The ABO's of Incompatible Organ Transplantation

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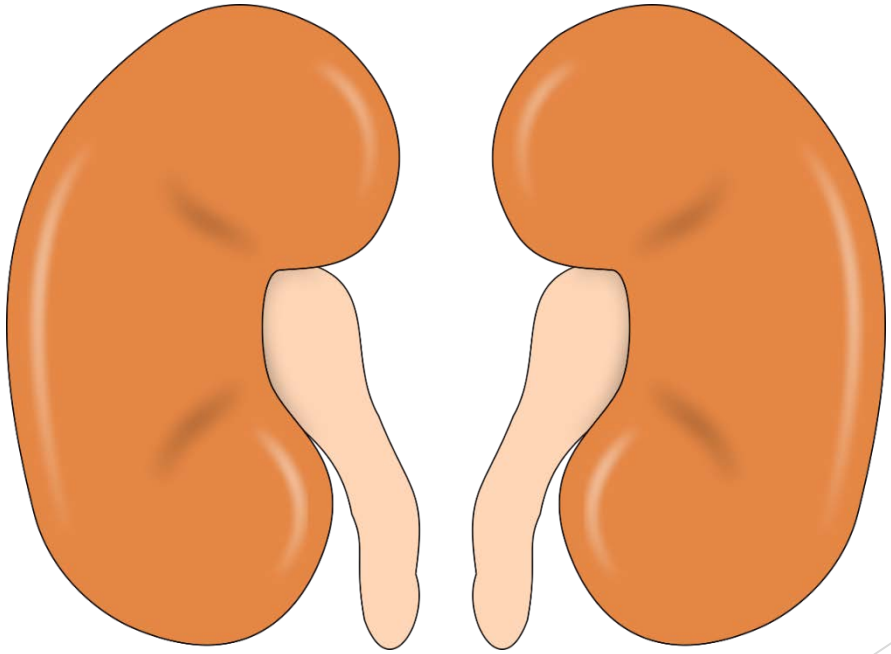
End Stage Renal Disease

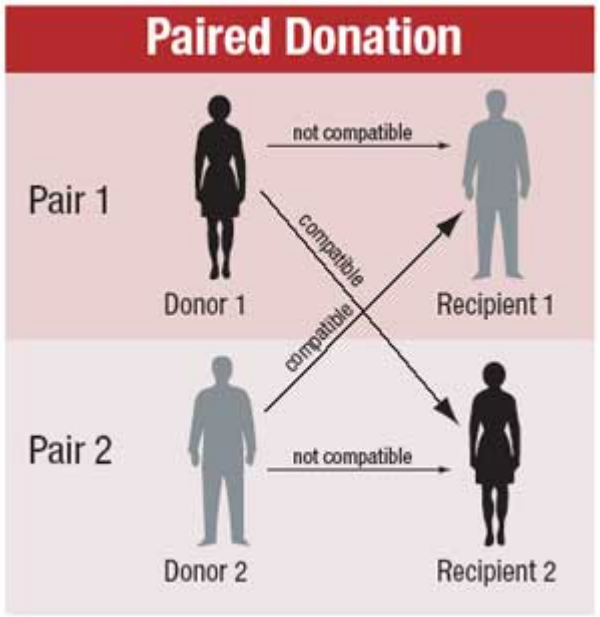
- Over 100,000 Americans currently waiting for kidney transplant
- Average wait time is over 5 years
- Leading causes of renal failure:
  - Type 2 Diabetes
    - 39% Emory; 32% US
  - Hypertension
    - 32% Emory; 21% US
- 9% increase in new patients added to Emory's transplant list in 2016



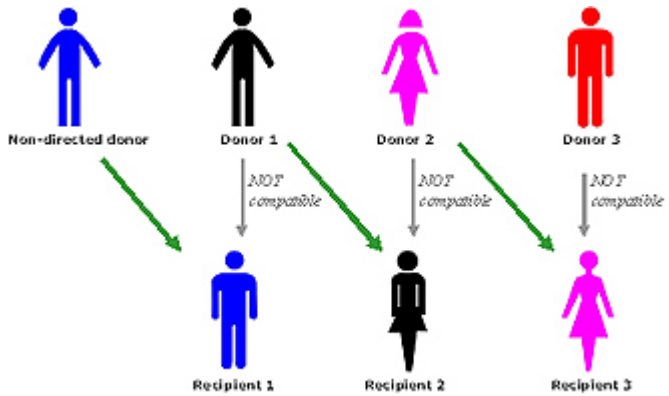
Deceased and Living Donors

- Most kidneys for transplant come from deceased donors
- A recipient may have a living donor such as a family member
  - 2014: 2693 out of 17814 kidney transplants involved living donors
- If recipients have living donors that are not a match for them but are for another recipients, a paired donation may take place
- 2015: 54% of living donor transplants were unrelated

