Use and Comparison of Different Test Systems and/or Reagents in the Problem-Solving Process
Program #144801
Wednesday, January 15, 2014
2:00 pm-3:30 pm (ET) ~ 7:00 pm-8:30 pm (GMT)

The presentation will include a review of antibody detection methods, reagents used to perform serologic evaluation of a patient's sample and techniques to resolve serologically-difficult patients.

Objectives:

- Compare the different test media for antibody detection.
- List reagents used during antibody identification.
- State steps used for antibody resolution.

Director/Moderator:
Michele Hayes, MT(ASCP)SBB
Program Director, School of Medical Technology
Mount Nittany Medical Center

Faculty:
Joanne Kosanke, MT(ASCP)SBB<sup>CM</sup>
Director, Immunohematology Reference Laboratory
American Red Cross
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Use and Comparison of Different Test Systems and/or Reagents in the Problem-Solving Process

Joanne Kosanke, MT(ASCP)SBB
Director, IRL
American Red Cross, Central Ohio

What we will cover today

• Comparison of different test media for antibody detection
• Discussion of reagents used during antibody identification
• Example of steps used for antibody resolution

Test Media

• Low-ionic strength solution (LISS)
• Polyethylene glycol (Peg)
• Column agglutination (Gel)
• Solid phase
LISS: What does it do?

• 1964: Vox Sang 9:385-395
• \([\text{AgAb}]_{eq} = K_d [\text{Ab}]_{eq}\)
  – \(K_d\) = strength of bond between Ag and Ab
• Affected by: pH, temperature, salt concentration

LISS: What does it do?

• Ionized groups on Ab and Ag
• Lower molar strength than normal saline
• Effect: decreases the time for antigen-antibody reactions
• Must use equal volume of plasma/serum to LISS reagent

Comparison study

• 1979: Transfusion vol 19 p 81-85
  – Compared in-use test media: albumin, normal saline, low-ionic strength salt solution, and polymerized albumin
  – To determine ‘maximum sensitivity in the shortest incubation time without loss of specificity’
1979: Study results

- Test procedures differed from today
  - 2% cell suspensions were prepared in the media
  - 2 drops cells + 0.1 mL serum
  - RT incubation included for normal saline and LISS testing
  - All methods read at 37°C and IAT

1979: Study results

- Negative control population: 170 donors
  - 6 positive
  - 5 were cold agglutinins
    - Anti-IH only in LISS
    - Anti-M reacting with all M+ cells in LISS, but only M+N- cells reacted in normal saline
  - 1 IAT antibody: anti-Fy\(^b\) on identified in LISS

1979: Study results

- Positive population: 33 patients
  - ID using LISS and polymerized albumin
  - LISS ‘wins’ at IAT
    - One anti-c
    - Three anti-Jk\(^a\)
  - Polymerized albumin ‘wins’ at 37°C
    - Direct agglutination at 37°C
1979: Study results

- Incubation times with known antibodies of 2+ IAT strength
  - 5 min incubation: 50% vs 81%
  - 15 min incubation: one anti-D and one anti-Fy\(^a\) only detected by LISS-IAT
- Their conclusion: 10-15 minutes incubation required with no loss of sensitivity

Peg: What does it do?

- Water soluble polymer
- Polymers replace water molecules
- Effect: concentration of antibody and antigen with increased chance for collision and antigen-antibody complex formation

Comparison Study

- Immunohematology 1995;11:11-13
- Compared popularly used LIS with the newcomer Peg for antibody detection and identification
- Immediate transfusion reaction prompted the study
1995 Study

• Studied known samples
  – 50 known positive by LISS-IAT
  – 50 known negative by LISS-IAT
• Parallel studies
  – Prospectively: 151 samples

1995 Study

• Results
  – Retrospective:
    • LISS and Peg detected all clinically-significant antibodies
  – Prospective:
    • Peg detected 35 clinically-significant, 15 not
    • LISS detected 15 clinically-significant, 33 not

Testing by Peg

• Do no spin after 37C
• Use of anti-IgG to reduce clinically-insignificant antibodies
• Do not use if plasma precipitates
Gel: What does it do?

• Columns with incubation wells
• Gel = dextran acrylamide
• Gel inhibits agglutinated cells from traversing the column
• Gel
  – Measured volumes of cells and plasma
  – Columns can be read at a later time

Solid Phase: What does it do?

• Single layer of red cells are fixed to the microplate
• Test sample is added, incubated, washed
• Indicator cells are added
• Results:
  – Positive = diffuse pattern in well
  – Negative = pellet of cells in well

Comparison Study

• Immunohematology, 2006 vol 22 p196-202
  – In search of the Holy Grail: comparison of antibody screening methods
  – Find the balance
Comparison Study

- Issitt et al in 1997
  - 1184 samples for comparison study
  - 193 positive
    - 14 Peg only – 5 false positive
    - 73 solid phase only – 46 false positive

Issitt et al

- 193 positives out of 1184
  - 6% positive by Peg
  - 11% positive by Solid Phase
- Benign autoantibodies
  - 3 detected by Peg only
  - 7 by both
  - 22 by solid phase only

2005 Study

- Specifically compared MTS Gel and Capture-R
  - # of antibodies tested by each differed
  - Wanted antibodies: 2.4 vs 2.5
  - Passive anti-D: 14% vs 42%
Best Test Media

