

# Reactivity Everywhere! Where do you go?

## *Our Mission:*

*To provide blood and support services  
that meet the needs of patients, donors,  
physicians and hospitals.*

# Case 1

- 3/3 patient admitted to hospital for venous stress due to Cellulitis
- Hgb 8.1
- WBC 8.9
- Not transfused in last 3 months

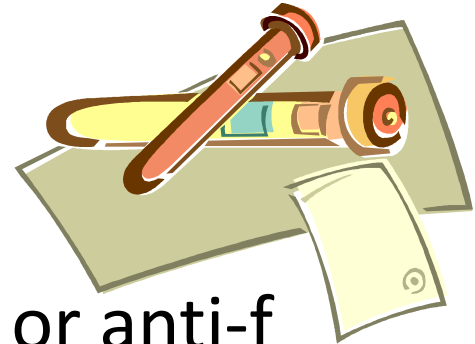


# March 6

## Sample Arrives in IRL



- Hgb 6.0
- WBC 11.55
- Hospital reports possible anti-e or anti-f
- A negative
- DAT Positive
  - 3+ IgG, complement negative
- Not recently transfused
  - First impression is this sounds like an auto antibody



# Why we're thinking WARM Auto

- DAT is positive, but no recent transfusions
- Patient is Rh Negative
  - Most likely e+
- Hospital is reporting a possible anti-e

# Consistent with Warm Auto Antibody

[illegible]

# Ficin Treated Red Cell Panel

	Rh						Kell		Duffy		Kidd		MNSs				Test Results	
	D	C	c	E	e	f	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	M	N	S	s	30' 37	IAT
1	+	+	0	+	0	0	0	+	+	+	+	+	+	0	+	0	0	0
3	+	0	+	+	0	0	0	+	0	+	+	+	0	+	0	+	0	0
7	0	0	+	0	+	+	+	+	0	+	0	+	+	+	0	+	1+	2+
9	0	0	+	0	+	+	0	+	0	+	+	0	+	+	+	0	1+	2+
10	+	+	0	0	+	0	0	+	+	+	0	+	0	+	0	+	1+	M+

Anti-e detected could still be  
Warm Auto Antibody

# Negative Acid Eluate!

[illegible]

# Time for the Ether

## Potential Health Effects

### Inhalation:

Irritant. **General anesthesia by inhalation can occur.**

Continued exposure may lead to respiratory failure or death.

Early symptoms include irritation of nose and throat, vomiting, and irregular respiration, followed by dizziness, drowsiness, and unconsciousness.

### Ingestion:

Irritating to the mucous membranes. Ingestion of 1 or 2 ounces may be fatal. Because of volatility the stomach becomes distended, which may cause belching. Other symptoms can include vomiting, unconsciousness, and coma.

### Skin Contact:

Irritating to the skin and mucous membranes by drying effect. Can cause dermatitis on prolonged exposure. May be absorbed through skin.

### Eye Contact:

May cause irritation, redness and pain. Prolonged exposures to high concentrations of vapor can cause eye damage.

### Chronic Exposure:

Repeated exposures may be habit forming. Prolonged exposures may result in headache, drowsiness, excitation, and psychic disturbances. Teratogenic effects are possible.

### Aggravation of Pre-existing Conditions:

Persons with pre-existing skin disorders or eye problems or impaired liver, kidney or respiratory function may be more susceptible to the effects of this substance. Alcoholic beverage con

- Ether Eluate negative
- Now everyone is dizzy

Screening Cell	Peg IAT
I	0
II	0
III	0





# Discussion

## What we Know

- DAT Positive IgG
- LISS negative
- Anti-e in FICIN
- Eluate negative (Acid and Ether)

## What we need to know

1) Why is the DAT Positive?

- Requested Drug List to be faxed to IRL

2) What is the nature of the anti-e?

- Auto or allo?