Paired Donation



Sometimes it gets even more complicated: Never Ending Altruistic Donor (NEAD) chain



Patient Survival Post-transplant

	Living Donor	Deceased Donor
1 year post-transplant	98.75%	96.5%
3 years post-transplant	96.02%	91.74%

Shortage of available organs

- 2011: 5000 patients died while waiting on a kidney.
- ABO incompatibility was once an absolute contraindication for transplant, but now…
- 30% of kidney transplants performed in Japan are ABO incompatible.
- From 1995 – 2010, less than 1% of kidney transplants performed in the US were ABO incompatible; but things are changing…

Case Study #1

- 70 year old AA male
- ESRD (End Stage Renal Disease) secondary to DMII (Diabetes)
- On hemodialysis since 2012: current schedule MWF
- Patient only had one kidney left due to previous nephrectomy for renal cell carcinoma
- Patient was entered into the National Kidney Paired exchange program
- Received kidney on 1/6/2016

Case Study #1

- Donor was 69 year old male from Indiana (organ was shipped to GA)
- Patient is now doing well although his baseline creatinine is slightly elevated.
- Patient has not developed any antibodies to donor HLA antigens.
- Also….
  - Patient is group B
  - Donor is group A2

ABO Incompatible Kidney Transplantation (ABOiKT)

Outcomes now comparable to ABO compatible transplantation

Most common scenario involves A2 or A2B kidney for B recipient, possibly with preconditioning

Preconditioning principles:

- B cell depletion
  - Splenectomy – rarely used
  - Anti-CD20 (Rituximab)
- Antibody depletion
  - Plasma exchange with or without immunoadsorption column
- Antibody measurement
  - Titration

“I was told there would be no math!”

Most Lab results (Chem; Heme; Coag; etc)

Chol: 232

MCV: 83

INR: 1.2

Glucose: 180

PTT: 32

Hgb: 13.5

Numbers that are reproducible lab to lab

Most blood bank results are words

Also reproducible lab to lab

Group and type: A pos

Antibody screen: positive

Antibody identification: Anti-Jka

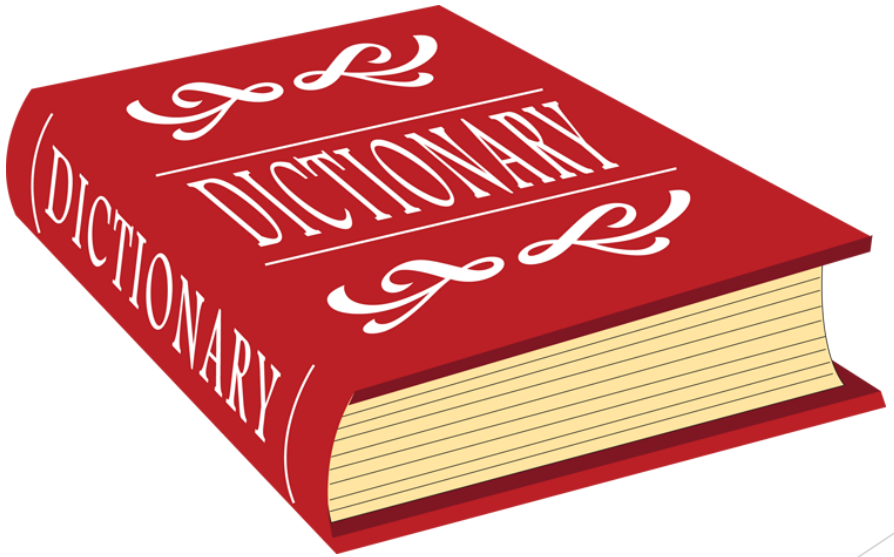
DAT: positive with C3

Eluate: panagglutinin

Occasional numbers:

*FY*B* (-67c) / *RHCE**ce (733G)

Kleihauer Betke - 1% fetal cells



Then there are titers (titrations)….

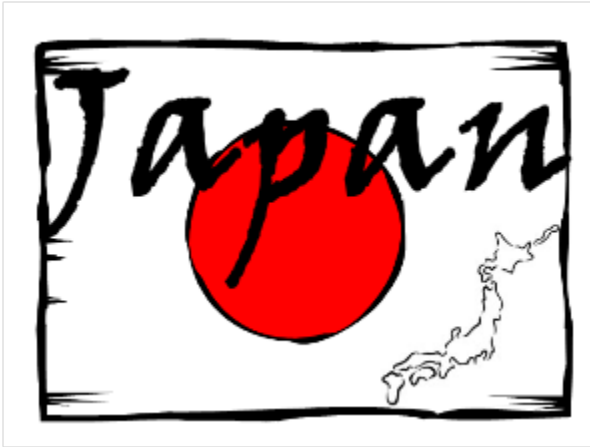
Reported as reciprocals or ratios… numbers!

