UnityPoint Health Pekin
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Effective Date: 11/05/18
Date Reviewed/ Date Revised: 11/05/18

DIRECT ANTIGLOBULIN TEST (DAT)

(NOT CORD BLOOD)

Test Code: DATSCN

I. PRINCIPLE

UPH-Pekin laboratory technicians will utilize this procedure for the Direct Antiglobulin Test on all specimens *except cord bloods*.

The test principle is a hemagglutination test. Anti-Human Globulin Anti-IgG, -C3d; polyspecific acts as a link between the antibody and/or compliment coating of neighboring red blood cells and induces agglutination. Uncoated red blood cells will not agglutinate. Anti-Human Globulin Anti-IgG acts as a link between the antibody coating of neighboring red blood cells and induces agglutination. Uncoated red blood cells will not agglutinate.

II. CLINICAL SIGNIFICANCE

The Direct Antiglobulin Test (DAT) is used to determine if red cells are coated in vivo with immunoglobulin (IgG), complement, or both. The Direct Antiglobulin Test is useful for:

A. Diagnosis of autoimmune hemolytic anemia.
B. Investigation of drug induced red cell sensitization.
C. Investigation of transfusion reaction.

III. SPECIMEN

A. EDTA or citrate anticoagulated whole blood samples must be used for the DAT.
B. Specimens should be tested as soon as possible after collection.
C. If testing is delayed, EDTA specimens should be stored at 2 to 8°C, citrated specimens (donor segments) at 1 to 6°C.
D. Plasma may be separated from red cells and frozen.
E. Stored samples should be allowed to reach room temperature prior to testing.
F. Use of samples older than ten days should be avoided unless there is no other alternative since antibody reactivity has been shown to decrease in older samples.
G. Blood specimens exhibiting gross hemolysis or contamination should not be used.

IV. REAGENT

A. 0.9% normal saline (buffered)
B. Anti-Human Globulin (Anti-IgG, -C3d; Polyspecific)
C. IgG coated red cells (Coombscell-E)
D. Anti-Human Globulin (Anti-IgG; Monospecific)
E. Biotestcell 1, 2, or 3

V. INSTRUMENTATION/EQUIPMENT

A. Dade Immufugell-Centrifuge
B. Helmer UltraCWII Cell Washer
C. 12x75mm test tubes
D. Plastic disposable pipettes
E. Agglutination viewer

VI. QUALITY CONTROL

A. The reactivity of all reagents should be confirmed by testing with known positive and negative red blood cells on each day of use.
B. To confirm the reactivity or specificity of Anti-Human Globulin Anti-IgG, -C3d; Polyspecific and Anti-Human Globulin Anti-IgG test each with IgG coated (Coombscell-E) and non-coated (Biotestcell 1, 2, or 3) red blood cells.

VII. PROCEDURE:

A. Polyspecific Coombs QC: Label one 12 x 75 mm test tube as a positive control and one as a negative control. Add two drops of polyspecific Anti-Human Globulin to each tube. Add one drop of Coombscell-E to the positive tube and one drop of Biotestcell 1, 2, or 3 to the negative tube. Centrifuge for 20 seconds, or for the optimum calibrated spin time, at 1000 RCF. Examine for agglutination. Record results on Reagent Quality Control Worksheet.
B. Patient: Place one drop of a 3-5% saline suspension of red cells in 12 x 75 mm test tube labeled with first and last initial of patient being tested. (Lengthen the minimum letters to differentiate patients with the same initials, if necessary.)
C. Wash three times with normal saline either by hand or in automatic cell washer (If washing by hand, decant last wash completely).
D. Add two drops of polyspecific Anti-Human Globulin and mix.
E. Centrifuge for 20 seconds, or for the optimum calibrated spin time, at 1000 RCF.
F. Examine for agglutination both macro and microscopically. Negative reactions may also be examined with an agglutination viewer.
G. Add one drop of Coombscell-E control to all tubes with negative results.
H. Centrifuge for 20 seconds, or for the optimum calibrated spin time, read and record results. If the cells are now agglutinated, the negative result is valid.

