Southeastern Area Blood Bankers

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It’s in the Details

Problems with Testing You Never Saw Coming

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Quotient Biodiagnostics

QUOTIENT BIODIAGNOSTICS

I have come to realize that the “extras” around the testing – saline, tubes, temperature, washes, etc. – which seem so fundamental,
can really throw a kink in the works.

Q

"Essential processes are all composed of tasks or activities, some of which actually contribute to the process, while others exist just because "we've always done it that way"… some of these activities add value to the process and some of them don't"

Walters, Lisa M. Introducing the Big Q: A Practical Quality Primer. AABB Press, 2004. Page 107

QUOTIENT BIODIAGNOSTICS

“To manage a system of processes effectively, the facility must understand how its processes interact and what cause–and–effect relationships exist between them”

AABB Technical Manual, Seventeenth Edition. AABB Press, 2011. Pg. 4.

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Incidents That Stimulate Change

- Mistakes made in your own lab
- Mistakes made in other facilities
- Citations from inspections, your own or others
- Published cases
- Published research

Antigen typing

	Manufacturer Quotient **Anti - N**	Manufacturer Other Manufacturer **Anti - N**
Instructions	1 drop reagent 1 drop of 2-4% red cell suspension; Mix well Incubate 5 min @ 20-25 C Spin / read	1 drop reagent 2 drops of 2% red cell suspension; Mix well Incubate 30 min @ 20-30 C Do Not Spin / read
Positive Control M+N+ cell	3+	3+
Negative Control M+N- cell	1+	Neg

Investigation

- Major difference in testing was length of incubation
  5 minutes versus 30 minutes

- Technologists generally removed reagents from the refrigerator and used them immediately.

- Quotient Anti-N sera was not being tested at the correct incubation temperature. Package insert states “False positive or false negative results can occur due to… improper reaction temperature…”

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Antigen typing

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Positive Control M+N+ cell	3+	3+
Negative Control M+N- cell	Neg	Neg

- Recent AABB inspection on the West Coast.

- Citation for performing testing using an anti-sera that was supposed to be tested at room temperature, which was defined in the package insert as 18-24 ^{o}C . The laboratory was warm. Inspector asked about the room temperature. Thermometer read 24.5 ^{o}C

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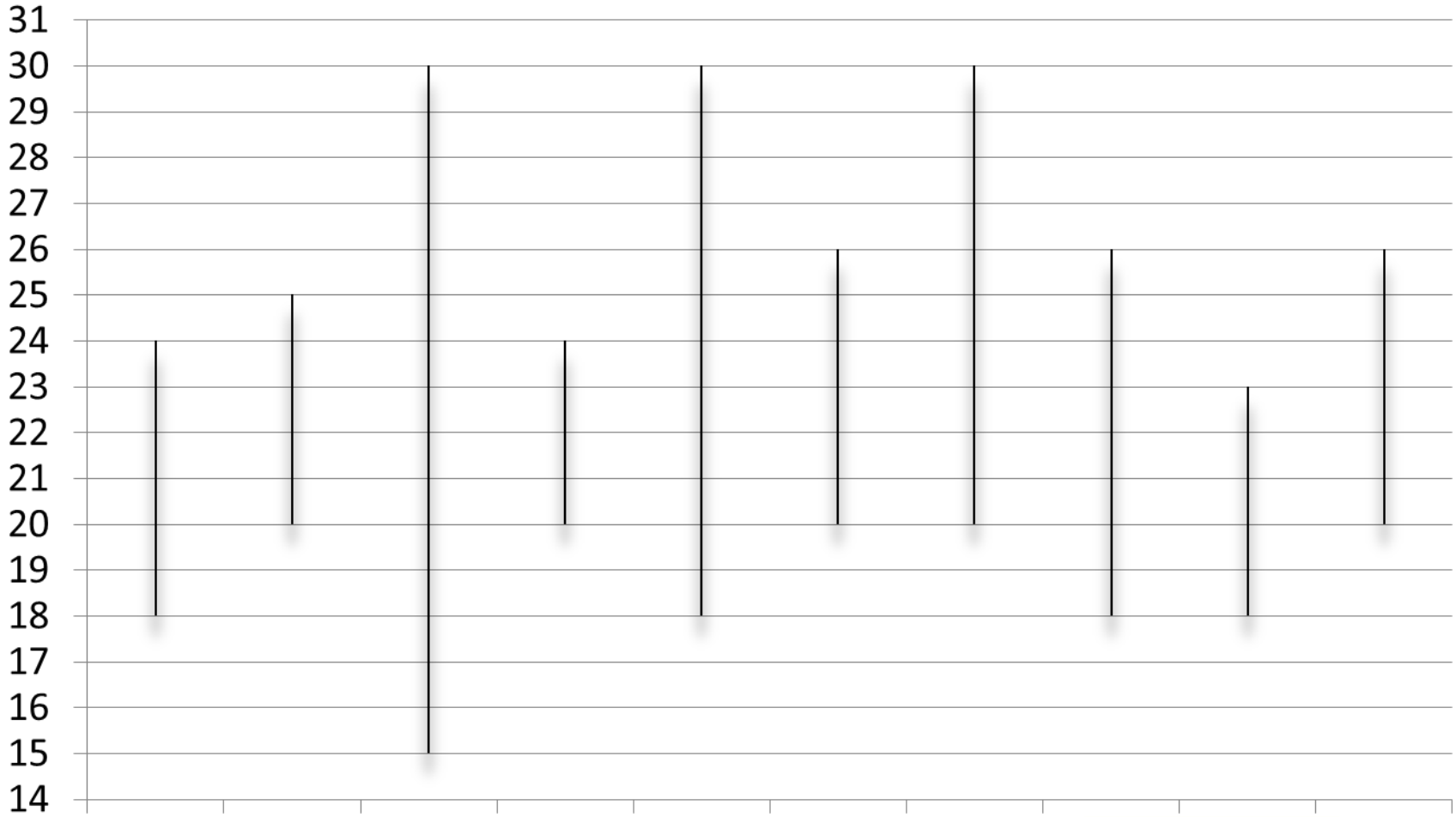
“Room Temp” Temperature Ranges



