SPECIFICATION SPN220/3

Investigation and Clinical Management of Patients with a positive DAT with and without Haemolysis

This Specification replaces SPN220/2.1	**Copy Number**
	Effective **13/04/17**

Summary of Significant Changes

Hyperhaemolysis section removed, as this is now covered in SOP4743.
Paragraph on Paroxysmal nocturnal haemoglobinuria deleted.
Subtitle (page 5) changed to: “haemolytic anaemia with cold-reacting antibodies”.
Significant modifications to the section on CHAD have been made.
Updated references added.
One-stage DLT deleted, covered in SOP1150.
Author name changed.

Purpose

To ensure that a uniform RCI Clinical Policy for the investigation and clinical management of patients with a positive DAT with and without haemolysis is implemented throughout the NHSBT.

Definitions

Applicable Documents

SOP4742 – Investigating samples with a positive DAT

SOP1149 – Direct antiglobulin test

MPD1071 – Processing samples in RCI

ESD121 – BCSH Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories 2012

SOP1150 – Donath Landsteiner Test

SOP1151 – Drug induced haemolytic anaemia techniques

SOP4728 – ABO and D grouping in RCI

MPD1057 – RBC genotyping of samples by fluogene in RCI

Significance of a Positive DAT

The direct antiglobulin test (DAT) is generally used to determine whether red cells have been coated, in vivo, with immunoglobulin, complement, or both. A positive DAT, with or without shortened red cell survival, may result from:-

1. Autoantibodies to intrinsic red cell antigens, e.g. Autoimmune Haemolytic Anaemia

2. Alloantibodies in a recipient's circulation, reacting with antigens on recently transfused donor red cells which may or may not be associated with symptoms, signs and biochemical changes of an acute or delayed haemolytic transfusion reaction (AHTR or DHTR).

3. Alloantibodies in donor plasma / derivatives that react with recipients' red cells for example:
   a) Transfusion of Group O platelets with high titre anti- A,B to Group A/B recipient
   b) Intravenous Immunoglobulin (IVIg) may contain ABO antibodies / anti-D[1].

4. Alloantibodies in maternal circulation that cross the placenta and coat fetal red cells which may or may not be associated with symptoms, signs and biochemical changes of haemolytic disease of the fetus and newborn (HDFN).

5 Antibodies produced by passenger lymphocytes in transplanted organs.[2]
Passenger lymphocytes of donor origin produce antibodies directed against ABO or other antigens on the recipient's cells, causing a positive DAT.

6. Drug-induced haemolytic anaemia (e.g. Methyldopa-type, Penicillin type), usually IgG+/-C3d.

7. DAT positive with complement components only
   a) About 10% of patients with warm AIHA have red cells with a positive DAT due to C3 coating alone.[3] These AIHAs can be caused by warm-reacting IgM antibodies, or IgM+IgG antibodies.[4]
   b) Cold haemagglutinin disease / Paroxysmal Cold Haemoglobinuria.
   c) Drug Induced (Drug-dependent-Immune complex type).

8. Non-antibody immunoglobulins associated with red cells in patients with Hypergammaglobulinemia.[5] Multiple Myeloma or recipients of antilymphocyte globulin (ALG) or antithymocyte globulin (ATG).[6]

9. Elevated levels of IgG or complement have been noted on the red cells of patients with sickle cell disease, β-thalassemia, renal disease, autoimmune disorders (including systemic lupus erythematosus), AIDS, and other diseases with elevated serum globulin or blood urea nitrogen (BUN) levels.[1-7]

10. A positive DAT, without clinical manifestation of immune mediated red cell destruction, has been reported in 1:7000 healthy blood donors.[8]

    Interpretation of positive DATs must include the patient's history, clinical data, and the results of other laboratory tests.

Laboratory investigations of Patients with Positive DAT in warm antibody induced Autoimmune Haemolytic Anaemia (WAIHA).

WAIHA may occur as idiopathic or secondary to other diseases (chronic lymphocytic leukaemia, lymphoma, systemic lupus erythematosus, ulcerative colitis, myelodysplastic syndromes). MPD1071 describes how samples are processed in RCI.

1. **Determine ABO, Rh and Kell phenotype.** if the patient's RBC are heavily coated with antibodies (strong positive DAT): warm saline wash or chloroquine diphospate treatment can be used (SOP4728). Alternatively, Rh DCcEe, M N S s, K, k, Fy^a, Fy^b, Jk^a, Jk^b can be predicted with a high level of accuracy by molecular testing (MPD1057).