I. If results are positive using polyspecific Anti-Human Globulin, repeat procedure using monospecific IgG Anti-Human Globulin (the QC is done every morning with Immucor corQC kit).

VIII. REPORTING RESULTS

A. Positive – cell agglutinated or hemolyzed.
B. Negative – no agglutination or hemolysis.
C. Enter reactions and interpretation as POS or NEG in Sun Quest (Blood Order Processing).
   1. Report the results of the IgG- Anti-Human Globulin DAT by adding on DAT/IgG (Q key) to the Polyspecific (Broad Spectrum) DAT in Sun Quest (BOP). Record your reactions and interpretations.
   2. If DAT is positive and the patient has been transfused in the last three months, or the doctor wants further testing, send to UPH-Methodist Lab for elution testing (test code-ELUT).

IX. PROCEDURAL NOTES/PROBLEM-SOLVING TIP

A. Low frequency antigens may not always be present on reagent red blood cells and a double dose of antigen may be required to detect very weakly reacting antibodies. Therefore, negative reactions with the screening red blood cells do not always indicate the absence of unexpected antibodies.
B. Insufficient or inappropriate washing can lead to false negative or false positive reactions. Small amounts of residual patient sera/plasma can neutralize the Anti-Human Globulin Anti-IgG, -C3d; Polyspecific.
C. Some conditions that may cause false positive results are:
   1. Contamination of sample or reagents
   2. Autoantibodies
   3. Improper storage or preparation of red blood cells
   4. Antibodies to antibiotics or other reagents
   5. Cold antibodies
D. Positive reactions may be seen from individuals who have received Rh Immunoglobulin.
E. Negative reactions will be obtained if the sample contains antibodies present in concentrations too low to be detected by the test method employed. No test method is capable of detecting all red cell antibodies.
F. The performance characteristics with frozen/deglycerolized and enzyme treated red blood cells have not been established.

Some drugs associated with immune hemolysis and/or positive DATs due to drug-induced antibodies:

DRUG	THERAPEUTIC CATEGORY
Acetaminophen	Analgesic, antipyretic
Aminopyrine	Analgesic, Antipyretic
Amphotericin B	Antifungal, antibiotic
Ampicillin	Antibacterial
Antazoline	Antihistamine
Apazone (azapropazone)	Anti-inflammatory analgesic
Buthiazide (butizide)	Diuretic, antihypertensive
Carbenicillin	Antibacterial
Carbimazole	Thyroid inhibitor
Carboplatin	Antineoplastic
Carbromal	Sedative; hypnotic
Catergen	Diarrheal astringent, treatment of hepatic disease
Cephalosporins **First Generation:** Cefadroxil (Duricef) Cefazolin (Ancef, Kefzol) Cephalexin (Keflex) Cephalothin (Keflin) Cephapirin (Cefadyl) Cephradine (Anspor) **Second Generation** Cefaclor (Ceclor) Cefamandole (Mandol) Cefmetazole (Zefazone) Cefonicid (Monocid) Cefotetan (Cefotan) Cefoxitin (Mefoxin) Cefuroxime (Zinacef, Kefurox, Ceftin) Cefuroxime axetil (Ceftin) **Third Generation** Cefixime (Suprax) Cefoperazone (Cefobid) Cefotaxime (Claforan) Ceftazidime (Fortaz, Ceptaz, Pentacef, Tazicef, Tazidime) Ceftizoxime (Ceftizox) Ceftriaxone (Rocephin)	Antibacterials