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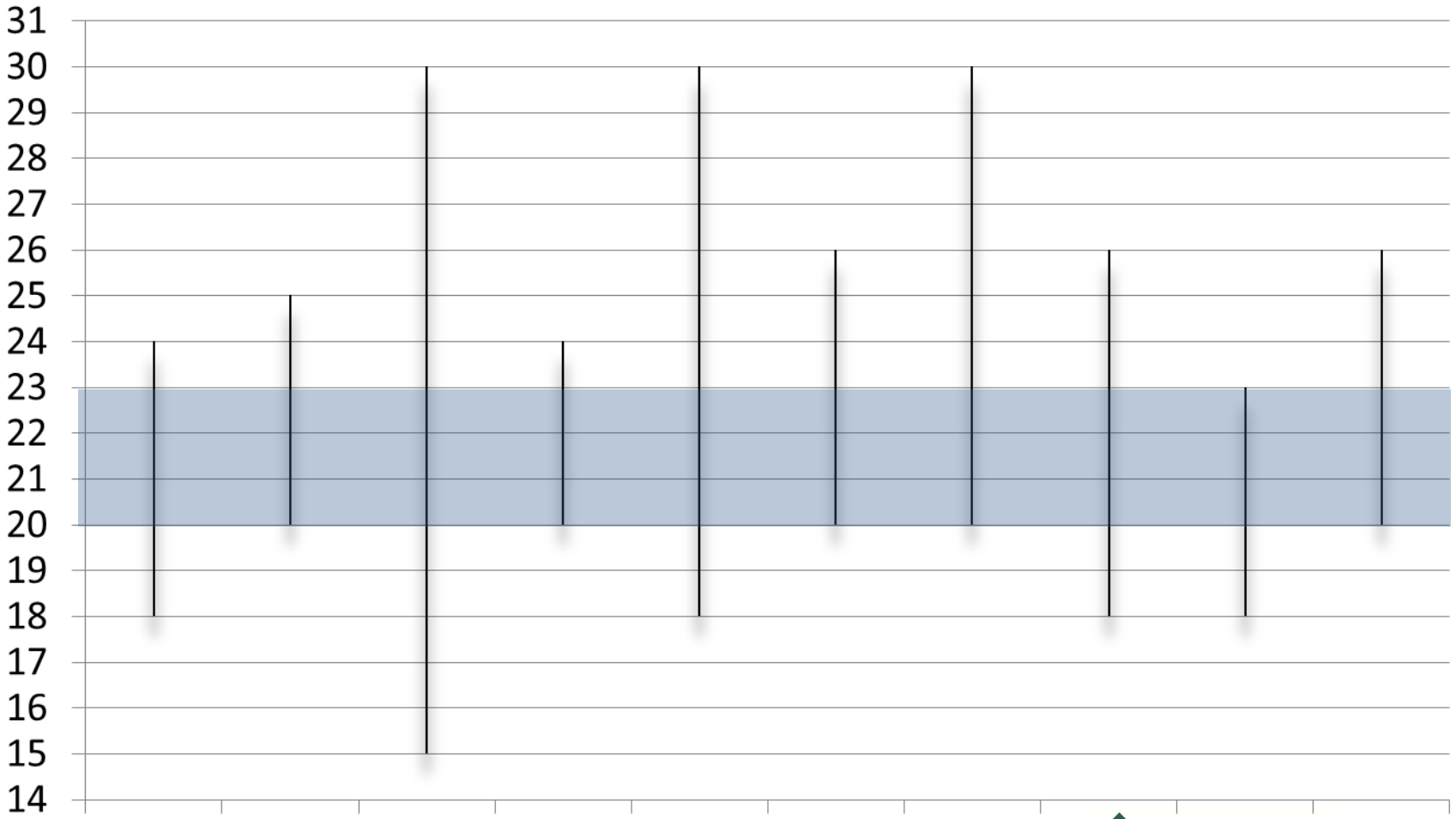
“Room Temp” Temperature Ranges



