



Titers and ABO Incompatible Kidney Transplants

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Objectives For ABO Incompatible Titer

Review and discuss the following:

- The purpose of a titer for incompatible kidney transplant
- Titer methodologies (i.e. Tube vs. Gel)
- Titer protocol used at Carter BloodCare and how it's reported to the physician



ABO Incompatible Kidney Titer



"Okay, Mom ... I'm sorry I re-gifted one of the kidneys you gave me."

ABO Incompatible Kidney Titer

09/03/2015

Carter BloodCare IRL receives an order for :
A titer for an ABO incompatible
kidney transplant....

What?!!



ABO Incompatible Kidney Titer

Background:

ABO Incompatible Kidney Transplants

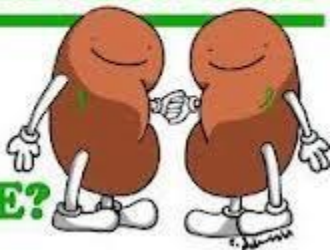
- 1980s- study from Belgium indicated graft survival was 75%
- Japan –late 1980s
- U.S.- 1990s
- Europe- 2000

ABO Incompatible Kidney Transplants

- ❑ **High number of end-stage renal patients**
 - Number of available kidneys is low
 - May use live donors
- ❑ **5-year graft survival rates were comparable between**
 - ABO compatible kidney transplants and ABO incompatible kidney transplants

YOU HAVE A PAIR

**WHY
NOT
SHARE?**



ABO Incompatible Kidney Transplants

□ Challenges

- Antibody Mediated Rejection (AMR)
 - Antibodies against HLA
 - Antibodies against alloantibodies to donor endothelial surface antigens

ABO Incompatible Kidney Transplants

□ Challenges, con't

- Antibodies against the A, B antigens
 - Determining a titer protocol
 - IgM anti-ABO response
 - IgG anti-ABO response (considered more significant)
 - DTT is used to determine the IgG titer

ABO Incompatible Kidney Titer

- ❑ **ABO antibody levels will determine (titer result)**
 - The best time to perform the surgery
 - The effectiveness of desensitization
- ❑ **Titer for the A1, A2 and B antibodies of the patient may be performed depending on the ABO type of the donor**
- ❑ **Monitoring of the titer will be necessary pre- and post-op**

ABO Incompatible Kidney Titer

When do we get a titer order?

- ❑ Pre-surgery for a baseline titer
- ❑ Depending on the titer, we may see multiple titers before and after apheresis procedures if applicable (always stat)
 - The goal is to achieve a titer of <8 or 16 (some say 32 or 64)
 - **Maintain a titer of <8 or 16 post surgery**



ABO Incompatible Kidney Titer

Titer Methodology

□ Traditional Tube Titer (saline)

- Less expensive
- Subjective: difficult to standardize
- Time consuming
- Validated system in place in our IRL lab
- Physicians familiar with tube end points

ABO Incompatible Kidney Titer

Titer Methodology

□ Gel titer (i.e. Ortho)

- Consistent: "more reproducible"
- Expensive
- Need additional materials (i.e. calibrated pipettes, gel system, etc.....)
- End points?



ABO Incompatible Kidney Titer

Titer Methodology

□ Titer by Flow

- Beads carry A or B trisaccharides
- Need equipment and expertise
- Consistent
- Very Expensive!!!



Carter BloodCare

ABO Incompatible Kidney Titer

Titer Methodology at Carter BloodCare-Tubes

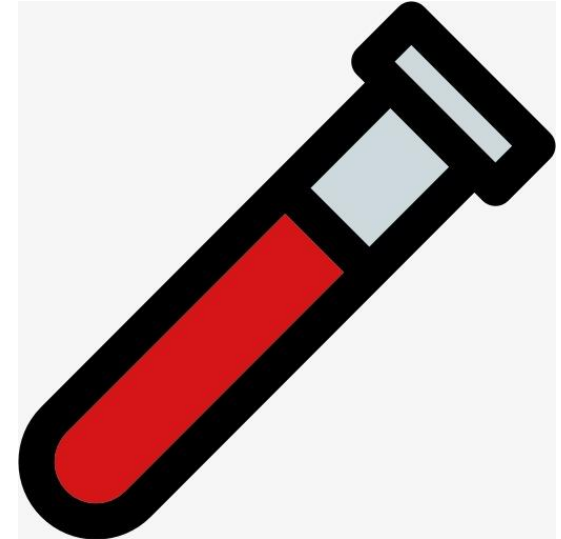
- We already had a validated system
- Most widely used method for ABO incompatible kidney transplants
- Employees are trained to tubes
- Physician is comfortable interpreting & treating with tube end points