2. **Direct antiglobulin test:** BioRad cards are used on all investigations. This might detect IgA only AIHA, if using polyspecific anti-human globulin (AHG) or AIHA due to low affinity autoantibodies (SOP1149).

3. **Antibody identification:** by standard methods (indirect antiglobulin test) + auto control. Variation in strength of reactions may indicate the presence of both allo and autoantibodies. If DAT positive, there is no history of transfusion during the previous 3 months, no evidence of AIHA and no free antibody is present, no other tests are required.

4. **Autoadsorption technique:** if reactions obtained by IAT are uniformly weak with all panel cells it is unlikely that there is a significant underlying alloantibody and adsorptions are unnecessary. Autoadsorptions are performed using the patient's red cells treated with a reagent called ZZAP which contains dithiothreitol and cysteine-activated papain. Autoabsorption should not be performed if the patient has been transfused within the past 3 months to ensure allogeneic red cells are not present. Extended blood group serological or molecular typing will assist in the identification of alloantibodies.

5. **Differential adsorption technique:** the red cells employed in the differential adsorption technique must be carefully selected to include all clinically significant blood groups and generally performed only in the following circumstances:-

   - pan-reacting antibodies are detected and the patient has been transfused within the last three months
     or
   - if there are insufficient red cells (due to a low hct and urgent transfusion requirement) to perform auto-adsorption
     or
   - if auto-adsorption fails to remove (or weaken significantly) pan-reactive antibodies.

6. **Red cell eluate study to be undertaken:**

   a) If there is history of transfusion within 1 month with an increase in the transfusion requirement and/or a change in the serology (SOP4742).

   b) Evidence of haemolysis with no demonstrable allo or autoantibody in the serum. Eluate should be tested, even if DAT negative, if immune haemolysis is strongly suspected [9] (discuss with laboratory).

c) When adsorption studies are inconclusive, especially in patient transfused within the past 3 months.

d) Post ABO mismatched haemopoietic cell transplantation.

Examples of positive DAT and no elutable antibody are seen in cases with:

1) penicillin type induced haemolysis

2) patients with circulating immune complexes

3) patients with high paraprotein / gammaglobulins

If a specific antibody is eluted, the patient's red cells should be checked if possible for the antigens concerned. The presence of alloantibodies in an eluate suggests:

1) delayed transfusion reaction

2) haemolytic disease of the newborn

3) "mimicking" autoantibodies [10,11]

Policy for selection/provision of Cross-Matched Blood in WAIHA cases

The chief value of transfusion is to gain time for other therapies (e.g. Corticosteroids) to work. Blood must be transfused cautiously and for appropriate indications. Rapid haemolysis e.g. Hb <5g/dL or life threatening symptomatic anaemia (angina, respiratory distress, cerebral ischaemia, progressive cardiac decompensation associated with reticulocytopenia constitutes a medical emergency).

1. Blood selected for patients with clinically significant allo-antibodies must lack the corresponding antigens.

2. Select K negative blood of the same ABO group and of Rh type compatible with that of the patient eg:[11]

Patient Rh phenotype	**Select**
rr	rr
R_1r	E Neg (R_1R_1 rr or R_1r)
R_1R_1	R_1R_1
R_1R_2	Any Rh phenotype
R_2r	C Neg (R_2R_2 rr or R_2r)
R_2R_2	R_2R_2

This will avoid stimulating the production of Rh/K alloantibodies and will prevent transfusion reactions from such antibodies already present but masked by the autoantibodies.

It is preferable to select blood using this principle even if the patient's autoantibody shows specificity within the Rh system.[12,13] The only exception would be if there was active ongoing haemolysis with clear-cut single Rh specificity (e.g. anti-e), in which case it might be considered that the advantage of possible increased red cell survival would outweigh the potential for stimulating alloantibody production.[14]

3. Crossmatched units that are serologically incompatible but considered appropriate for the patient should be issued as **"SUITABLE FOR".**

4. If transfusion of ABO Rh, K compatible red cells does not result in expected rise in Hb, extend the phenotype (or predicted phenotype if molecular methods are being employed) to include Ss, Kidd and Duffy and contact the NHSBT consultant to consider/discuss with hospital clinician the option of further transfusion, with IVIg / steroid cover.[15]

5. In the situation where the need for blood is urgent and there is insufficient time to perform autoadsorption/differential adsorption test, select ABO, Rh phenotype compatible, K negative blood. These are the most common specificities of alloantibodies found in AIHA.[16] In extreme circumstances, transfusion under IVIg / steroid cover should be considered.[15]

   The decision to transfuse these units in life threatening emergency requires adequate consultation between hospital clinician and NHSBT consultant.