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“Room Temp” Temperature Range

“Sweet Spot”

20 – 23 °C

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Unusual Quotes from Anti-sera Inserts

- “Do not use any optical aid to examine the test results.”
- “Do not examine microscopically.”
- “Optical Aid (opt) The use of an optical aid for agglutination reading must be validated by the user.”
- “Do not centrifuge at any time.”
- “Centrifuge 60 seconds at 800-1000 x g.”
- “Adding 2 drops of reagent may enhance reactivity.”

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Unusual Quotes from Anti-sera Inserts

- “reactions of 1+ or weaker (test or control cells) should be investigated before a phenotype is assigned, as they may be an indication that the environmental temperature, centrifugation speed or time, or the volume of reagent or cell suspension used are not optimum or that the reagent is deteriorating.”
- “NOTE: Hemolysis, if obtained, should not be interpreted as a positive test. Hemolysis may indicate that the reagent has become contaminated with bacteria.”

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Unusual Quotes from Anti-sera Inserts

- “Washed red blood cells may demonstrate better reactions”
- “Add 1 drop of an approximately 3-4% suspension of the red blood cells to be tested (previously washed at least 1 time and resuspended in saline)”
- “Incubating for the upper end of the time range may enhance reactivity.”
- “Anti-X must be used only with red blood cells suspended in isotonic saline.”

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Unusual Quotes from Anti-sera Inserts

- Materials Required:
  10x75 mm or 12x75 mm glass test tubes

- "Resuspend the cells completely and examine *macroscopically* without the use of magnifying devices, i.e., hand lens, agglutination viewer or microscope, for agglutination. Use of such devices may lead to misinterpretation of negative reactions as positive reactions."

Unusual Quotes from Anti-sera Inserts

- “Add 1 volume of red blood cells suspended to 2-4% in unbuffered isotonic saline….
  As this reagent reacts optimally at pH 8.5 and is extremely sensitive to pH, test red blood cells should be suspended in unbuffered medium. All red blood cells suspended in buffered medium e.g. Alsever’s solution, should be washed at least once and resuspended in unbuffered saline prior to use.”

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Unusual Quotes from Anti-sera Inserts

- “The optimal isotonic saline for most antibodies is pH 7.0 – 7.2. Use of isotonic saline with a low pH may cause decreased sensitivity.”

