Objectives

• Explain how the concepts of sensitivity and specificity apply to blood bank
• Discuss studies showing how sensitivity and specificity have affected operations in the blood bank and patient outcomes
• Demonstrate that non-specific antibody reactivity may be an indicator of future antibody development.
Acknowledgement

Some information shamelessly stolen from:

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Randy has no knowledge of this presentation and any blame, criticism, or foul comments stemming from sitting through the forthcoming presentation should be directed at me.
Let’s Do Some Sensitivity Training

• Cultural Sensitivity Training
• Racial Sensitivity Training
• Gender Sensitivity Training
• Religious Sensitivity Training
• All very Important…

Not what we are going to be talking about today
Sensitivity Training: Definitions

- **True Positive**: Positive assay result that was expected to yield a positive result.
- **True Negative**: Negative assay result that was expected to yield a negative result.
- **False Positive**: Positive assay result that was expected to yield a negative assay result. (includes detection of clinically insignificant antibodies)
- **False Negative**: Negative assay result that was expected to yield a positive assay result.
- **Sensitivity**: True Positive Rate (finds all positive results)
- **Specificity**: True Negative Rate (negatives are negative)
Laboratory Sensitivity Training

**Sensitivity**

\[
\text{Sensitivity} = \frac{\text{True Positives (TP)}}{\text{True Positives (TP) + False Negatives (FN)}} \times 100
\]

**Specificity**

\[
\text{Specificity} = \frac{\text{True Negatives (TN)}}{\text{True Negatives (TN) + False Positives (FP)}} \times 100
\]

**Ideal Antibody Screening Test = 100% Sensitive and 100% Specific**
“Truth” Lies With the Victors

• What defines a TP, TN, FP, or FN?
  — “Gold Standard” always wins...at least at first........
  • Is that a legitimate method to demonstrate a new
    systems sensitivity and/or specificity?

Example:

test system 1 (current/gold standard) = Negative Ab Screen
  test system 2 (new) = Positive AB screen

Is this a TP, FP, or FN reaction?
Didn’t FDR Take Us Off The Gold Standard?

• Yes, in June 1933
  – Consider that the educational part of the presentation
• Usually the “Gold Standard” is simply the laboratory system currently in use
  – Not necessarily best system...just system of record
  – Again, which system is “telling the truth?”
Example Answer

• Based on the system of record ("Gold Standard")
  – This would be a FP reaction
  – But is it truly?
    • Did we ID an antibody in new test system?
    • Can we dismiss the new system as hypersensitive [lack specificity]?

\[
\text{Sensitivity} = \frac{\text{True Positives (TP)}}{\text{True Positives (TP)} + \text{False Negatives (FN)}} \times 100
\]
What if the New System Is Better Than Gold Standard?

• There is no shortcut to the process of comparing it to the existing “gold standard.”
• The sample labeled as positive antibody screen by the new test (negative on the “gold standard”) will be categorized as a false positive (FP).
• If on follow-up, a significant number of these patients actually develop antibodies, then the new test is in fact detecting antibodies earlier than, and is better than, the gold standard.
• In some instances, there may be other strategies available to determine whether the new test is in fact better. [beyond this presentation]

Sacket, DL, et al.
Difference Between Sensitive and Hypersensitive

• Antibody screening and ID testing is not 100% sensitive or 100% specific
  – This is the art of blood banking.....or.....
  – Why we think we are soooood special
• Sensitive enough = We find all significant antibodies present and we can ID them
• Hypersensitive = We find other stuff too
Path To Increased Sensitivity

- Enzymes 1947
- LISS 1964
- Gel 1985
- Capture R 1986
- PEG 1987
- ABS 2000 1998
- ProVue 1997
- Galileo 2004
- Echo 2007
Fatal HTR (non-ABO)

- Historically 1:2-3 million* (reporting an issue)
- Currently 1:1.8 million
- Reality = no change (as best can be reported from past data)

Increased Sensitivity

Decrease in fatal HTR (non-ABO)
What About DHTRs?


