded. The patient had been transfused 9 weeks earlier, when pre-transfusion testing revealed anti-K and a non-specific cold autoantibody, confirmed by a reference centre. The on-call BMS screened the plasma and crossmatched the units using a normal ionic-strength saline (NISS) tube technique (2:1 serum to 3% cell ratio), rather than their routine automated BioVue technique, because the presence of the cold antibody interfered with the reactions obtained using BioVue. However, they did not use an antibody identification panel. The two units were compatible and transfused and a third unit was requested. The reactions to the third unit were recorded as 'sticky', but this was assumed to have been caused by the cold antibody and the unit was transfused. The patient spiked a temperature during the transfusion of this 3$^{rd}$ unit, but no investigations were undertaken. Approximately 3 days later, a junior house officer queried the febrile episode as a transfusion reaction and another sample was taken and sent to the reference laboratory for investigation. Anti-Jk$^a$ was identified (in addition to anti-K and a non-specific cold antibody) in both the plasma and in an eluate made from the patient's cells. The pretransfusion sample was still available, and antibody screening and crossmatching results gave the same reactions as they had done during pretransfusion testing. An identification panel was still not performed. The Jk$^a$ antigen status of the transfused units was not recorded.*

These two cases were difficult to categorise, since it is likely that the Kidd antibodies would have been detectable pre-transfusion, had the appropriate tests been performed. Had this been the case they would have been categorised as IBCT. In addition, there is no strong evidence of haemolysis in either case, which makes them serological rather than haemolytic transfusion reactions. Furthermore, the latter case was only detected because of belated investigation of a febrile reaction during transfusion and isolated febrile reactions are not reportable to SHOT.

BCSH guidelines[19] recommend that an identification panel be performed every time a new sample from a patient with a positive antibody screen is tested. The pre-transfusion sample was only kept for a very short time in case 8 (probably less than 3 days), and in case 25, although still available, was not fully retested. It is particularly surprising that an identification panel was not performed in case 25, since the patient had been recently transfused, the crossmatch was weakly incompatible and the patient suffered a febrile reaction during the transfusion. BCSH guidelines[18] also recommend a serum:cell ratio of 4:1 for a NISS tube technique to obtain maximum sensitivity.

## COMMENTARY

- Kidd and/or c antibodies accounted for approximately 72% of all new antibodies, and were implicated in 83% of all patients in whom antibodies were found.
- 67% of patients developed more than one antibody. This highlights the importance of a thorough antibody investigation in patients who have already developed a red cell antibody as they are likely to be good responders.
- There were 3 cases (5, 8 and 25) where the patient had a known antibody, but an antibody identification panel was not performed prior to transfusion,; in one of these cases there was a history of recent transfusion. This reflects non-compliance with BCSH guidelines,[19] which state "If the patient is known to have a red cell alloantibody, the serum/plasma should be checked on each occasion of testing to exclude the development of further alloantibodies".
- There is no evidence that laboratories are not following guidelines with respect to timing of samples in relation to the transfusion.
- Retrospective testing of the pre-transfusion sample was only undertaken in 25% of cases. There is a suggestion that some laboratories are discarding the samples very soon after transfusion.
- It would appear that some laboratories are using screening cells that have not been validated for the IAT technology used. Data from UK NEQAS and the Welsh Assessment of Serological Proficiency Scheme (WASPS) exercises (99E7/00R9 and W10/03, respectively) has shown that a cell suspension lower than that recommended by the manufacturer can result in weak antibodies being missed.

## RECOMMENDATIONS

- Investigation of a suspected DHTR should include retesting of the pre-transfusion sample (where still available) by different or more sensitive techniques. This may involve referral to a reference centre.

  ***Action: Hospital blood transfusion laboratories***

- Automated systems or changes to IAT technology should be validated using a range of weak antibodies to ensure appropriate sensitivity.

  ***Action: Hospital blood transfusion laboratories***

- Consideration should be given to issuing antibody cards to all patients with clinically significant red cell antibodies. These should be accompanied by patient information leaflets, explaining the significance of the antibody and impressing that the card should be shown in the event of a hospital admission or being crossmatched for surgery.[19] Laboratories should be informed when patients carrying antibody cards are admitted.

  ***Action: The CMO's NBTC and its counterparts in Scotland, Wales, and Northern Ireland***

- There is a need for a review, co-ordinated by a professional national body, of how long specimens should be kept post-transfusion. The review needs to consider the relative risks and benefits of storing specimens beyond the time that they are suitable for use in further crossmatching tests.

  ***Action: BBTS and BCSH***