METHODS FOR ANTIBODY SCREENING AND IDENTIFICATION

GOALS FOR ANTIBODY SCREENING INCLUDE

- DETECT AS MANY CLINICALLY SIGNIFICANT ANTIBODIES AS POSSIBLE
- DETECT AS FEW CLINICALLY INSIGNIFICANT ANTIBODIES AS POSSIBLE
- COMPLETE PROCEDURE IN A TIMELY MANNER

AABB STANDARDS REQUIRE 5.13.3

- METHODS OF TESTING SHALL BE THOSE THAT DEMONSTRATE CLINICALLY SIGNIFICANT ANTIBODIES
- WHEN CLINICALLY SIGNIFICANT ANTIBODIES ARE DETECTED, ADDITIONAL TESTING SHALL BE PERFORMED
- SAMPLES USED FOR PRE-TRANSFUSION IN RECENTLY TRANSFUSED, PREGNANT, OR UNKNOWN HISTORY – 3 DAY RULE APPLIES.

HOW DO WE DEFINE CLINICALLY SIGNIFICANT ANTIBODIES?

1. ASSOCIATED WITH HDN
2. HEMOLYTIC TRANSFUSION REACTION
3. WITH NOTABLY DECREASED SURVIVAL OF TRANSFUSED RBCS

PATIENT INFORMATION

We need as much information as possible including:
- Race
- Clinical Diagnosis
- Current Medications
- Transfusion/Pregnancy History
- Serological Results
- AGE

TRANSFUSION/PREGNANCY HISTORY

- Patients with no known history of transfusion or pregnancy more likely to have clinically insignificant antibodies and/or auto antibodies
- Patients with a history of transfusion or pregnancy are more likely to have a clinically significant antibody but may also have clinically insignificant antibodies
- Recent Transfusion History (within 3 months):
  - Rule out possible delayed hemolytic transfusion
  - Patient phenotyping may not be possible
DAT

- Determines whether antibody is allo, auto OR both
- Medication list is crucial when investigating positive DAT results

Current Medications

Drugs are known to cause antibody identification problems:

- Numerous drugs may cause a positive direct coombs test (DAT)
- Intravenous immunoglobulin (IVIG) may cause unexpected antibodies to be present in the serum. This may be the cause when an Rh positive patient has Anti-D in their serum.
- Rh Immune Globulin:
  - Anti-D can be detected in the maternal circulation for up to 6 months after administration
  - Include the date of the most recent injection

WORK SMARTER NOT HARDER

THE MORE PIECES OF THE PUZZLE YOU KNOW, THE FASTER AND EASIER IT IS TO SOLVE THE PUZZLE!

3 METHODS OF TESTING

1) TEST TUBE
2) GEL
3) SOLID PHASE

REAGENT RED CELLS MUST EXPRESS THE FOLLOWING ANTIGENS

- D, C, E, c, e, M, N, s, P1, LEA, LEB, K, k, FYA, FYB, JKA, JKB

TEST TUBE METHOD: GOOD WITH NOT SO GOOD

1) METHOD USED FOR YEARS
2) INEXPENSIVE
3) EQUIPMENT COMMON TO BLOOD BANK
4) GOOD BACK-UP METHOD
5) NO AUTOMATION CAN DO BLOOD TYPE QUICKER

- GRADING CAN BE INCONSISTENT: OVER-READ/UNDER-READ
- LABELING TUBES: TIME AND LABOR
- ERROR PRONE – TUBE MIS-LABEL: FAILURE TO ADD REAGENT, INADEQUATE WASHING
- NOT AS SENSITIVE AS GEL AND SOLID PHASE
TEST TUBE ENHANCEMENT MEDIA

- **SALINE AND ALBUMIN** not used very much. Can be used for increased serum to cell ratio but additional time required.

- **LISS** must follow package insert. 1 drop cells + 2 drops serum/plasma + 2 drops liiss. Order is important. If liiss added to RBCs prior to serum/plasma may lead to slight hemolysis of RBCs.