- Using SHOT data from 2007 – 2012
- Non-Capture® rate of DHTR = 1:33,350
- Capture® rate of DHTR = 1:64,500

**Conclusion:**
- Increased sensitivity may lead to a decrease in DHTR
Liu C, Grossman B. Antibody of undetermined specificity: frequency, laboratory features, and natural history. Transfusion 2013;53:931-938. (Barnes Hospital, Washington University St. Louis)

Frequency Analysis (Part I)

- 7-09 to 12-11 Manual or automated (ProVue) gel, 2 cell screen
- Positive Antibody Screen Rate 4.37% (6058/138,510)
  - Hoag (SP) 4.1%
- Antibodies Detected 8121 (6058 patients)
  - Alloantibodies 71.6% n=5813
  - Autoantibodies 5.1% n=418
  - Passive D 5.5% n=448
  - No Specificity 17.7% n=1442
- Rate per positive screen 23.8% 1442/6058
  - Hoag 25.2%
- Rate per sample 1.0% 1442/138,510
  - Hoag 0.9%
Antibody Development (Part II)

- First quarter of 2012: **174 non-specific antibodies**
- Female/Male: 2:1
- Only Antibody Detected: 76% (n=132)
- DAT Positive: 34% (n=59)
  - Polyspecific: 29
  - Anti-C3: 3
  - Anti-IgG: 27
    - Panagglutinin in Eluate: 8
    - Negative Eluate: 19

- 45 non-specific patients with follow-up testing
- Disappeared 14/45 (31%)
- Persisted in repeat sample (median 8 days) 31/45 (69%)
- 16% (7/45) subsequently developed 10 new alloantibodies
  - anti-E 3
  - anti Jk(b) 2
  - anti-D 1
  - anti-C 1
  - anti-Le(a) 1
  - anti-s 1
  - warm auto 1

- **Routine methods**
  - automated solid phase screen
  - gel antibody identification
- **9 cases in 3 months**
  - Positive screen, inconclusive gel panel
  - Solid phase panel
    - Jk(a) n=7
    - Jk(b) n=2
    - Homozygous Kidd cells positive in PEG tube

- Conducted 6 months retrospective analysis
- 32,831 automated solid phase screens in 6 months
  - New positives with inconclusive gel panel n=83 (0.25%)
  - Review showed “potential Kidd” specificity n=57 (0.17%)
    - That is 2/3 of the cases........
- Changed practice – solid phase antibody ID if gel testing inconclusive

- Converted from manual gel to automated solid phase
- Positive Antibody Screen: gel panel “confirmatory test”
- 495 patients: positive solid phase, negative gel panel
- 188 returned for follow-up testing

<table>
<thead>
<tr>
<th>Solid Phase</th>
<th>Gel</th>
<th>Positive Solid Phase Negative Gel Follow-up n=188</th>
<th>Negative Solid Phase Controls n=397</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>17.0%</td>
<td>4.3%</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>not tested</td>
<td>79.8%</td>
<td>95.7%</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>3.2%</td>
<td>0.8%</td>
</tr>
<tr>
<td><strong>Specific Antibodies</strong></td>
<td></td>
<td>9.6%</td>
<td>3.5%</td>
</tr>
<tr>
<td><strong>Non-Specific Antibodies</strong></td>
<td></td>
<td>7.4%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Non-specific reactivity can be a predictor of subsequent antibody formation.
Meade T, Armstrong SM, Sanford K. The development of alloantibodies in patients that demonstrated capture-R solid phase phenomenon (abstract). Transfusion 2012;52:142A. (Virginia Commonwealth University Medical Center, Richmond VA)

- June 2006 – December 2011
  - 228 (3%, 109 patients) of 6718 antibody workups had pan-reactivity via “automated solid phase” technique.
  - 55/109 patients had follow-up testing
    - Antibody screen negative 27.3% (n=15)
    - Pan-Reactive remained 61.9% (n=34)
    - New Alloantibody 10.9% (n=6)
      - 3 transfused
- Pan-reactivity found in solid phase may be a predictor of subsequent antibody formation.
Resulting Non-Specific at VCUMC

• Non-Specific
  – No discernable pattern in SP (screen and panels)
  – Not pan-reactive in SP
  – Nothing ID’ed using PEG
  – Warm Reacting Antibody of Undetermined Specificity....”WU”
  – Perform full AHG crossmatches
Resulting Pan-Reactive SP at VCUMC

• Pan-Reactive
  – All SP cells positive (screen and Panels)
  – All other testing (PEG) negative
  – Called “Solid Phase Phenomenon” and not treated as an antibody ID
  – Perform full AHG crossmatches while present
  – When no longer present, electronic crossmatch eligible
Summary

- Increased Sensitivity = Increased non-specific results
- Good or Bad? Eye of the beholder
  - Catch nearly everything (no system is 100% sensitive)
  - Increased sensitivity doesn’t appear to be related to a decrease in fatal HTR (non-ABO)
  - May cause a decrease in DHTRs
- Non-specific reactivity associations
  - Precursor to development of clinically significant antibodies?
    - 10-16% vs. 4% in controls
  - Higher incident in females, especially if currently pregnant
Sensitivity Training Takeaway

Tough being the new guy (instrument, methodology, etc.)
Questions???

Questions are guaranteed in life; answers are not.