Blood Bank---
Reviewing the basics

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Objectives:

- Define the most commonly performed Blood Bank tests and related terminology.
- Discuss sample collection and routine pre-transfusion testing.
- Evaluate unexpected results and options for transfusing patients in urgent situations.

Sample Collection & Patient ID

Patient Misidentification----Leading cause of transfusion fatalities

From CAP Website: "*Mistransfusion occurs from*

- *-misidentification of the intended recipient at the time of collection of the pretransfusion testing sample,*
- *-during laboratory testing and preparation of units to be issued, and*
- *-at the time of transfusion.*

- *Misidentification at sample collection occurs approximately once in every 1,000 samples, and*
- *in one in every 12,000 transfusions the recipient receives a unit not intended for or not properly selected for him/her."*

From CAP Today, January 2009

Dr. Richard Friedberg stated:

“ If the label’s wrong from the get-go, everything else we do about accuracy and reliability and precision goes out the window.”

From the Q-Probes study: one in 2,500 samples has the WRONG BLOOD in TUBE.

AABB Requirements for patient sample identification and labeling in Stds. 25th ed.

Std. 5.11: Samples and Requests

- "Identifying information for the patient and the blood sample shall correspond and be confirmed at the time of collection using two identifiers."

Patient sample ID and labeling-*continued*

AABB Std. 5.11.2

- "Blood samples should be identified with an affixed label bearing sufficient information for unique identification of the patient, including **two independent identifiers."**

- 5.11.2.1: " The completed label shall be affixed to the tubes before the person who drew the specimen leaves the side of the patient."

CAP-TRM.30575: Does the facility have a plan to implement a system to reduce the risk of mistransfusion for non-emergent red cell transfusions?

Options:

- Collect and test second sample
- Mechanical barrier
- Electronic ID system
- Another System to reduce risk

Sample Age: AABB Std 5.13.3.2

"If the patient has been transfused in the preceding 3 months with blood or a component containing allogeneic red cells, has been pregnant within the preceding 3 months, or the history is uncertain or unavailable, a sample shall be obtained from the patient within 3 days of the scheduled transfusion. Day 0 is the day of draw."

Sample Age-*Continued*

- Extended time limited by the type of sample collected.

- Refer to manufacturers instruction for reagents used for compatibility testing.

  - Time period for use of EDTA varies from 7 to 14 days.

Patient History: AABB 5.13.5.

"There shall be a process to ensure that the historical records listed below have been reviewed and compared to current records and that discrepancies have been investigated and appropriate action taken before a unit is issued for transfusion."

Patient History, *continued*

-Evaluation of patient information

-Diagnosis

-Race

-Medication

-Transfusion history

-Transplant

-Pregnancy

ABO Typing Reagents

- Monoclonal--only commercial source
- Check manufacturers instructions for clone information when performing investigations
- Anti-A,B vs. Anti-A + -B
- Weak A Ag expression readily detectable by routine monoclonal Anti-A reagents.
- New clone of anti-B does not routinely detect Acquired B.

Reagent Red cells for reverse grouping

-Requirement to be Rh negative (D-)

-No requirements for other red cells antigens; they could be M+ or Le(a+)

-Pool of at least 2 donors

Common causes of ABO discrepancies

- Most discrepancies due unexpected antibody are due to Anti-A_1
  - 2nd most common is due to other cold reactive antibodies (anti-M, Cold autoantibody)

- Most subgroups of A are routinely detectable
  - -Seldom noticed unless there is a serum grouping issue.

Case 1

CELL TYPING ANTI			SERUM GROUPING	
-A	-B		A_1 cell	B cell
3+	0		2+	4+

-Where is the discrepancy observed?

-Which is the most probable cause of the results observed?

-What can be done to resolve it?

-What blood type should be issued in an emergency if discrepancy not resolved?

Case 1-*continued*

CELL TYPING ANTI		SERUM GROUPING				
-A	-B	A_1 cell	A_2 cell	B cell	O cell	AC
3+	0	2+	0	4+	0	0

-Weaker that expected reaction with A_1 cell

-Subgroup of A ?

-Testing of A_2, O red cell and Auto Ctrl provide more info.

-Until resolved, transfuse

Red Cells : group O

Plasma: group AB

Case 2

CELL TYPING ANTI			SERUM GROUPING	
-A	-B		A_1 cell	B cell
3+	0		2+	4+

-Where is the discrepancy observed?

-Which is the most probable cause of the results observed?

-What can be done to resolve it?

-What blood type should be issued in an emergency if discrepancy not resolved?

Case 2-*continued*

CELL TYPING ANTI		SERUM GROUPING				
-A	-B	A_1 cell	A_2 cell	B cell	O cell	AC
3+	0	2+	3+	4+	3+	0

-Weaker that expected rxn. with A_1 cell

-Subgroup of A or?

-Testing of A_2, O red cell and AC suggest the presence of cold reactive antibody

-Red Cells : group A
Plasma: group O

Case 3

CELL TYPING ANTI			SERUM GROUPING	
-A	-B		A_1 cell	B cell
0	0		2+	4+

-Where is the discrepancy observed?