# Anti-e detected in PEG

	Rh						Kell		Duffy		Kidd		MNSs				Test Results
	D	C	c	E	e	f	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	M	N	S	s	Peg IAT
1	+	+	0	+	+	0	0	+	0	+	+	+	0	+	0	+	M+
2	+	+	0	0	+	0	0	+	+	0	0	+	+	+	+	0	1+
3	+	0	+	+	0	0	0	+	+	+	0	+	+	0	+	+	0
4	+	0	+	0	+	+	0	+	0	0	+	+	+	0	0	0	2+
5	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	2+
6	0	0	+	+	+	+	0	+	+	+	+	0	+	0	+	0	1+
7	0	0	+	0	+	+	+	+	0	+	+	0	+	0	0	+	1+
8	0	0	+	0	+	+	0	+	+	0	+	0	0	+	0	+	2+
9	0	0	+	0	+	+	0	+	0	+	+	+	+	0	0	+	2+
10	+	+	0	0	+	0	+	+	0	+	0	+	+	+	+	+	M+
11	+	0	+	+	+	+	0	+	+	0	+	+	+	0	+	+	M+

# Rule Out Panel all other common antibodies

	Rh						Kell		Duffy		Kidd		MNSs				Test Results
	D	C	c	E	e	f	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	M	N	S	s	Peg IAT
1	+	0	+	+	0	0	0	+	0	+	+	0	+	0	+	0	0
2	+	0	+	+	0	0	0	+	+	0	0	+	0	+	+	+	0
3	+	0	+	+	0	0	+	+	0	+	0	+	+	0	0	+	0
4	+	0	+	+	0	0	0	+	0	+	+	+	0	+	0	+	0
5	+	w	+	+	0	0	+	+	0	+	+	0	+	0	0	+	0
6	+	w	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0
7	+	+	0	+	0	0	0	+	+	0	+	+	+	+	0	+	0
8	+	+	0	+	0		0	+	+	0	+	+	0	+	0	+	0

# Patient Phenotype

rr, K- Fy(a+b-) Jk(a-b+) S- s+

- Some e+ patients lack e epitopes
- Can make a clinically significant anti-e
- Would require e-negative blood



# How should we want to transfuse

They are phenotypically  
matched units

D-, C-, E-, e+, K-, Fy(b-) Jk(a-), S-

2 choices

1. Send phenotypically  
matched (e+) nonreactive  
with LISS
  - Risks transfusion reaction  
due to e
2. Sent e- units that are not  
phenotypically matched  
nonreactive in PEG
  - Exposure to E  
antigen; not hospital's  
preference

# Drug List has arrived

- Aspirin
- Balsm-Trypis-Caster Oil
- Famotidine
- Heparin
- Hydralazine
- Insulin
- Nystatin
- Piperacillin/Tazobactam
- Pravastatin
- Sodium Chloride
- Acetaminophen
- Alum-Magnesium Hydroxide-Simethicone
- Diazepam
- Docusate Sodium
- Fentanyl Citrate
- Hydrocodone
- Ondansetron HCL
- Polyethylene
- Zolpidem Tartrate
- Vancomycin

## ◆ APPENDIX 17-1

Drugs Associated with a Positive DAT and/or Immune Hemolytic Anemia

Drug		Method of Detection	
Aceclofenac		+Drug	
Acetaminophen		+ Drug	
Acyclovir		DT	
Aminopyrine		DT	
Amoxicillin		DT	
Amphotericin B		+ Drug	
Ampicillin		DT	+ Drug
Antazoline		+ Drug	
Azapropazone	AA	DT	
Butizide		+ Drug	
Carbimazole	AA	DT	+ Drug
Carboplatin	AA	DT	+ Drug
Carbromal		DT	
Cefamandole		DT	
Cefazolin		DT	
Cefixime		DT	+ Drug
Cefotaxime		DT	+ Drug
Cefotetan	AA	DT	+ Drug NIPA
Cefoxitin	AA	DT	+ Drug
Ceftazidime	AA	DT	+ Drug
Ceftizoxime		DT	+ Drug
Ceftriaxone		+ Drug	
Cefuroxime		DT	
Cephalexin		DT	
Cephalothin		DT	+ Drug NIPA
Chloramphenicol	AA	DT	
Chlorinated hydrocarbons	AA	DT	+ Drug
Chlorpromazine	AA	+ Drug	
Chlorpropamide		+ Drug	

(Continued)

## ◆ APPENDIX 17-1

Drugs Associated with a Positive DAT and/or Immune Hemolytic Anemia (Continued)