Are titers reproducible lab to lab?

Journal: Xenotransplantion; March2006

2003 (1st survey)

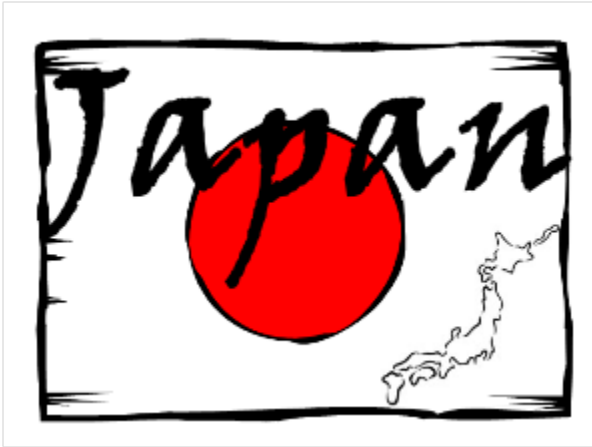
- Serum from 6 healthy volunteers sent to 29 labs for anti-A/B titers to be performed by their customary methods:
  - IgM levels varied by 32 fold
  - IgG levels varied by 256 fold



Journal: Xenotransplantion; March2006

2004 (2nd survey)

- Serum from 6 healthy volunteers sent to 38 labs for anti-A/B titers to be performed by a standard protocol:
  - Variation reduced to below 8 fold



Journal: Transplantation; Dec 2007

Sweden and Germany

- Samples from 21 healthy blood donors sent to three labs for anti-A/B titer using their own methods:
  - Results differed by 3 dilutions
- The same samples were repeated using gel method and the same testing cell:
  - Results differed by 1 dilution

Journal: Transfusion; November 2016

United Kingdom

- 98 patients had hemagglutinin titers performed in 8 local facilities by their own methods and subsequently frozen and sent to the central laboratory where repeat titers were performed by the gel method:
  - 43 of the patients had a greater than 1 dilution difference between the local and central lab

AABB – Fenwal Scholarship Award 2012
Precision of Antibody Titration in Gel vs Tube

- 42 samples with RBC antibodies
- Samples to be tested by tube and gel for titer and score
- Each sample tested three times by each method
- Results in gel showed slightly more consistency:
  - No difference in titer when repeated
  - 7% difference in score when repeated
- Tube testing showed more variation:
  - 17% difference of more than 1 dilution for titer
  - 28% difference in score

College of American Pathologists Proficiency Testing Program: Titer Surveys 2014 - 2016

Anti-D Tube: 37/IgG	Most responses	Range of responses	Anti-D: IgG Gel	Most responses	Range of responses
2014: ABT 02	256	32 – 2048		512	256 - 2048
ABT 05	256	8 - >2048		512	512 - 2048
2015: ABT 02	32	8 - >2048		128	32 - 512
ABT 05	128	16 - >2048		512	256 - 1024
2016: ABT 02	256	8 - >2048		512/1024 (tie)	256 - >2048
ABT 05	16	Neg - 512		64	16 - 256

College of American Pathologists Proficiency Testing Program: Titer Surveys 2014 - 2016

Conclusions:

- Gel produces a narrower range of variation than tube.
- Gel results are 1 – 2 dilutions higher than tube.

Organ Procurement and Transplantation Network (OPTN)

Final Rule effective 2/1/2015

- In order for a group B candidate to be matched with to a blood type A(non-A1) or AB(non-A1B)…the candidate must have an IgG antibody titer value of less than 1:8
- OPTN may allow certain transplant centers to establish their own threshold. (Emory's threshold is 1:8 or less: titers to be repeated every 90 days to maintain eligibility.)
- But, if no one can get the same result….

Houston, we have a problem….

I know…let's develop a standard protocol!

AABB – Fenwal Scholarship Award 2015
Impact of Uniform Methods on Inter-laboratory Antibody Titration Variability

- New York Blood Center – contributor was Beth Shaz, MD
- 2008: CAP and Biomedical Excellence for Safer Transfusion (BEST) Committee introduce

"The Uniform Method"

- ABT survey for anti-D:
  - Uniform tube = 30 min / 37 degrees/ anti-IgG (endpoint = w+)
  - Uniform gel = IgG card / 15 min (endpoint = 1+)

But…did it work?

AABB – Fenwal Scholarship Award 2015
Impact of Uniform Methods on Inter-laboratory Antibody Titration Variability

Conclusion:

"Application of uniform methods did not show statistically significant interlaboratory reduction in titer variance consistently except on rare occasion."

Factors contributing to interlaboratory inconsistency:

- Diluent
- Incubation time
- Cut off reading
- Testing cell
- Methods:
  - Tube
  - Gel
  - and ?????