   Comment. No critically ill patient with autoimmune haemolytic anaemia should die through lack of blood.

6. Once alloantibodies are excluded after adsorption studies at a reference laboratory, the hospital may select the same ABO, Rh and K phenotype units and use an immediate spin cross match technique for compatibility testing prior to issue of blood.[16] The laboratory results and the advice provided to the hospital by the reference laboratory should be well documented.

   A sample obtained within 72 hours of the previous transfusion is acceptable for serological investigation for AIHA to exclude additional alloantibodies and to select suitable units.[17,18]

DAT negative AIHA

DAT-negative AIHA has been reported in about 6% of all warm AIHA. There are several possible mechanisms: a) low affinity IgG antibodies which may dissociate from the red cells during washing for DAT, b) low number of IgG molecules on the red cells, undetectable by standard DAT but demonstrable by more sensitive techniques such as solid phase enzyme-linked anti-globulin test, flow-cytometry, c) AIHA caused by non-IgG (e.g. IgA) antibodies.[19] Sachs et al reported that 4.6% DAT-negative patients suspected to have immune haemolysis had red cell auto- or allo-antibodies detectable by elution.[9]

Haemolytic anaemias due to cold-reacting autoantibodies

Cold agglutinin disease

The acute transient form of CAD is usually associated with mycoplasma pneumoniae infection or infectious mononucleosis, a chronic form usually seen in the elderly, can be idiopathic or

associated with lymphoma, Waldenstrom's macroglobulinaemia and chornic lymphocytic leukaemia.[20]

DAT is typically positive for C3d, and negative (or, occasionally, weakly positive) for IgG.[20]

Cold autoantibody is usually IgM, but examples of cold reacting IgA autoantibodies (e.g. anti-Pr) have also been reported. [21] IgM Antibodies usually have anti-I specificity (anti-i in the post-infectious form of CHAD), but autoantibody specificity is not diagnostic of the underlying condition of CHAD and clinical relevance depends on the thermal range of the antibody.

Testing for clinically significant cold agglutinins

A practical screening procedure is to incubate the patient's serum with normal red cells suspended in saline, at room temperature for 30-60 minutes. Negative test practically rules out clinically significant antibody. If the screening test is positive, further tests are necessary. To distinguish between clinically significant cold agglutinins, and those that are not antibody titration and/or its thermal amplitude (highest temperature at which the antibody binds to red cell) can be tested. Clinically significant cold agglutinins usually have a 1:500 – 1:1000 titre at +4^{o}C. Cold Haemagglutinins of thermal amplitude >30^{o}C (detected by tube NISS direct agglutination technique) are considered to be a reasonable indicator of clinical significance. [22]

Laboratory testing problems that cold agglutinins may cause

Samples for cold agglutinin titre/thermal amplitude should be transported to laboratory at 37^{o}C. As this is often not practical, EDTA-anticoagulated samples should be re-warmed at 37^{o}C for 1 hour before testing. [22]

The thermal range of the antibody is more important than the agglutination titre for clinical purposes. (ref a and b).

Cold autoantibody may agglutinate all cells within the reverse ABO group leading to discrepant ABO typing. If these findings are correctly interpreted as a non-specific cold reaction and the tests are repeated at 37^{o}C the reactions will disappear.

Potent cold reactive autoantibodies reacting by IAT at 37^{o}C may mask the underlying alloantibodies active at 37^{o}C. The patient's plasma can be treated with DTT (DTT is used to treat serum to prevent IgM antibodies acting as agglutinins, by disruption of J chains) and post DTT treated sample should be tested at 37^{o}C by IAT technique using monospecific anti IgG reagent. Alternatively, alloadsorptions at 4^{o}C may be performed. Donor red cells should be transfused through a blood warmer. [24] Acute forms of CHAD with infections often have abrupt but short clinical course. The chronic form may require therapy. Corticosteroids, IVIg and splenectomy are often, but not always, ineffective in CHAD. Rituximab has been tried as 1st line and Fludarabine +/- Rituximab as 2nd line. [25]

Positive DAT by complement/IgG and IgM (mixed-type AIHA)

In approximately 7% of cases with AIHA, both warm IgG autoantibody and cold IgM autoantibody are simultaneously detected in the patient's serum. These cases are referred to as mixed-type AIHA. The diagnosis of mixed-type AIHA requires thorough serologic studies. DAT is positive

with both IgG and C3d. Demonstration of both warm IgG autoantibody and cold IgM autoantibody reacting at high thermal amplitude (>30C) is essential for diagnosis.[23]

Mixed-type AIHA can be further classified into idiopathic or secondary, the latter often being associated with systemic lupus erythematosus or lymphoma, or occurring after haemopoietic cell transplantation.