ABO Incompatible Kidney Titer

Tube Method

Materials:

- 0.01M DTT
- A1, A2, B cells (2-4%)
- Saline
- Pipettes/Tips
- Lots of Test Tubes



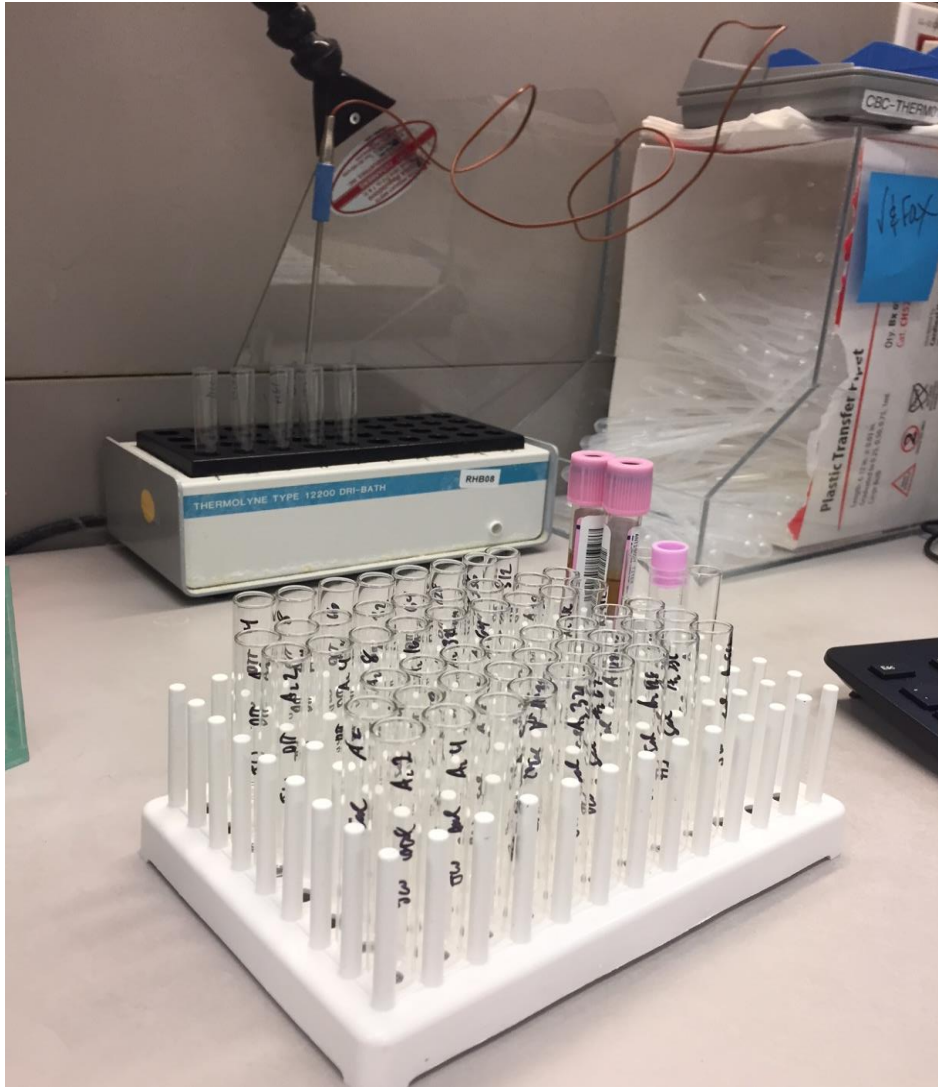
ABO Incompatible Kidney Titer

Method:

1. 1ml of patient plasma + 1ml of saline
2. 1ml of patient plasma + 1ml of DTT
3. Controls for the DTT are tested:
 - Negative control using anti-P₁
 - Positive control using anti-Fy^a
 - Saline control using anti-P₁