Saline

- Isotonic Blood Bank Saline
  Isotonic NaCl solution
  0.85 – 0.9% weight to volume
  pH range of 6.0 – 7.5

- Phosphate Buffered Blood Bank Saline
  Isotonic NaCl solution
  0.85 – 0.9% weight to volume
  pH range of 7.0 – 7.3 (may range as high as 7.5)
  Buffered with phosphate

Saline

- Braun Saline
  0.9% weight to volume
  Sterile saline solution
  Used for irrigation of wounds and IV solutions
  pH range of 4.5 – 7.0 (goal seemed to be 5.0 or 5.6)
  Available in small volumes of 250, 1000 or 3000 mL

Saline

- Bruce, M. et.al. “A serious source of error in antiglobulin testing.” Transfusion, 1986; 26:177-181

- Abstract: “The investigation of a failure of proficiency showed that certain saline solutions are inappropriate for use in blood group serology tests. In particular, it was found that solutions of unexpectedly low pH and/or those autoclaved and stored in plastic containers could severely compromise the sensitivity of the antiglobulin test when used as wash solutions. “

QUOTIENT BIODIAGNOSTICS

Saline

- Bruce, M. et.al. “A serious source of error in antiglobulin testing.” Transfusion, 1986; 26:177-181

- Abstract: “The observed loss of sensitivity ranged from a reduction in titration score to a complete failure in the detection of clinically significant blood group antibodies……improved standardization and sensitivity could be achieved by using phosphate-buffered saline pH 7.0-7.2…. It is recommended that unbuffered saline solutions of pH less than 6.0 should not be used for serological testing.”

QUOTIENT BIODIAGNOSTICS

Saline

- Bruce, M. et.al. “A serious source of error in antiglobulin testing.” Transfusion, 1986; 26:177-181

- Evaluated 26 saline solutions from 10 blood banks from multiple sources
- Range of saline pH 4.8 – 8.4
- Some examples of Anti-D, Anti-S, Anti-s, Anti-Fy^a, Anti-Jk^a, Anti-Mi^a and Anti-V^w failed to react or reacted weakly, when AHG testing was performed using saline with pH of $\leq$ 6.5

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Table 2. *The effect of saline solution Ai on the detection of antibodies by the antiglobulin test*

Antibody Specificity	No. tested	Percent Reduction	
		Average	Range
D*	10	16	0–100
Kell*	5	4	0–18
Fy^{a}*	5	38	20–52
Jk^{a}*	4	30	0–100
Jk^{b}*	1	64	—
S*	9	60	6–100
s*	3	85	29–100
U	2	25	11–38
Mi^{a}	1	100	—
V^{w}	5	46	0–100

* Tests involved heterozygous red cells.

Saline

- Bruce, M. et.al. “A serious source of error in antiglobulin testing.” Transfusion, 1986; 26:177-181

- Low pH decreased AHG reactivity by two separate mechanisms.

  - Dissociation of bound IgG during washing

  - Exposure of gamma globulin to acid causes these molecules to swell symmetrically as the pH is lowered, producing conformational changes which renders the IgG molecules incapable of reacting with anti-IgG.

Saline

- Noted that there must be additional factors beyond pH alone, because one saline with a pH of 6.0 consistently produced a 29% reduction in the antiglobulin test reactivity of anti-S. The reduced reactivity could not be mitigated by adjusting the pH.

- Again, from the abstract, they concluded that "improved standardization and sensitivity (in serological tests) could be achieved by using phosphate-buffered saline pH 7.0-7.2 "

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Why?

- Red cells have negative charge at pH = 7
- Antibody molecules have weakly positive charge at pH of 7-7.5
- Enhances attraction between antigens and antibodies during first stage of agglutination / sensitization
- Acid elution - decrease pH, disassociate antibody

Saline

- Rolih, S. et.al. “Antibody detection errors due to acidic or unbuffered saline.” Immunohematology, 1993 Vol 9, pg. 15-18

- Study to replicate the findings from 1986, using unbuffered and buffered saline, and to determine if pH had an effect on solid phase testing.
  pH range tested was 5.5 – 8.0.

QUOTIENT BIODIAGNOSTICS

Saline

- Rolih, S. et.al. “Antibody detection errors due to acidic or unbuffered saline.” Immunohematology, 1993 Vol 9, pg. 15-18

- Conclusion: “We also support the suggestion of these authors that saline solutions used in antibody detection tests be considered as important as the potentiating media, reagent red cell type, or antiglobulin reagent employed. Thus the pH of saline should be strictly controlled at 7.0 – 7.5 when either solid phase testing or hemagglutination testing is performed.”