Drug		Method of Detection	
Cimetidine		DT	+Drug
Ciprofloxacin		+Drug	
Cisplatin		DT	NIPA
Cladribine	AA		
Clavulanate			NIPA
Cyanidanol	AA	DT	+ Drug
Cyclofenil	AA		+ Drug
Cyclosporin		DT	
Diclofenac	AA	DT	+ Drug
Diethylstilbestrol		+ Drug	
Diglycoaldehyde			NIPA
Dipyron		DT	+ Drug
Erythromycin		DT	
Etodolac			+ Drug
Fenoprofen	AA		+ Drug
Fluconazole		DT	+ Drug
Fludarabine	AA		
Fluorescein		DT	+ Drug
Fluorouracil			+ Drug
Furosemide			+ Drug
Hydralizine		DT	
Hydrochlorothiazide		DT	+ Drug
Hydrocortisone		DT	+ Drug
9-Hydroxy-methyl-ellipticinium			+ Drug
Ibuprofen		+Drug	
Imatinib mesylate		DT	
Insulin		DT	
Isoniazid		DT	+ Drug
Levodopa	AA		
Levofloxacin		DT	+Drug
Mefenamic acid	AA		



# Drugs that cause positive DATs and/or DIHA

- Hydralazine
- Acetaminophen
- Insulin
- Vancomycin
- **Piperacillin**





<div> <div>Rh</div> <div>(D,E,c,E,e)</div> </div>	<div> <div>Catechin, diclofenac, glafenin, hydrochlorothiaide,</div> <div>ibuprofen, moxalactam, nomifensin,</div> <div><b>piperacillin</b>, quinine, rifampin,</div> <div>streptomycin, sulindac, tolmetin</div> </div>
I	Chlorpheniramine, fluorouracil, Isopaque, nitrofurantoin, nomifensine, rifampin, thiopental
P	Elliptinim, melumine iothalamate, nomifensine
Kell	Glafenine, trimethoprim
Lutheran	Elliptinium, rifampin
Kidd	Chlorpropamide
MNS	Streptomycin
H	Sulfamethoxazole

# How to distinguish between anti-e or Piperacillin?

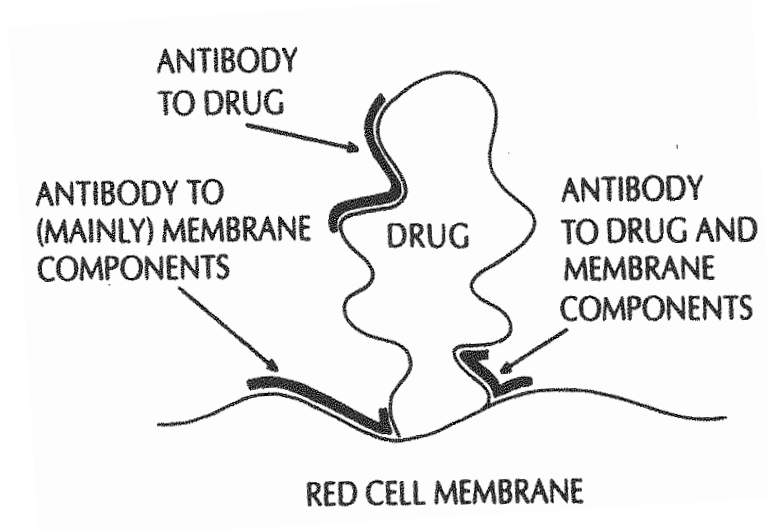
- Perform a drug study?

- Result won't be as helpful as you might think...

1. Study by ARC showed that when testing with drug coated cells – 91% of their donors already have piperacillin antibody!

# Test Panel Drug Adsorption

Neat Plasma	Drug coated RBC's	Uncoated RBC's
15 ' 22C	1+	0
30' 37C	0	0
IAT	M+	0



# How to distinguish between anti-e or Piperacillin

## – Perform a drug study?

- Result won't be as helpful as you might think...
- 1. Study by ARC showed that when testing with drug coated cells – 91% of their donor already have piperacillin antibody!
- 2. “Immune complex” method – recommended method for testing for piperacillin antibodies requires combination of drug and plasma (1:1)

# Test Panel “Immune Complex”

	e+ cell			Ficin tested e+ cell		
	30' 22C	30' 37C	IAT	30' 22C	30' 37C	IAT
Plasma + drug	2+	2+	2+	4+	4+	4+
Plasma + PBS	0	0	0	0	0	0
Drug + PBS	0	0	0	0	0	0
Eluate + Drug	0	0	1+	1+	2+	2+
Eluate + PBS	0	0	0	0	0	0