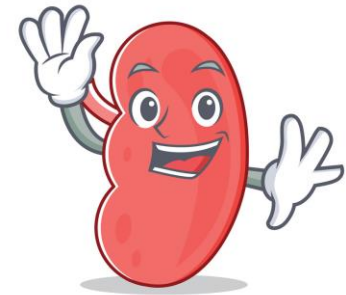
ABO Incompatible Kidney Titer



ABO Incompatible Kidney Titer

Methods:

1. All tubes incubate at 37°C for 30 minutes
2. Perform titration studies (titer to 512) and controls:
 - DTT treated sample
 - Control (saline) sample



ABO Incompatible Kidney Titer

Methods:

1. DTT treated titer sample

- Label tubes for A1, A2, B titer
- Add 2 drops of the appropriate dilution to each tube
- Add 1 drop of appropriate red blood cells

ABO Incompatible Kidney Titer

Methods:

1. Saline control titer sample

- Label tubes for A1, A2, B titer
- Add 2 drops of the appropriate dilution to each tube
- Add 1 drop of appropriate red blood cells

ABO Incompatible Kidney Titer

Methods:

- 1. Incubate all tubes at room temp for 30 minutes, centrifuge, shake**
- 2. Incubate all tubes at 37°C for 30 minutes, wash, add anti-IgG, spin, shake**
- 3. Total time: 2.5 - 3 hours minimum**

ABO Incompatible Kidney Titer

Interpretation:

Titer is the highest plasma dilution that gives 1+ positive reaction

Tube	2	4	8	16	32	64	128
RT (saline)	2+	2+	1+	1+	0	0	0
RT (DTT)	1+	1+	0	0	0	0	0
IAT (saline)	2+	2+	1+	1+	1+	1+	0✓
IAT (DTT)	2+	2+	1+	1+	0✓	0✓	0✓

ABO Incompatible Kidney Titer

Interpretation:

- Reactivity with the saline and no reactivity with DTT- IgM
- Reactivity with saline and DTT= IgG and IgM antibody
- Reactivity with DTT= IgG antibody

ABO Titer Report-

Anti-B	Reactive by						vs.- B cells
Anti-	Reactive by						
Anti-	Reactive by						
Anti-	Reactive by						
Anti-	Reactive by						
Anti-	Reactive by						

ADDITIONAL DETAILS

ABO Incompatible Titer Results (By Testing Phase)	IgG Antibody Present (DTT Treated Sample)	Total (IgG & IgM) Antibodies Present (Saline Treated Sample)
Room Temperature (RT)	4	16
Indirect Antiglobulin Testing (IAT)	16	64



ABO Incompatible Kidney Titer

Case 1 September 2015 -

- An ABO incompatible kidney transplant surgery is to be scheduled
- Patient is O positive & Donor is B positive
- ABO incompatible titers will be needed to monitor the patient
- A baseline titer will be needed
- Titers before and after plasma pheresis will be ordered, before and after surgery



ABO Incompatible Kidney Titer

Case 1

09/03/15- 1st titer (anti-B)

Titer 1	RT	AHG
IgM + IgG	32	64
DTT (IgG)	4	32

Post Apheresis

Titer 2	RT	AHG
IgM + IgG	16	16
DTT (IgG)	Negative	16



ABO Incompatible Kidney Titer

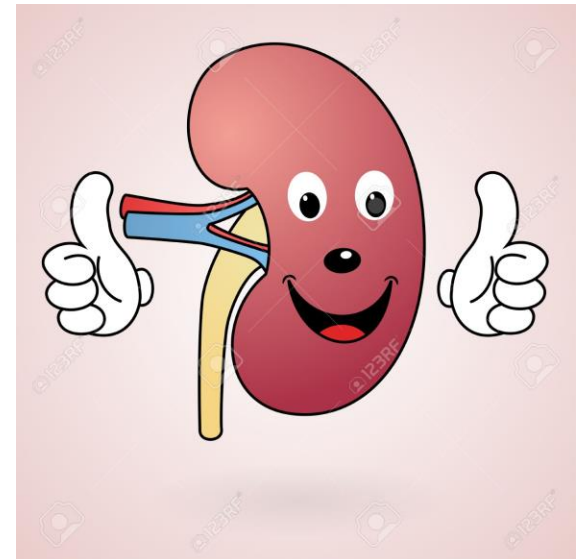
Case 1

10/26/15- 1st titer (anti-B) (41 titers later) –Transplant performed

Titer 1	RT	AHG
IgM + IgG	8	16
DTT (IgG)	2	16

Post Apheresis

Titer 2	RT	AHG
IgM + IgG	16	16
DTT (IgG)	Negative	16



ABO Incompatible Kidney Titer

Case 1

Monitoring the titer Post-Op (34 titers performed)