-Which is the most probable cause of the results observed?

-What can be done to resolve it?

-What blood type should be issue in an emergency if discrepancy not resolved?

Case 3-*continued*

CELL TYPING ANTI		SERUM GROUPING				
-A	-B	A_1 cell	A_2 cell	B cell	O cell	AC
0	0	2+	0	4+	0	0

Weaker that expected rxn. with A1 cell; A2 negative

Subgroup of A ?

Testing of A2, O red cell and AC suggest possible A sub group.

Red Cells : group O
Plasma: group AB

Routine Rh (D) typing testing requirements

AABB STD 5.13.2:

“Rh type shall be determined with anti-D reagent. The test for weak D is unnecessary when testing the patient.”

Anti-D (Rh_o) Antisera

- ◆Monoclonal reagents widely used
- ◆Human polyclonal reagents still available
- ◆Check manufacturers instructions for clone information when performing investigations.
  - ◆Very useful information when resolving discrepancies.

Anti-D: Unusual findings

- Individuals previously typed D-; now type D+
- I.S. reactivity that may not persist at 37C or IAT
  - i.e. Crawford, Rho^{Har}

Clones in Commercial Monoclonal Anti-D Reagents

	Immucor	Ortho
Anti-D		
	Gamma-clone	BioClone
	Gama401	MAD2
	F8D8	HUMAN
	Immucor Series 4	Gel Card
	MS201	
	MS26	MS201
	Immucor Series 5	
	TH28	
	MS26	

D typing and Pregnancy

AABB STD 5.20.2: “Women who are pregnant or have been pregnant recently shall be considered for Rh Immune Globulin administration when all the following apply:

1) The woman’s test for D Antigen is negative. A test for weak D is not required.

2) The woman is not actively immunize to the D antigen.”

Case # 4

Patient typed as Rh negative during 2 pregnancies between 1985 and 1990.

Now:	Anti-D	Rh Ctrl
–	2+	0

Is additional needed?

Case #4- *Continued*

Was the correct patient collected?

- Recollect and repeat typing.

Could the Rh typing be a false positive?

- Results are valid. Testing performed at I.S. and Rh Control is negative.

- Monoclonal anti-D is specific for D antigen.
  - If testing performed with polyclonal anti-D, the presence of antibody to a low frequency antigen is possible. Perform testing with a 2nd source of anti-D.

Direct Antiglobulin Test-DAT

Definition: *Test used to demonstrate in-vivo coating of red cells with antibodies or complement, in particular IgG and C3d.*

Antiglobulin reagents

- Monoclonal
- Blends (rabbit IgG and monoclonal C3)
- Gel card contains Rabbit IgG
- Solid phase indicator cell is coated with monoclonal Anti-IgG

DAT- Testing reminders

Strict adherence to critical test steps needed!

- *Wash RBCs at least 3 times without delay*
- *Spin immediately after adding AHG*
- *Resuspend and read immediately after spin.*

When DAT is positive, what next?

- *Perform testing with anti-IgG and anti-C3 reagents to determine the sensitizing protein*

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Elution

Definition: *Method to free and recover antibody from sensitized red cells.*

When to perform an eluate:

- DAT is positive with IgG
- DAT is negative- in certain instances when trying to explain decrease red cell survival

Time period to perform?

- “Recently transfused” patients, which is commonly defined as transfused within the last 14 days. In other labs, this period is extended to 21 days.

Albumin

- Not considered to be an antibody enhancement reagent
- Does not provide commonly accepted level of sensitivity
- Due to the molecular size, Rh antibodies may react stronger in albumin compared to *saline* methods.

LISS-additive for tube testing

Advantages:

- **Detects most potentially clinically significant blood group antibodies.**

- **Less sensitive than PEG for detection of warm reactive autoantibodies.**

- **May facilitate identification of antibodies demonstrating reactivity at 37°C phase of testing. Anti-Le^a, -Le^b, -M and -N and other direct agglutinins may be identified using this method.**

LISS-additive for tube testing

Disadvantages:

- **Less sensitive than gel or PeG/IAT in detection of some clinically significant blood group antibodies and warm reactive autoantibodies.**

- **Enhanced detection of most cold reactive autoantibodies.**

GEL: Column agglutination technology

***Advantages*:**

- **Detects most potentially clinically significant blood group antibodies.**
- **Uses smaller volumes of serum and reagents.**
- **Reactions are stable for up to 24 hours.**
- **Standardized procedure for improved consistency.**

GEL: Column agglutination technology

***Disadvantages*:**

- **Somewhat less sensitive for detection of some alloantibodies.**
- **Enhanced detection of warm reactive autoantibodies.**
- **Requires special equipment and reagent preparation.**

Solid Phase: *Advantages*

- **Provides stable, well-defined endpoints of the reaction.**
- **Enhanced sensitivity makes the detection of weak alloantibodies easier.**
- **Ease of use.**
- **No predilution of reagents is required.**
- **Standardized procedure for improved consistency when manufacturer's panels are tested.**

Solid Phase: Disadvantages

- Requires a centrifuge that can spin microplates.
- Requires a 37°C incubator for microplates.
- Requires a light source for reading the final results (non-automated method).
- Increased sensitivity may detect weak autoantibodies that other systems do not.