Fourth Generation Cefepime (Maxipime)	
Chaparral	
Chlorpropamide	Antidiabetic
Chlorpromazine	Antipsychotic
Cisplatin	Antineoplastic
DRUG	**THERAPEUTIC CATEGORY**
Cladribine (chlorodeoxyadenosine)	Antineoplastic
Clavulanate potassium	β-lactamase inhibitor/antibacterial
Cyanidanol	
Cyclofenil	Gonad-stimulating principle
Cyclosporine	Immunosuppressive
Diclofenac	Anti-inflammatory
Diethylstilbestrol	Estrogen
Diglycoaldehyde	Antineoplastic
Dipyrone	Analgesic, antipyretic
Elliptinium acetate	Antineoplastic
Erythromycin	Antibacterial
Etodolac	Anti-inflammatory analgesic
Fenfluramine	Anorexic
Fenoprofen	Anti-inflammatory, analgesic
Fludarabine	Antineoplastic
Fluorescein	Injectable dye
Fluorouracil	Antineoplastic
Glafenine	Analgesic
Hydralazine	Antihypertensive
Hydrochlorothiazide	Diuretic
Ibuprofen	Anti-inflammatory
Insulin	Antidiabetic
Interferon	Antineoplastic, antiviral
Isoniazid	Antibacterial, tuberculostatic
Levodopa	Antiparkinsonian, anticholinergic
Mefenamic acid	Anti-inflammatory
Mefloquine	Antimalarial
Melphalan	Antineoplastic
6-Mercaptopurine	Antineoplastic
Methadone	Narcotic analgesic
Methicillin	Antibacterial
Methotrexate	Antineoplastic, antimetabolite

Methyldopa	Antihypertensive
Moxalactam (latamoxef)	Antibacterial
Nafcillin	Antibacterial
Nomifensine	Antidepressant
p-Aminosalicylic acid	Antitubercular
Penicillin G	Antibacterial
Phenacetin	Analgesic, antipyretic
Piperacillin	Antibacterial
Podophyllotoxin	Antineoplastic, cathartic
Probenecid	Uricosuric
Procainamide	Cardiac depressant, antiarrhythmic
Propyphenazone	Analgesic, antipyretic, anti-inflammatory
Pyramidon	Analgesic, antipyretic
Quinidine	Cardiac depressant, antiarrhythmic
Quinine	Antimalarial
DRUG	**THERAPEUTIC CATEGORY**
Ranitidine	Antagonist (to histamine H2 receptors)
Rifampin (rifampicin)	Antibacterial, antitubercular
Sodium pentothal	Anesthetic
Stibophen	Antischistosomal
Streptomycin	Antibacterial, tuberculostatic
Sulbactam sodium	B-lactamase inhibitor/antibacterial
Sulfonamides	Antibiotics
Sulfonylurea derivatives	Antidiabetic
Sulindac	Anti-inflammatory
Suprofen	Anti-inflammatory, analgesic
Suramin	Antitrypanosomal, antifilarial
Temafloxacin	Antibacterial
Teniposide	Antineoplastic
Tetracycline	Antibacterial, antirickettsial, antiamebic
Thiopental	Anesthetic
Tolbutamide	Antidiabetic
Tolmetin	Anti-inflammatory
Triamterene	Diuretic
Trimellitic anhydride	Used in preparation of dyes, resins, etc
Zomepirac	Analgesic, anti-inflammatory

X. REFERENCES

A. Anti-Human Globulin, Anti-IgG, -C3d; Polyspecific (Rabbit/Murine Monoclonal), Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany, 187824/13, Rev. 08/2014..
B. Anti-Human Globulin, Anti-IgG (Rabbit), Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany, 187822/13, Rev. 08/2014.
C. AABB, Technical Manual, 19th Edition, American Association of Blood Banks, 2017, Bethesda, MD 20817.

POLICY CREATION :		***Date***
Author:	***Sharrol Brisbin, MT (ASCP)***	***11/18/2004***
Medical Director:	***Kathryn Kramer, MD***	***11/18/2004***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
11-5-18	Kathryn O. Kramer MD	
SECTION MEDICAL DIRECTOR		

REVISION HISTORY (began tracking 2011)			
Rev	Description of Change	Author	Effective Date

Lead	**Date**	**Coordinator/ Manager**	**Date**	**Medical Director**	**Date**
	11-5-18				