Solid phase titrations?

(Not available in US…yet)

Case Study #2

- 43 year old AA male
- DMII and HTN diagnosed at age 19
- End stage renal disease currently on hemodialysis schedule TThSat
- Received kidney on 12/20/15
- Donor was deceased 4 year old AA male (both donor kidneys were transplanted)
- New process that allows adult recipients to receive kidneys from pediatric donors

Case Study #2

- Currently patient has excellent renal function despite low levels of HLA antibodies against donor antigens
- Patient: group B
- Donor: group A2
- IgG Anti-A titer (10/8/15): 1:4

Typing Donors for A1

OPTN rules for ABO typing of deceased donors:

- Testing must be performed only on pretransfusion samples
- If a deceased donor is blood type A, a subtyping must be performed.
- A second subtyping is to be performed either on a second sample or by a second lab.

(Lifelink has one determination done at the donor hospital and one at a reference lab.)

Typing Donors for A1

"If you get the ABO right, you're almost there."

Unattributed source

Lectins:

- Carbohydrate binding proteins present in the seeds of many plants
- Anti-A1 lectin is made from *Dolichos biflorus* seeds

Typing Donors for A1

True or False

Anti-A1 (*Dolichos biflorus* lectin) reacts with the A1 antigen on red cells.

Typing Donors for A1

Issitt:

“The raw lectin has anti-A but not anti-A1 specificity.

It is easy to find a dilution at which the lectin will agglutinate A1 but not A2 cells.

The ability of diluted Dolichos lectin to agglutinate A1 but not A2 cells is related to the number of copies of the A antigen on those cells.”

Typing Donors for A1

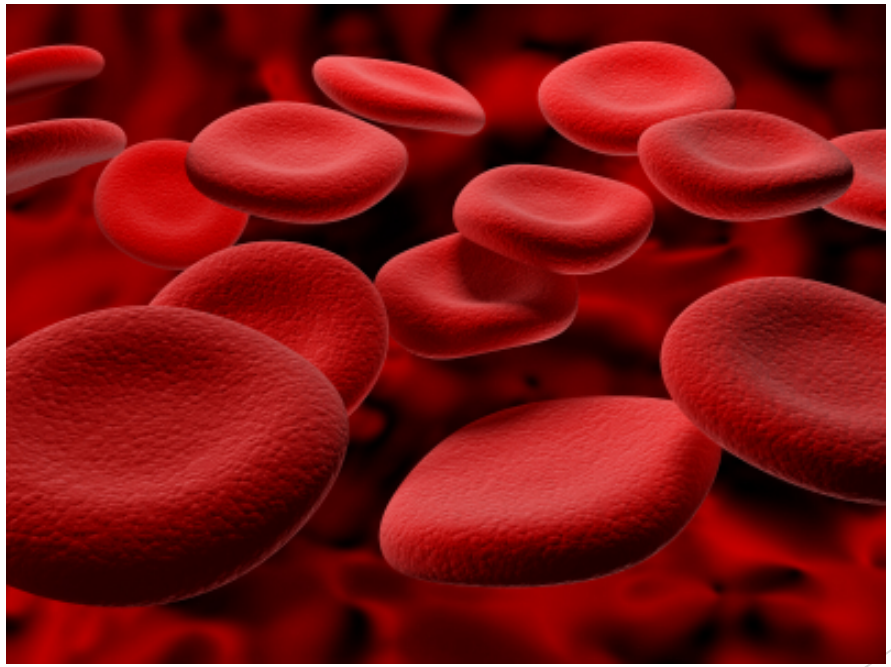
Number of A antigen sites:

A1: 1,000,000

(80% of population)

A2: 220,000

(20% of population)



Typing Donors for A1

Insert for one vendor's Anti-A1 lectin:

"A1 and A1B should produce reactions of at least 2 – 4+."

but…

"Reactions of 1+ or weaker should be investigated before it is assumed that the red cells carry a weakened expression of A"

Typing Donors for A1

OPTN statement:

"From the perspective of transplant safety for the use of non-A1 organs, any RBC reaction with anti-A1 lectin…should be regarded as A1 reactive by the transplant center unless proven otherwise."

Typing for A1: Case Study #3 (if you absolutely, positively have to know…)

- 73 year old AA male
- Carcinoma of lung with brain metastases; admitted for possible stroke
- Blood typing results:
  - Anti-A: 0
  - Anti-B: 0
  - A1 cells: 0
  - B cells: 3+
    - Anti-A,B: 2+
    - Anti-A1: 0

Typing for A1: Case Study #3 (if you absolutely, positively have to know…)

Sent to IRL for ABO sequencing:

- Genotype: *ABO*O.01.01, ABO*AW.30.01*
- 1 allele = O
- 1 allele = Ax

Thank you!

- Dolichos biflorus

- Questions?