PAROXYSMAL COLD HAEMOGLOBINURIA (PCH)

Pathophysiology

PCH is caused by an IgG biphasic autoantibody, usually with anti-P specificity and commonly seen as acute condition in children. This antibody binds to the RBC in the cold, but activates complement and causes haemolysis at 37ºC. Cases may be idiopathic or can be secondary to acute viral infection in children. Chronic PCH is a very rare condition affecting adults and ***may be*** associated with congenital or tertiary syphilis or lymphoma.

Clinical features

The most common clinical features include haemoglobinuria, jaundice and pallor preceded by viral infection.[26] Acute, transient PCH is characterised by a sudden onset of acute intravascular haemolysis, most frequently seen in young children, following a viral illness. It has been reported following measles, mumps and chicken pox, but in many cases, no specific viral infection can be identified.[27,28] Relative reticulocytopenia has been noted in some patients with PCH at the time of presentation.[27] Parvovirus induced PCH has been reported in association with reticulocytopenia.[29] Erythrocyte P antigen is a viral receptor for Parvovirus infection of red cell precursors. Awareness and correct diagnosis of PCH is important, as haemolysis is transient in nature and can be halted by keeping the patient warm.

Serological investigation and pretransfusion testing

PCH is caused by an IgG autoantibody, and the biphasic nature of the antibody has been demonstrated by either the Direct or Indirect Donath-Landsteiner Test (DLT).

Direct Antiglobulin Test

The DAT is positive for complement only. The biphasic IgG molecules will already have dissociated *in vivo.*

Antibody Screening

BCSH guidelines (ESD121) recommend the use of IAT only at 37ºC for pre-transfusion antibody screening, without an additional screening technique, since IAT 37ºC methods used alone can detect the majority of clinically significant alloantibodies.

Negative antibody screen by the standard IAT at 37°C is a common finding in a suspected case of PCH because of the low thermal amplitude nature of the autoantibody. If the antibody investigation is carried out at a lower temperature (15ºC), in suspected PCH cases, either with enzymes or treated red cells in a direct agglutination technique or by IAT, in suspected PCH cases, pan reactive cold antibodies may be detected as the majority of autoantibodies show anti-P specificity with thermal amplitude range up to 15-24°C. Usually the antibody titre is low (less than 64) even when investigated at 4°C.[30] *Anti-P specificity can be confirmed at the reference laboratory.*

Note: A low serum complement level and/or a low antibody level can result in a negative outcome to the tests. Negative results, therefore, do not rule out PCH. *Donath-Landsteiner Test (DLT) is the confirmatory test for PCH.*

Donath-Landsteiner Test (DLT)

The nature of the biphasic antibody can be demonstrated by DLT. The DLT can be performed as either a Direct or Indirect procedure. False results are not uncommon in the Direct DLT for one or more reasons stated below:[31]

1) Low antibody level
2) Low complement level (complement is consumed during the haemolytic process)
3) Due to the presence of C3dg on the patient's red cells (resistant to complement-mediated haemolysis).

Two-stage indirect DLT (see SOP1150)

Globoside is the most abundant red cell membrane glycolipid, and is present in the serum of all P+ individuals to variable amounts. Addition of ABO compatible fresh serum therefore, as a source of complement, could result in cross-reaction with anti-P and can lead to a false negative indirect DLT.[32] This can be overcome by the two-stage indirect DLT.

In the two-stage indirect DLT, ABO compatible, fresh serum is only added to the red cell-serum suspension following the initial 1 hour incubation at 0°C. This prevents antibody inhibition during the cold phase, and allows maximum sensitisation of the red cells.[32]

Note*: Indirect DLT sample handling (collection and separation of serum from whole blood) should be strictly at 37°C to prevent autoadsorption which may cause false negative result. False negative is common with one-stage indirect DLT and should proceed to* two-stage indirect DLT if indicated*. In some cases the Donath-Landsteiner antibody is only detectable using enzyme (papain) treated cells in the two-stage indirect Donath-Landsteiner test.*

Transfusion Support

Awareness, a high index of suspicion of PCH and a correct diagnosis are important, as keeping the patient warm at an ambient temperature of 30°C will assist recovery. In rare severe cases, however, when blood transfusion may be required, ABO, Rh and K compatible units should be selected for the crossmatch, and the use of blood warmers may provide some protection against a

haemolytic reaction.[24] P- (pp or Pk) blood is not readily available, but should be considered if there is no response to transfusion of P+ cross-match compatible blood. In such a case, the reference centre should be contacted to explore the availability of P- units at the National Frozen Blood Bank. Steroids may be helpful.

