**Direct Antiglobulin Testing and Mixed Fields**

**A Self-Study Module**

This module is designed as a self-teaching opportunity. You work at your own speed, ask questions as you need to, and complete the exercise with increased knowledge of DATs and Mixed Field findings in Transfusion Service.

**Let’s Get Started with Definitions and Applications**



Go to Lab Test Online to read a general description of DAT and its application to HDFN, Transfusion Reactions, autoimmune hemolytic anemias, etc.

* <http://labtestsonline.org/understanding/analytes/antiglobulin-direct/tab/test>

**Using the information in Lab Test Online, answer the following questions:**

* List 3 diseases that may have a POS DAT
* List two other applications for DAT

**Now that you have defined DAT and how it is applied in the laboratory, let’s look at specifics about the DAT itself.**

Why can’t you detect a POS DAT?

* IgG below the threshold of detection, <150 molecules / RBC
* IgA or IgM not detected by conventional reagents
* Low affinity IgG washed off cells in conventional 4 x wash (try cold LIS wash)
* Technical Error
	+ Used a cell suspension that was not fresh – antibodies are eluting off into the suspension medium
	+ Cell suspension is too heavy or too light
	+ Delayed reading tubes after cell washer finished – antibodies are eluting off into the suspension medium
	+ Delayed reading tubes after anti-IgG/Polyspecific were added and spun – anti-IgG reacts quickly but also disperses quickly. Snapshot moment to catch the action before it is gone!

**Write a short scenario describing a laboratory situation that might lead to technical error. *This is fiction so have fun with it.***

**Is a DAT an Auto Control? Is an Auto Control a DAT?**

They are separate tests that are not interchangeable.

Every cell taken to the AHG phase is having a DAT performed on it. *If the screening cells or panel cells had a POS DAT, we would be demanding new cells!!! If an antigen negative donor unit is unexpectedly incompatible at AHG, a DAT should be performed on the donor unit before assuming the only explanation is that the patient has a new antibody.*

So, that means the **Auto Control is a DAT**. But **it is also more than a DAT** because it is also an opportunity for antibody to increase its binding concentration during incubation, resulting in a POS Auto Control that may be stronger in reactivity than the DAT.

**A DAT is therefore NOT an Auto Control.** Their results are interconnected and should be evaluated individually **and** as part of the antibody identification process.

**OK, we have discussed the test, its applications, and its shortcomings. Now let’s look at when the DAT is POS.**

**How do we grade DAT testing?** *(For SQ)*

**If the DAT is Mixed Field, how would you document your findings in SQ?**

**What reagents are used in DAT Testing?**

**When is a control required and what reagent is used in the control?**

**Are these results valid and reportable?**

*If invalid, write your course of action to produce valid results below.*

***Scenario 1***

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **Immediate Spin** | **Check Cells** | **Plan of Action** |
| **Polyspecific** | 3 | ND |  |
| **Anti-IgG** | 0 | 3 |
| **Anti-C3** | 0 | 2 |
| **Control** | 0 | ND |

*These results should not be resulted to ORCA until an investigation of the discrepancy between Polyspecific 3+ and anti-IgG/anti-C3 = NEG gives a satisfactory answer.*

***Scenario 2***

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **Immediate Spin** | **Check Cells** | **Interpretation** |
| **Polyspecific** | MF  |  |  |
| **Anti-IgG** | MF |  |  |
| **Anti-C3** | MF |  |  |
| **Control** | 0 | ND |  |

*These results should not be resulted to ORCA until an investigation of the mixed field discrepancy gives a satisfactory answer. An eluate may be indicated.*

***Scenario 3***

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **Immediate Spin** | **Check Cells** | **Interpretation** |
| **Polyspecific** | 2 |  |  |
| **Control** | 1 |  |  |

*These results should not be resulted to ORCA until an investigation of the positive control gives a valid test result.*

***Scenario 4***

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **Immediate Spin** | **Check Cells** | **Interpretation** |
| **Polyspecific** | 3 | ND |  |
| **Anti-IgG** | 2 | ND |  |
| **Anti-C3** | 0 | 2 |  |
| **Control** | 0 | ND |  |

*These results can be resulted to ORCA. An eluate may be indicated.*

What do these cells have that the free cells don’t have?

Antigen? Antibody?

**Mixed Field**

Why didn’t they pick me? I wanna join the group!

What has she got that I haven’t got?

**Mixed Field**

Unexpected findings in laboratory testing, once identified, must be resolved before releasing results or making blood component selection. Mixed field findings may have many explanations and require differing levels of investigation and response by the Transfusion Service.

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| **Definition** |
| **Mixed field is defined as agglutinates surrounded by free red cells*** **Agglutinates may be of varying sizes from weak+ to 4+.**
* **Volume of free red cells varies also.**
* **The agglutinated cells have an epitope in common that is detected by the antibody source.**
* **The unagglutinated cells lack the epitope that is detected by the antibody source.**
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**Mixed Field** (continued)