# How to distinguish between anti-e or Piperacillin

## – Perform a drug study?

- Result won't be as helpful as you might think...
- 1. Study by ARC showed that when testing with drug coated cells – 91% of their donor already have piperacillin antibody!
- 2. Immune complex method – recommended method for testing for piperacillin antibodies requires combination of drug and plasma (1:1)
  - » Sample we have already has piperacillin in it and we know that it's positive
- 3. Could test the eluate and drug against cells
  - Piperacillin antibodies do not always react in the eluate
- 4. Drug study wasn't ordered (good)
  - Time consuming, expensive, results vary, wouldn't learn anything new

Requested a sample before drug was  
administered

Summary of the results

**NEGATIVE!!!!!!**

**NEGATIVE!!!!!!**

**NEGATIVE!!!!!!**

# Summary of results

DAT



Panel

PEG

Negative

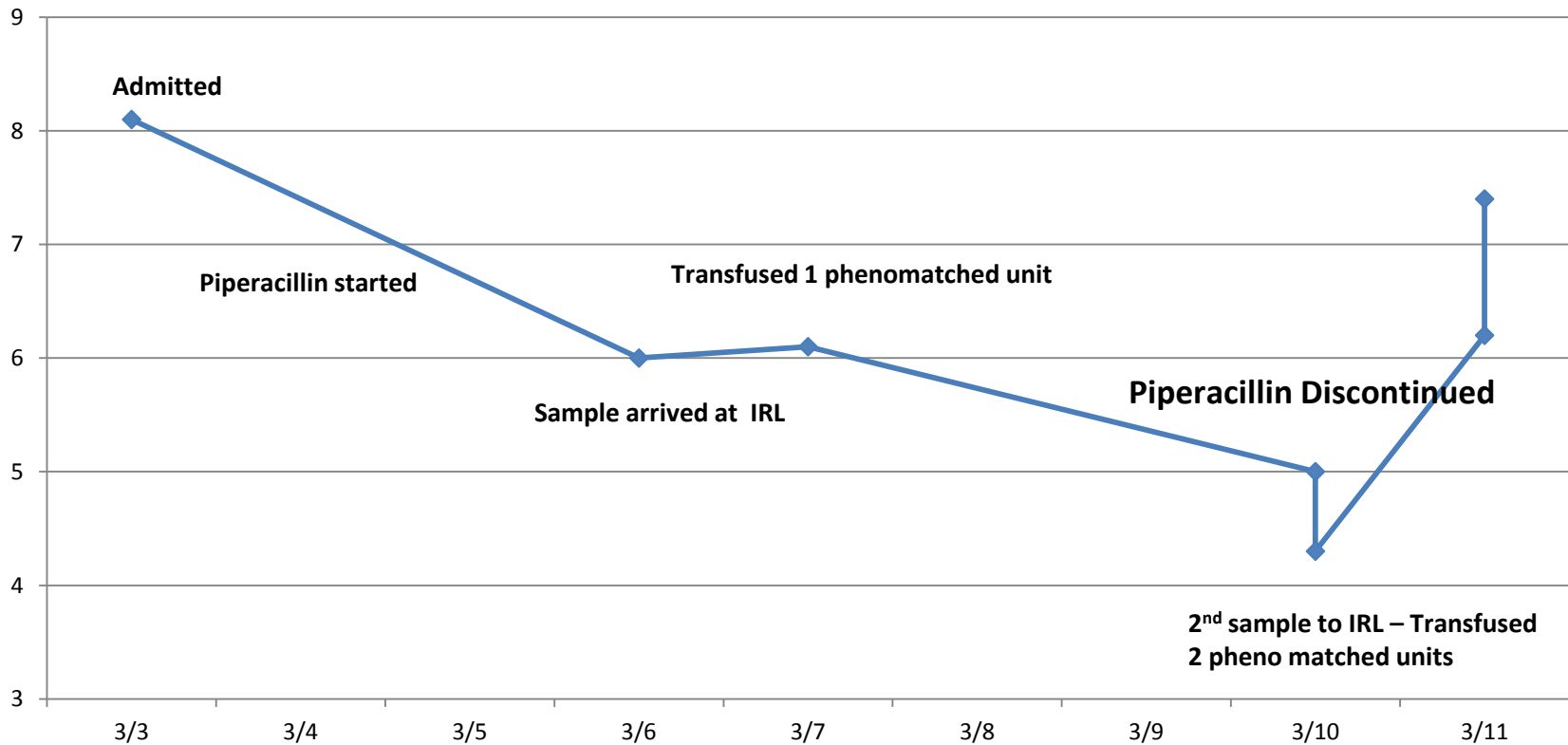
Negative

- Sent 2 phenotypically matched units nonreactive at LISS IAT
  - D-, C-, E- , K- , Fy(b-) Jk(a-), S-