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Saline – pH changes

- Once unbuffered containers of saline are opened, and exposed to the air, they become more acidic because of transfer of carbon dioxide from the atmosphere to the liquid.

- It is advisable to date a cube of saline upon opening. Thirty days is suggested.

Monoclonal antibodies

- Produced from a single immune cell line
- All antibody molecules are identical
- Highly specific and generally high titre
- No variation from batch to batch
- Large quantities can be made
- Majority are of murine (mouse) origin
- Some (anti-Ds) are of human origin

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Polyclonal antibodies

- Usually of human origin
- Same specificity but produced by different clones of antibody producing cells
- Each molecule is NOT identical
- Each batch needs to be standardized to ensure same reactivity
- Each batch needs to be tested to ensure no other contaminating antibodies are present

The Age of Monoclonal Anti-sera

- Monoclonal anti-sera are not like polyclonal sera
- Monoclonal sera have been grown and manufactured. They may have unusual incubations, unusual spin times, specific room temp requirements, specific saline requirements
- Monoclonal antibodies are all from the same clone and directed at the same epitope – they have no tolerance. To see the specificity, you have to follow the directions, exactly.

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Plastic versus Glass Tubes

"Widely accepted, although little is documented"

The use of plastic rather than glass is especially a concern when using monoclonal antibodies, where to a certain extent every antibody is identical. This means that the overall charge on the antibodies is within a very narrow range. If the antibody charge is incompatible with the charge on the plastic (the surface of which is more open and charged than glass), the antibody can be completely adsorbed.
Glass is much more inert.

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Plastic versus Glass Tubes

Alba Bioscience experience

- One monoclonal antibody that requires a different filter because when processed with the company's filter of choice, the antibody is completely removed during filtration, due to binding to the membrane and housing of the filter.
- Also seen sharp decline of potency of some monoclonal antibodies when stored in plastic.

Processes to Prove “In Control”

- Room temperature probe hooked up to electronic monitoring system (along with refrigerators and freezers) to monitor room temperature in the IRL.
- Thermometers at each desk, and recording the room temperature each time ran a room temperature test.
- ARC - checking the package insert directions before testing – each and every time
- Only use Phosphate buffered saline. Recording a new 30-day expiration at opening.

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Processes to Prove “In Control”

Increased Validation of Laboratory Equipment

Cell Washers – great expansion of validation process

- adequate wash volume; monthly measurement
- decant cycle; residual saline left on cell button
- appropriate spin times for each type of test, (well formed button that is easily dispersed)
- annual performance of cell button test
- maintenance: changing pump tubing, or cleaning with bleach and rinsing multiple times

QUOTIENT BIODIAGNOSTICS

Non-reactive / weak coombs control cells

Issitt PD, Anstee DJ. Applied Blood Group Serology, Third Edition. Durham, NC: Montgomery Scientific Publications, 1998. Pg. 119-120.

- Washing of red cells prior to adding antiglobulin serum must be very thorough, as it actually constitutes a series of dilutions in which the unbound proteins remaining from the original serum or plasma are diluted in successive lots of wash saline.
- It is known that as little as 2mg of IgG/ml remaining at the end of the wash cycle can cause neutralization of antiglobulin serum when it is added.

QUOTIENT BIODIAGNOSTICS

Non-reactive / weak coombs control cells

Issitt PD, Anstee DJ. Applied Blood Group Serology, Fourth Edition. Durham, NC: Montgomery Scientific Publications, 1998. Pg. 119-120.

TABLE 6-1 Cell Washing Before the Addition of Antiglobulin Serum Expressed in Terms of Remaining Non-Bound IgG

	0.1 ml Serum/Saline left on cells		0.05 ml Serum/Saline left on cells		0.01 ml Serum/Saline left on cells	
Saline Wash Volume	2ml	3ml	2ml	3ml	2ml	3ml
µg IgG/ml remaining*						
After 1 wash	750	500	375	250	75	50
After 2 washes	37.5	16.66	9.375	4.16	0.375	0.166
After 3 washes	1.875	0.55	0.234	0.07	0.0018	0.0005

*Starting level 15,000 µg IgG/ml

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Maintenance of Cell Washers

Rygiel SA, Issitt CH, Fruitstone MJ. "Destruction of the S antigen by Clorox" (abstract). Transfusion 1983; 23: 410.