- **PEG** (Polyethylene glycol)

LISS

- Low ionic strength solution
- Used in routine tube method
- Inexpensive
- Requires technical skills to read
- Some anti-k do not react well with
- Uses cell washer

PEG

- Concentrates antibody
- Increases sensitivity and antibody uptake
- Removes water from test environment
- No 37C reading
- Uses cell washer
- Can not be used in high level of globulin (multiple myeloma)
- Clinically benign autoantibodies are more likely to be detected
- Greatly enhances weak Kidd antibody reactivity

ENZYMES

- Not routinely used in antibody detection but in antibody identification

- Most common: ficin and papain - red cell antigens destroyed. Include M, N, S, s, Fya, Fyb

- Red cell antigens enhanced. Include Rh, P, I, Kidd, Lewis

GEL TESTING ADVANTAGES

1. Elimination of washing, no shaking of tubes, no control check cells
2. Requires few procedural steps with less hands on time
3. Small amount of patient specimen
4. Reactions are stable and can be read later
5. Has both sensitivity and specificity
6. Gel is better at detecting mixed field reaction than solid phase
7. Can be automated
8. Ease of use

GEL TESTING PROCEDURE

- 50 ul of .8% suspension donor/screen cell plus 25 ul serum/plasma (very little sample needed)
- Card is incubated 15 min at 37C
- Centrifuged for 10 min
- Grade and record results (no washing step!!)
- Can be read the next day

GEL TESTING DISADVANTAGES

- Expensive to start up
- Gel can enhance antibodies that are not clinically significant (ex anti-Ch)
- Can increase detection of clinically benign autoantibodies
- Even though IgG test, IgM cold antibodies can agglutinate cells directly and cause strong positive reaction. (ex anti-IH)
**ORTHO PROVUE GEL AUTOMATION**

- **NUMBER OF ERROR OPPORTUNITIES**
  - TUBE 88
  - GEL 36
  - ORTHO PROVUE 4

- **DI SPENSE VERIFICATION**

- **AUTOMATIC MONITORING OF REAGENT EXPIRATION AND GEL CARD INTEGRITY**

- **PROCESS STEPS**
  - TUBE 30
  - GEL 9
  - PROVUE 3

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**CAN THE MTS ANTI-IgG GEL CARD BE USED FOR CLIA XMATCH REQUIREMENTS FOR ABO COMPATIBILITY ???**

1. **ORTHO LETTER FROM JAN 28, 2011**
   - **NO**
   - ANTI-IgG GEL CARD DOES NOT CONTAIN FDA APPROVED LABELING FOR IgM ANTIBODIES, IT IS NOT ADEQUATE TO DEMONSTRATE ABO INCOMPATIBILITY

2. **AABB ASSESSOR UPDATE**
   - FEBRUARY 2011 THE IgG GEL CARD IS A LOW IONIC TEST SYSTEM. REPORTS STATE THAT ABO INCOMPATIBILITIES DUE TO IgM ANTIBODIES HAVE BEEN MISSED WHEN WEAK AND IN TEST OF LOW IONIC STRENGTH

3. **CAN A FACILITY VALIDATE THE PROCESS OF USING THE GEL ANTI-IgG CARD TO DEMONSTRATE ABO INCOMPATIBILITY ???**
   - FDA REPLIES **NO**

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**APPROVED METHODS TO DETECT ABO INCOMPATIBILITY**

1. TUBE IMMEDIATE SPIN XMATCH
2. MTS BUFFERED GEL IMMEDIATE SPIN XMATCH
3. COMPUTER/ELECTRONIC XMATCH, WHEN ALL ELIGIBILITY FOR ELECTRONIC XMATCH ARE MET

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**PRINCIPAL OF SOLID PHASE**

- **ANIGEN COATED WELL PLUS LISS PLUS PLASMA OR SERUM**
- **INCUBATE**
- **WASH**
- **ADD INDICATOR CELLS**
- **CENTRIFUGE**
- **READ**
**SOLID PHASE TESTING**

- Uses phosphate buffered saline pH 6.5 to 7.5
- For manual work station a CSW100 washer is used. Wash 3 times then turn plate 180 degree and wash 3 more times to ensure proper washing
- Reagents must be at room temperature
- LISS changes from purple to slate blue with addition of serum/plasma

**SOLID PHASE POSITIVE SOLID PHASE**

**SOLUTIONS OF ERROR IN MANUAL SOLID PHASE TESTING**

- Not mixing indicator cells
- Indicator cells can get contaminated causing test failure
- Overcentrifuge - false negative
- Undercentrifuge - false positive
- Strips are in foil package with humidity indicator, if improper closure too much moisture in strips and they must be discarded (this is also problem in automation)
IMMUCOR AUTOMATION FROM 1998 TO 2007