Case # 5

18 y.o. male with sickle cell disease

-Admitted with acute chest syndrome

-No history of transfusion at your facility

Results of antibody screen with LISS:

	37C	IAT
SC I	0	2+
SC II	2+	4+
SC III	0	3+

Case # 5*-continued*

Comparison of antibody screening results: LISS vs. PEG:

		LISS		
–		37C	IAT	PEG/IAT
–	SC I	0	2+	3+
–	SC II	2+	4+	4+
–	SC III	0	3+	4+

Which method is most informative?

Case # 5*-continued*

Comparison of antibody screening results: LISS vs. PEG:

		LISS		
–		37C	IAT	PEG/IAT
–	SC I	0	2+	3+
–	SC II	2+	4+	4+
–	SC III	0	3+	4+

More about this case later.

Antibody identification (Ab-ID)

- Method to identify specificity (ies) of antibodies detected by antibody screen.

- Approaches vary based on
  - -staff expertise,
  - -available resources, and
  - -urgency of patient's transfusion need.

Ab-ID: Standard Panel vs. Selected panel

- Standard panel (full):
  - Designed to identify commonly encountered specificities.
  - Resolves most routine antibody problems.
  - Provides limited information for complex problems or when multiple specificities have been previously identified.
- Extended panels: 10 vs. 16 cell panels designed to help separate multiple specificities

Ab-ID: Selected cell panel

- Panel of cells selected using available information:
  - Ab screen results, pt. history, pt. phenotype

- Requires use of multiple panels and some experience and/or software to create

- Maximizes information obtained with the least amount of sample and testing.

Case # 5 continued……

- LISS
  - indicates the presence of >1 antibody
  - will probably identify one antibody specificity

- Additional testing: auto control? DAT?
  - Phenotype the patient's red cells?
  - Routine or selected cell panel?
    - Anti-E + -Fya are identified
  - Rule out of additional alloantibodies: PEG?

Automation

- Available instruments capable of performing most BB tests.

- Routinely used for basic testing: ABO-Rh, antibody screening. Ab–ID also available.

- Methodologies used:
  - Gel
  - Solid phase
  - Hemagglutination

Automation

Advantages:

- Minimizes hands-on time
- Positive sample identification
- Standardizes testing
- Records easily archived

Disadvantages:

- TAT can be greater than manual testing
- Flexibility for STAT testing varies
  - May detect unwanted antibody or antibody of undetermined specificity

In closing….

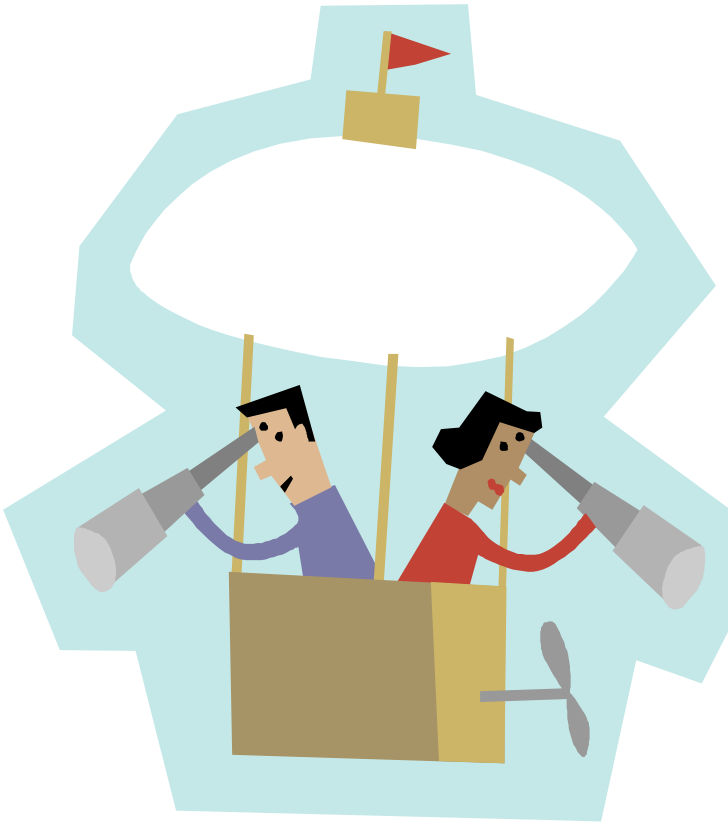
Be observant and inquisitive.

Use available resources to aid in problem resolution:

- patient, family, clinical staff, literature, manufacturers, colleagues, internet…….

Advancements occur everyday in Transfusion Medicine…..

Be an active participant!



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