- Sodium Hypochlorite (Clorox) oxidizes the methionine residue at position 29 in the Ss sialoglycoprotein resulting in the loss of S antigen expression
- At levels of 0.0005% Clorox in a 3% suspension, S+s+ RBCs become immediately non-reactive with anti-S; S+s- RBCs required 0.00075% Clorox in PBS

Git R Done

- 57 yr. old white female
- Diagnosis : Ovarian Cancer
- Anemia due to chemotherapy
- Patient has history of Anti-Fy^a, Anti-E, Anti-V

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Pre-transfusion Sample

- B Positive
- Antibody Screen Cell I 3+
  Cell II 0
- DAT Negative with Anti-IgG
- Antibody ID Anti-Fy^a only
- Anti-E, Anti-V not demonstrated at this time
- 2 units of E- Fy(a-) V- packed red blood cell units were cross-matched for outpatient transfusion on July 1.

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July 1

- 10:43 1st unit issued and transfused
- 12:53 2nd unit issued and transfusion started
- 14:20 Patient had chills and nausea (after 300 ml)

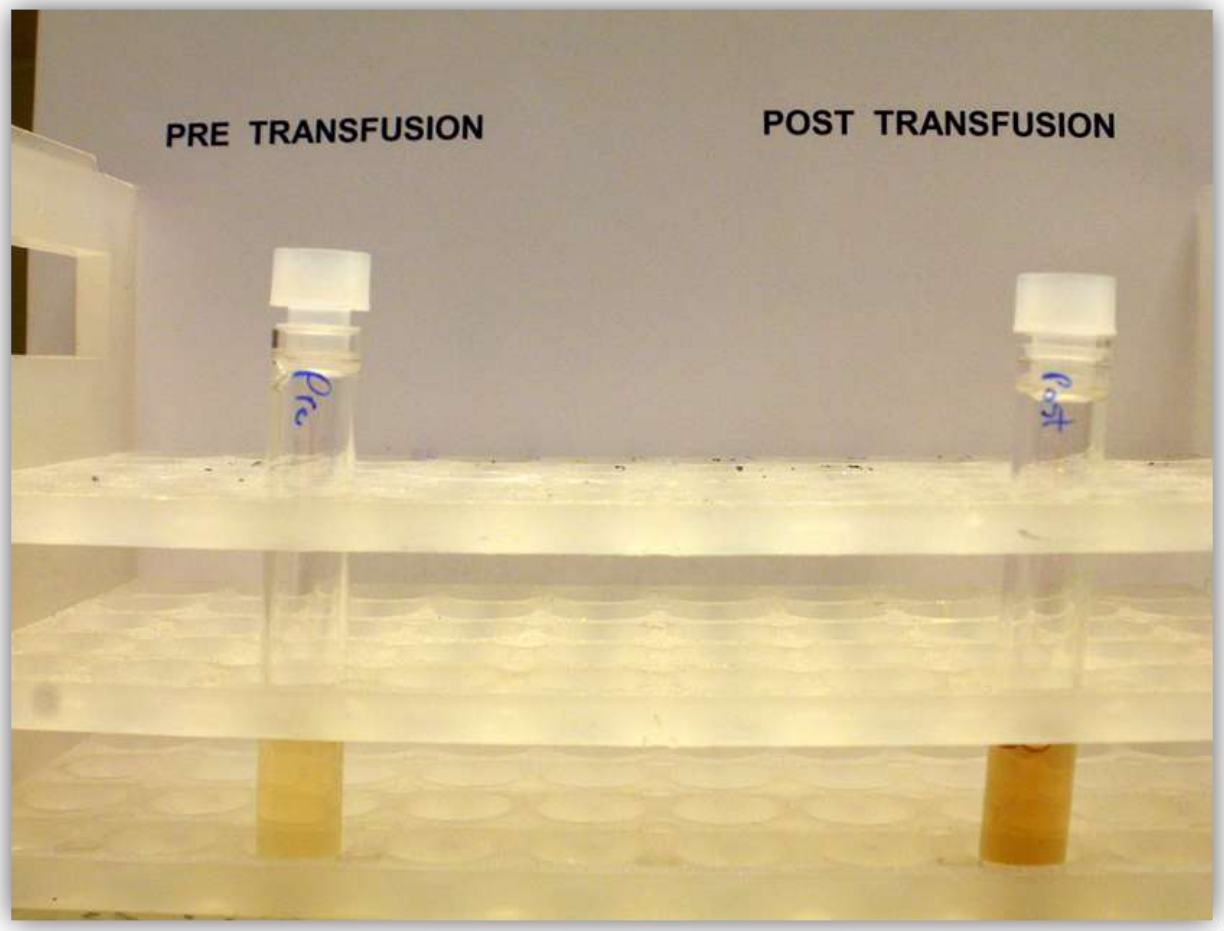
Pre	Post
Temp 97.5	Temp 97.7
BP 133/72	BP 191/86
Pulse 94	Pulse 101
Resp 18	Resp 18