GALILEO

- HAS A SAMPLE CAPACITY OF 224
- FLOOR MODEL
- CAN TEST POOLED, 2 CELL, 3 CELL, 4 CELL ANTIBODY SCREEN
- USES PBS AS DILUENT
- OPERATOR IS RESPONSIBLE FOR ASSIGNING STRIP SELECTION - IF INCORRECT LOST OF REAGENT
- READS TEST FRAME NOT INDIVIDUAL STRIPS
- DOES NOT DIFFERENTIATE MIXED FIELD. ALSO, FOR HEMAGGLUTINATION GRADED AS 1+ OR LESS IN TUBE, GALILEO CANNOT RELIABLY DETECT
- MAINTENANCE- DAILY APPROX 20 MINUTES, WEEKLY APPROX 20 MINUTES, MONTHLY APPROX 1 AND HALF HOURS TO CLEAN
- OPERATOR CAN CHANGE PARTS WITHOUT SERVICE CALL
- IMAGE ANALYSIS READER

SOURCES OF ERROR IN GALILEO SOLID PHASE AUTOMATION
- FAILURE OF OPERATOR TO ADD STIR BALL (this is what mixes reagent rbcs on-board)
- FAILURE TO SELECT PROPER STRIPS AND POSITION IN FRAME HOLDER
- PROBE HAS TEFLON COATING- FAILURE TO REMOVE CAP RESULTS IN PROBE DAMAGE
- SOFTWARE ISSUES WITH FREEZE OF SCREEN
- FAILURE OF OPERATOR TO REMOVE INDICATOR CELLS AFTER 24 HOUR EXPIRATION

ECHO (2007) INTENDED FOR SMALLER TRANFUSION SERVICES

ECHO

Actual size: 45” wide x 26” high
WHAT KIND OF TESTING CAN THE ECHO DO FOR YOUR BLOOD BANK?

- ABO/Rh Type
- Donor Confirmation
- ABO Retype
- Weak D
- Phenotype
- Antibody Screen (3-cell)
- Antibody Identification
- (primary panel O Plus panel and D neg panel)
- IgG DAT
- IgG Crossmatch

ECHO READS MICROSTRIP IDENTIFICATION, LOT #, EXP DATE AND SERIAL NUMBER

Every microstrip contains a two dimensional bar code that contains strip identification, lot number, expiration date, and a unique serial number.

THE TANGO

TANGO Optimo (BIOTEST)
TIME TO TANGO.....

- NEW SOLID PHASE TESTING- USES IgG INSTEAD OF INDICATOR CELLS. MICROPLATE WELL CONTAINS A LAYER OF PROTEIN A (HIGH AFFINITY FOR Fc PORTION OF ANTI-IgG)
- LOW RATE OF FALSE POSITIVES WITH SOLIDSCREEN II
- ABO/RH WELLS COATED WITH DRIED MONOCLONAL ANTISERA WHERE RED CELLS ARE SUSPENDED IN BROMELIN AT TESTING
- ON-BOARD REAGENTS 7 DAYS EXCEPT FOR BROMELIN
- 144 SAMPLE LOADING CAPACITY

TANGO OFFERS:

- Eight wells/strip
- Stips move through in paralell - 8x
- 12 strips/plate
- Removable strips and plates
- Pheno
- Rh
- D neg
- D neg panel
- Strip type

AND NOW THE “NEO” IN 2010
**NEO ANSWERS THE CALL**

- Speed and flexibility
- Blud Direct Technical Service from Monitor
- Still can load 224 samples
- New Stat Priority

**SOLID PHASE/ GEL/ LISS/ PEG**

- Sensitivity: Solid Phase < Gel < PEG < LISS
- Sensitivity of Gel was 98% compared to 92% for LISS
- If using PEG as “Gold Standard,” only 2/3 of reactions by Capture R (Solid Phase) considered true positives
- Personal experience: Anti-D from Rhig injection will be detected more often in Gel than PEG

**PUTTING THE AUTOMATION TOGETHER: TURN AROUND TIME (TAT)**

- **First ABO/RH**
  - Galileo 22 min
  - Echo 11 min
  - Provue 35 min
  - Tango 17 min
  - Neo 17 min

- **Antibody Screen**
  - Galileo 40 min
  - Echo 20 min
  - Provue 35 min
  - Tango 31 min
  - Neo T&S in 30 min

- **Specimens/Hour Type & Screen**
  - Galileo 60
  - Echo 16
  - Provue variable
  - Tango 12
  - Neo 61

**BEST TESTING METHOD ??? CLUES......**

- Affordable
- Provides patient safety
- User friendly
- Timely

**REMEMBER THERE IS NO SINGLE ANTIBODY SCREENING OR IDENTIFICATION METHOD THAT OPTIMALIALLY DETECTS ALL ANTIBODIES**

Thank you...
Questions