Titer	RT	AHG
IgM + IgG	<2	4
DTT (IgG)	<2	4

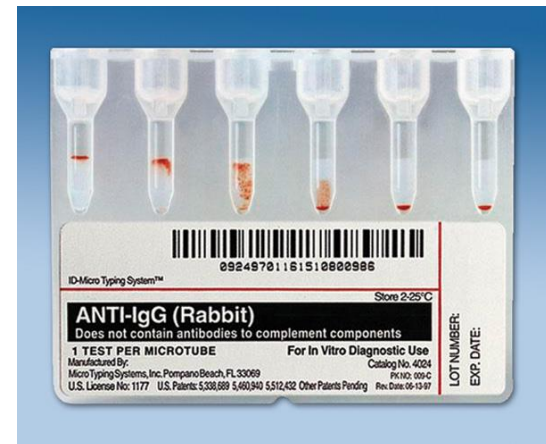
75 titers total performed from 09/03/15 - 01/19/16

ABO Incompatible Kidney Titer

Gel Method

Materials:

- IgG gel cards
- NaCl (buffer) gel cards
- .8% A1, A2, B cells
- Pipettes/tips
- Gel incubator/centrifuge
- Test tubes/PBS



ABO Incompatible Kidney Titer

Gel Method:

1. Perform titration studies with saline (PBS)
2. Prepare 0.8% cell suspension for the appropriate antigen (A1, A2, B)
3. Add 50ul of the cells to a gel card (buffer) and 50ul of the cells to a IgG gel card
4. Add 25ul of the diluted sample to the appropriate well of each card

ABO Incompatible Kidney Titer

Gel Method:

5. Incubate the buffer card at room temperature for 10 (or 15) minutes
6. Incubate the IgG card at 37°C for 15 minutes
7. Spin both cards for 10 minutes

ABO Incompatible Kidney Titer

Interpretation:

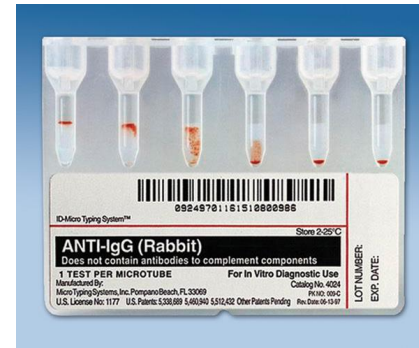
Titer is 16; the highest plasma dilution that gives 1+ positive reaction

Dilution	1	2	4	8	16	32	64	128
Strength	3+	2+	2+	1+	1+	0	0	0

ABO Incompatible Kidney Titer

What's Best?

Tube vs. Gel



ABO Incompatible Kidney Titer

Tube vs. Gel

- Gel is more sensitive? (2 fold higher per Aditya Birla Memorial Hospital, Pune, Maharashtra, India)
- Gel and Tubes are comparable? (Johns Hopkins)



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ABO Incompatible Kidney Titer

Pam's Study

Patient A	RT	AHG
Gel	4	16
Tube	16	16

Patient B	RT	AHG
Gel	2	8
Tube	4	8

ABO Incompatible Kidney Titer

Conclusion:

- ❑ ABO titers are imperative to determine when to perform an incompatible ABO kidney transplant and the effectiveness of desensitization (success)
- ❑ Multiple titers will be performed before and after treatment as well as after the surgery
- ❑ The titer methodology (tube vs. gel) will achieve similar results but gel titers have a shorter turn around time. More investigation is needed.....



Questions????



“How’s that kidney transplant going? ... I need him back on the field – pronto!”

ABO Incompatible Kidney Titer

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