Transfusion Reaction Workup

Post Transfusion Reaction Sample

- Collected July 1 at 16:45
- Plasma appearance = slight amber
- ABO/Rh = B Positive
- DAT = 1+ Anti-IgG
  = 0 Anti-C3b, -C3d
- Antibody ID in plasma = Anti-Fy^a only

Appearance of the Plasma



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Transfusion Reaction Workup

- Eluate: Anti-Fy^{a}
- Crossmatches repeated with post-transfusion sample and pre-transfusion sample

  1st unit crossmatch = compatible

  2nd unit = incompatible (2+) at AHG using anti-IgG

Blood Bank Medical Director suggested clinical monitoring and follow-up. Patient admitted to hospital.

Transfusion Reaction Workup

Repeat E and Fy^a antigen types

1^{st} unit E- V-Fy(a-)

2^{nd} unit E- V- Fy(a+) (2+)

Post Transfusion Urine collected day 2 (July 2) at 11:23

Color Brown

Blood Large (performed on unspun urine)

18-20 RBC/hpf

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Other Lab Values

		Hgb	Hct	BUN	Creat	LDH	Tbili	DBili
6/29		9.1	27.3	NT	NT	NT	NT	NT
7/1	16:45			20	0.92	211	4.1	1.6
7/2	11:56	9.0	27.0	16	0.94	NT	NT	NT
7/3	04:45	8.2	24.8	9	0.73	NT	NT	NT

Patient transfused 2 E- Fy(a-) V- packed RBCs (xmatch compatible)

7/4 11:25 9.8 29.8 Patient discharged to home

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Root Cause Analysis

- 1st unit was antigen typed and crossmatched by evening shift
- 2nd unit was antigen typed and crossmatched by overnight shift
- Overnight shift tech is one of 5 techs covering the entire lab
- She used the cell washer to wash the tubes for the Fy^a antigen type and also for the crossmatch
- After adding the Anti-IgG, she centrifuged the tubes and read the tubes after performing other tasks.
- Estimated time between adding Anti-IgG and reading tubes was "a few minutes".

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Root Cause Analysis

Question ?

Could the missed reactivity be due to the delay in reading the Anti-IgG?

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	Time elapsed between addition of Anti-IgG and reading reactions.				
Donor Cells	**Immediate Spin**	**30 sec**	**60 sec**	**2 min**	**5 min**
Pos Control Fy(a+b+)	**2+**	**2+**	**2+**	**1+**	**0**
Neg Control Fy(a-b+)	**0**	**0**	**0**	**0**	**0**
12FP12914 Fy(a+b-)	**2+**	**2+**	**2+**	**1+**	**1+^{w}**
12FQ03355 Fy(a+b-)	**2+**	**2+**	**2+**	**1+**	**0**
12KE24101 Fy(a-b+)	**0**	**0**	**0**	**0**	**0**

Lessons Learned

- Root Cause Analysis is helpful to determine when, where, who, what and how an error was made and to make changes to prevent future errors
- The manufacturers' instructions for use of all antisera must be read and followed carefully
- When performing IAT, don't delay reading tubes after adding Anti-IgG

Git R Done!

QUOTIENT BIODIAGNOSTICS

The greatest blunders, like the thickest ropes, are often compounded of a multitude of strands."

Victor Hugo, French novelist and poet

Mistakes, obviously, show us what needs improving.

Without mistakes, how would we know what we had to work on?

Peter McWilliams, Life 101

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