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| **So, Where Would We See Mixed Field?** |
| **Cell Testing** |
| **In General*** White cells, tissue cells, and contamination that doesn’t agglutinate will give the appearance of mixed field.
* Try washing the cell suspension one time. That usually removes the “junk”.
* Check your tubes for trash (cardboard dust, etc.)
 |
| **In ABO Typing Discrepancies*** Recent Transfusion of non-type specific RBCs
	+ Includes donor RBC or Granulocyte units, intrauterine transfusion, exchange transfusion
* Transplantation of non-type specific donor hematopoietic cells
* Fetal-maternal hemorrhage in either maternal or fetal/infant samples
* Tetragametic Chimerism or Dispermy Mosaicism are rare situations where 2 or more populations of cells are present in the patient/donor
* A3 and B3 Subgroups are defined by characteristic mixed field appearance; if you remove the agglutinates and add more antibody, the same mixed field appearance will recur again and again.
 |
| **In Rh Typing Discrepancies*** Recent Transfusion of non-Rh type specific RBCs
	+ Includes donor units, intrauterine transfusion, exchange transfusion
* Transplantation of non-Rh type specific donor hematopoietic cells
* Fetal-maternal hemorrhage in either maternal or fetal/infant samples
* Tetragametic Chimerism or Dispermy Mosaicism are rare situations where 2 or more populations of cells are present in the patient/donor
 |
| **In Antigen Typing Patients (Phenotyping) for Additional Antigens** (i.e., anti-E, anti-K, etc.)* Recent Transfusion of non-antigen specific RBCs
	+ Includes donor units, intrauterine transfusion, exchange transfusion
* Transplantation of non-antigen type specific donor hematopoietic cells
* Fetal-maternal hemorrhage in either maternal or fetal/infant samples
* Tetragametic Chimerism or Dispermy Mosaicism
 |
| **In Direct Antiglobulin Testing*** Recent Transfusion of donor cells which have an antigen directed against an antibody in the recipient’s circulation.
* Transplantation
* Fetal-maternal hemorrhage
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***Note:*** *Platelets, by definition, must have <2 mL of RBCs or a crossmatch is required. Therefore, it is unlikely that platelet transfusion would be responsible for mixed field.*

**Mixed Field** (continued)

|  |
| --- |
| **So, Where Would We See Mixed Field?** |
| **Plasma Testing** |
| **In Antibody Detection and Identification** * Anti-Sda
* Anti- Lua
 |

|  |
| --- |
| **Check Cells** |
| **Check Cells used to Validate Negative Antiglobulin Phase of Testing*** Always mixed field
* Tube contains test cells which did not agglutinate with antihuman globulin
* Check cells agglutinate validating the negative finding
* Mixed field is ignored in recording and reporting as it is an expected MF and our interest is in a POS reaction; we already recorded the NEG reaction
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**Scenario 1:** Pregnant patient has high titer anti-D. Frequent intra-uterine transfusions were performed following detection on ultrasound of anemia. PUBS sample tested APOS with DAT 4+. She delivered one month ago.

Baby sample is submitted for DAT, ABO/Rh, and Antibody Screen by the Pediatric Clinic. Describe *in general terms* expected results for this 1 month old and a possible explanation.

|  |  |  |
| --- | --- | --- |
| **Test** | **Expected Results** | **Why?** |
| **ABO forward** |  |  |
| **Rh typing** |  |  |
| **Antibody Screen** |  |  |
| **DAT** |  |  |

**Scenario 2: using the TEST environment, order a DAT. Enter MF results for Polyspecific and document. Bring a printout to the Technical Meeting.**

**Mixed Field** (continued)

**Frequently Asked Questions about Mixed Fields**

**Q: When would mixed field be unexpected or unexplainable?**

A: Once we start seeing mixed field agglutination it seems to be everywhere.

One place we shouldn’t see it (with rare exception) is when the cells are from a single source like panel cells or antibody screening cells (not from a pooled source). The rare exceptions are anti-Sda and anti-Lua which may demonstrate a mixed field agglutination appearance.

**Q: How far do I have to go to explain a mixed field finding before proceeding with selecting and issuing type specific blood products?**

A: Beyond a reasonable doubt.

**In ABD testing**, there are alternatives to selecting blood products based on universal donor type.

With **anti-Sda and anti-Lua,** both require AHG crossmatch with issuing AHG XM compatible RBCs

**In DATs**, the identification of the bound antibody is essential to selecting appropriate antigen negative donor units. There is a spot on the Urgent Blood Product Release has an applicable situation to mark: *Recipient demonstrates positive Direct Antiglobulin (Coombs) test. Investigation is incomplete. Blood appears serologically compatible.*

**Q: If the DAT is POS (Mixed Field or otherwise), why do I have to do an eluate if I already did an antibody ID on the patient plasma?**

A: The bound antibody may not be the same as the plasma antibody. When antibody production begins, it attaches first to antigen present in the body. It is only after all the antigen sites are full that excess antibody appears in the circulation and is detectable in the plasma.

**Q: How do I differentiate Mixed Field from WEAK?**

A: Sometimes you can’t. Mixed field should always be a consideration until proven impossible and/or improbable. For example, a weakly positive antibody screening cells doesn’t say MIXED FIELD to me as anti-Sda and anti-Lua are uncommon. I’m thinking weak reactivity so get out the PEG or other enhancement.

In a DAT, if I see a weak reaction, I immediately assume mixed field is probable so I get the transfusion history to rule out mixed field and a serologic transfusion reaction.

**Q: What things can I do to insure I recognize mixed field agglutination?**

**A:** Identify the tests mixed field may occur in and important to note. Look for it using the dissecting scope, if in doubt. Practice detection by making mixed field samples and testing them – how weak can you see?

**Q: What are the ramifications of missing a weak (and possibly mixed field) POS DAT?**

A: Unknown. Many incidences of cell destruction are subclinical. Extensive investigation of these patients probably would not improve outcome. Some cases are clinically significant. Other laboratory tests, clinical findings, and thorough investigation should provide an accurate diagnosis and treatment plan.