Drug induced Immune Haemolytic Anaemia

It may be evident from the medical history and serological investigation that drug-induced immune haemolytic anaemia should be considered. In cases where drug-induced immune haemolysis is suspected, please refer the case to Sheffield RCI laboratory (see list of drugs implicated in immune haemolytic anaemia).

NB: The presence of free autoantibodies in the serum may interfere with investigations for drug induced haemolysis. Please check with the Sheffield Blood Centre before agreeing to these investigations being undertaken.

Mechanisms of drug-induced immune haemolytic anaemia[33-36]

1. Drug Adsorption: Antibodies directed against certain drugs that bind to red cell membranes, (e.g. high dose IV penicillin/some cephalosporins): Positive DAT due to IgG coating. Antibody eluted from the red cells reacts with penicillin-coated red cells but not with uncoated red cells.

2. Non-specific adsorption: First generation cephalosporins alter the red cell membrane, which induces non-specific adsorption of proteins / immunoglobulins. Only positive DAT with no haemolysis.

3. Drug-dependent Immune-Complex Mechanism: Complement components due to formation of drug/anti-drug immune complexes (e.g. Quinidine / Phenacetin).

4. Drug-Independent Autoantibody Production: Serologically indistinguishable from idiopathic WAIHA. Red cells are coated with IgG, eluate/serum reacts with all cells tested. (e.g. L dopa, Procainamide, Mefenamic Acid, Fludarabine).

   Second and third generation cephalosporins are increasingly associated with severe AIHA. Some of these drugs may induce drug independent autoantibodies and others may also induce drug dependant antibodies through drug adsorption mechanism.

Drugs Implicated in Immune Haemolytic Anaemia

Acetaminophene	2
Acetylsalicylic acid	2
Ajmaline	2
Aminophyrine	2
Azapropazone	1
Buthiazide	2
Carbimazole	1
Carbromal	1,2
Cephalosporins	1
Cefotetan/Cefotaxime	
Cefoxitin/Ceftazidime	
Ceftriaxone/Cephalotin	
Cephalexin//Cephazolin	
Cephamanodole/Cephaloridine	
Latamoxef	
Chlorpromazine	2,3
Chlorpropamide	2
Cianidanol	2
Cimetidine	3
Cianidanol	2
Cimetidine	3
Cisplatin	1
catergen	1,2
Diclofenac	2
Dipyrone	1,2
DPT(diptheria,pertussis,tetanus)	3
Triple Vaccine	
Erythromycine	2
Fenprofen	2
Fludarabine	3
Glafenine	2
Glibenclamide	1
Hydralizine	2
Hydrochlorothiazide	2
Ibuprofen	3
Insulin	2
Isoniazid	1,2
Levodopa	3
Mefenamic acid	3
Mephalan	2
Methadone*	2
Methysergide	2,3
Nalidixic acid	2
Naproxen	3
Nomifensine	2,3
Penicillins	1,2
Amoxycillin/Ampicillin	
Flucloxacillin/Methicillin	
Nafcillin/Oxacillin	
Ticarcillin	
Unasyn (ampicillin ticarcillin sodium/sulbactum)	
Penacetin	2
Plethoryl	3
Probenecid	2
Procainamide	3
Quinine	1,2
Rifampicin	2
Stibophen	2
Streptomycin	1
Sulphonamides	1,2,3
Sulindac	2
Teniposide	2
Tetracycline	1
Thiopental	2
Tolbutamide	2
Tolmetin	2,3
Triamterene	2
TMA-Plasticiser	2
Valproic-acid	*
Zompirac	2

1. Documented or possible drug adsorption mechanism
2. Documented or possible immune-complex mechanism
3. Documented or possible autoimmune mechanism.

* Reported to cause a positive DAT without overt haemolysis

Ref: Clinical Practice of Transfusion Medicine, 3rd Ed., Churchill Livingstone
L.D.Petz, Drug Induced Immune Haemolytic Anaemia (Pg 494-496)

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Reviewed and approved by RCI colleagues: January 2017