- No one method will detect all antibodies of clinical importance
- Peg, Gel, and Solid Phase similar sensitivity
- Search for the best test media goes on

Reagents used in Ab ID

- Chloroquine diphosphate (CDP)
- EDTA-Glycine acid (EGA)
- Enzymes
- DTT

CDP or EGA

- Patient has a positive DAT due to IgG
  - Antigen typing with IAT-reactive antisera
  - Test with neat plasma to confirm autoantibody
  - Test back with adsorbed plasma to determine warm autoantibody removal by adsorption
CDP

• Quinoline derivative that splits ag-ab complexes
• Modified from an elution test to dissociation of IgG leaving red blood cells intact

CDP

• 4 volumes CDP to 1 volume cells
• 30 minute RT incubation
• Use anti-IgG to determine success (complement not removed from cells)

EGA

• EDTA
• Glycine: keeps rbc's from hemolyzing
• Acid: pH = 3
  – Low pH reverses ag-ab complexes
• TRIS buffer
EGA

• Mixture of EDTA and glycine acid are added to the red blood cells
• 1-2 minute RT incubation
• Buffer added
• Does not remove complement

EGA

• Denatures Kell system antigens and the high-prevalence antigen Er\(^a\)
• Something to know…
  – Abstract: Transfusion 2006; 132A
  – If unsuccessful, recover reticulocytes to EGA treat

Cell Separation

• Recover reticulocytes
  – Old cells are dense cells
• Sample: EDTA, fresh
• Fill microhematocrit tubes
Enzymes

- Cleaves protein at specific points
- Helpful when a plasma has multiple antibodies
- Helpful when an antibody to a high-prevalence antigen is present

Denatured by enzymes

- MNS, Fy\textsuperscript{a}, and Fy\textsuperscript{b}
- Ch/Rg
- JMH
- Y\textsuperscript{e}
- Ge\textsuperscript{2} and Ge\textsuperscript{4}
- In\textsuperscript{b}

Dithiothreitol (DTT)

- Denatures disulfide bonds
- 0.01 M DTT
  - Serum studies: IgM vs IgG
  - Cell studies: dispersal of autoagglutination
- 0.2M DTT
  - Cell pretreatment: denatures some blood group antigens
Denatured by DTT

- Knops
- Indian
- Dombrock
- YT
- Lutheran*
  *we haven’t been lucky

Steps for antibody resolution

- Serologic scenarios and application of reagents

Scenario One

- Antibody screen = positive (2+)
- Panel cells = positive (2+)
- Auto control = positive (2+)
Goal: to detect alloantibodies

- Type the patient for common antigens
- Verify the plasma reactivity is an autoantibody
- Adsorb the plasma to remove the autoantibody

Use EGA

- EGA treat cells
- Test for S, s, Fy^a, Fy^b, Jk^a, and Jk^b
- Test EGA-treated, DAT-negative cells with patient’s neat plasma by original test method
Resolution

• Use a different test method
  – If Gel is positive, test by Peg
  – If Peg is positive, test by LIS

• Adsorptions
  – Auto if not transfused
  – Allo if transfused

Scenario Two

• Antibody screen = positive (2+)
• Panel cells = positive (2+)
• Auto control = negative
Patient’s Phenotype

- Patient’s phenotype
  - D+C+E-c-e+
  - K-; S-s+; Fy(a-b+); Jk(a+b+)
Enzyme Sensitive

- Ch/Rg
- JMH
- Yt\(e\)
- Ge2 and Ge4
- In\(b\)

DTT Sensitive

- Knops
- Indian
- Dombrock
- YT (Yt\(e\))
- Lutheran

Scenario Two
Scenario Two

Ancestry Association

• Caucasian: k, Kp, Co, Lu, Yt, Vel, Lan, Sc1
• African: U, Js, Lu, Cr, Hy, At, Jo
• Asian/Hispanic: Di, Ge2/3, Lu, Jr, Ok, Jk3

Adsorption

• Phenotype patient for common antigens
• Adsorb the antibody against the high-prevalence antigen onto donor aliquot of cells lacking same antigens as patient
Elution

- Elute the antibody from the adsorbing cells
- Eluate = antibody to high-prevalence antigen with no ABO antibodies

Summary

- LISS, Peg, Gel, and Solid Phase
- EGA and CDP
- Enzymes and DTT
- Adsorptions
- Elutions
Upcoming Cellular Therapies Audioconference:

Donor Hemovigilance: What's In It For My Blood Center?
January 22, 2014
2:00 pm to 3:30 pm (ET); 7:00 pm to 8:30 pm (GMT)
Program # 144833

This audioconference will explore the use of Donor Hemovigilance data in a blood center. The speaker will provide real world examples of how the blood center is using the data collected through the Donor HART software to impact blood center operations and donor care. The session will include an overview of how to sign up for the program.

Objectives:

- Explain general awareness of donor vigilance and generate specific interest in submitting data to Donor HART.
- Review current activities associated with donor vigilance.
- Describe the steps to joining the Donor Hemovigilance Program.

Intended Audience: Physicians, Scientists, Managers/Supervisors, Nurses, Perfusionists

Event Level: Basic

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Director, AABB's Center for Patient Safety
AABB

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AABB would like to thank the members of the AABB Distance Learning Program Unit for their assistance in developing these programs:

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