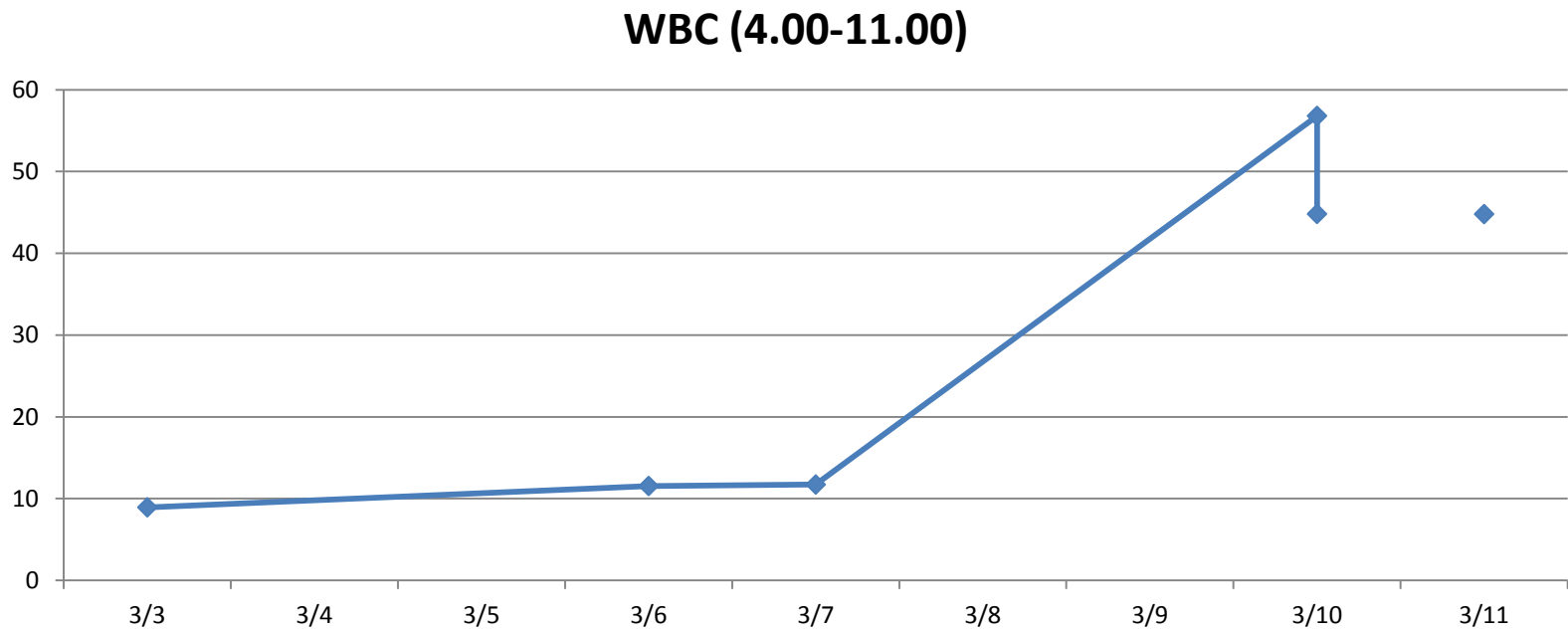


# Time Line

**Hgb (12.0-15.0)**



# Need for antibiotics



# Data from IRL last 19 months

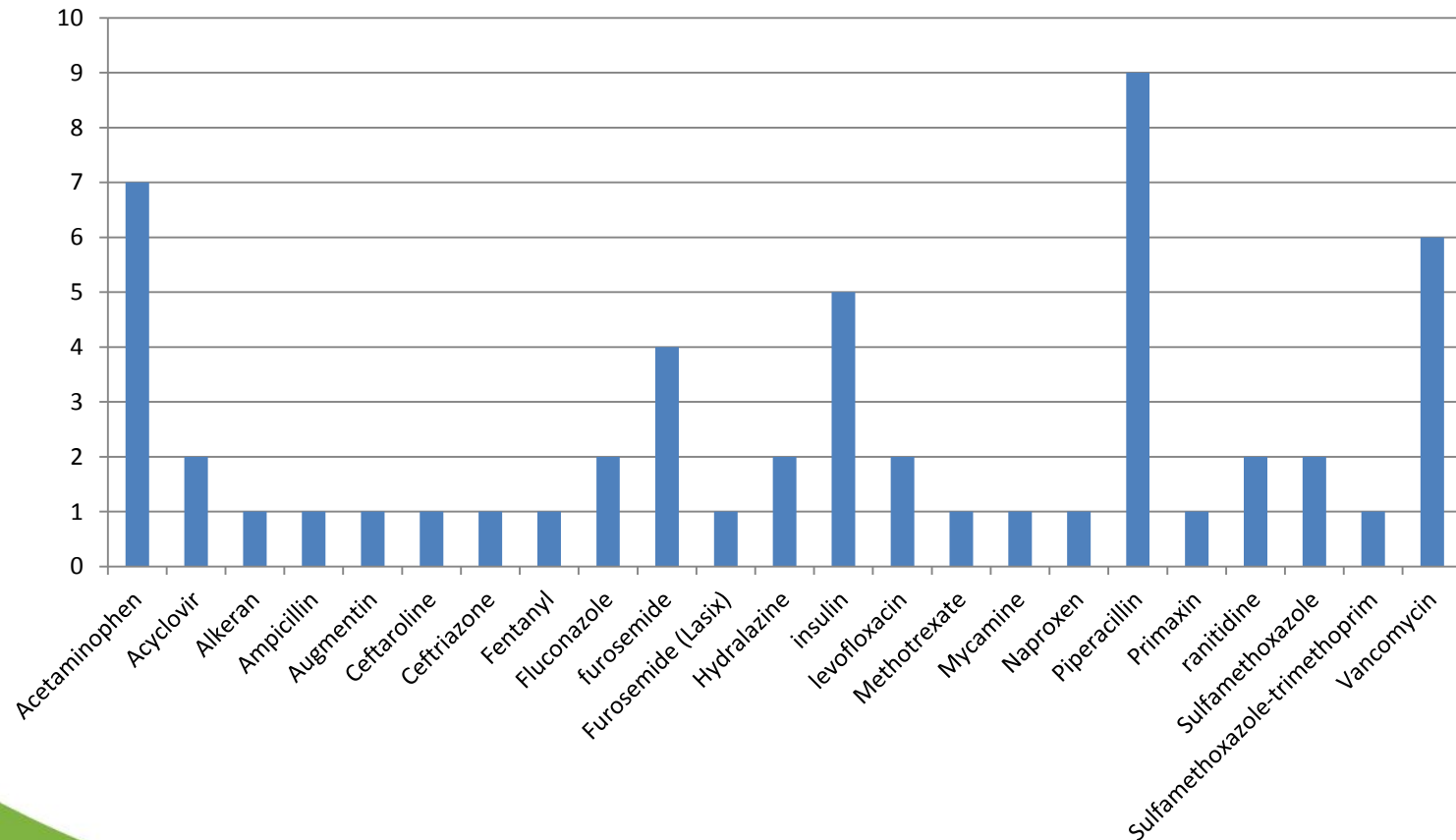
Positive DAT's	Negative Eluate	Patient on drug known to cause positive DAT	Cases where drug was suspected of hemolysis
527	55	26	3

<1% positive DAT's suspected to cause DIHA

## VERY RARE

# Data from IRL

Total



# Conclusion

- In our case study, we recognized that the reactivity we saw was most likely drug related and consistent with Piperacillin antibody
- The clinicians used that information along with other lab data and clinical evaluation to discontinue the piperacillin treatment and to switch to another antibiotic
- This saved the patient's life
- Important for blood bankers to recognize this phenomenon

# Transition Slide



# The Case of the Cold Nasty

## Sample Arrives in IRL



- 62 year old
- Female
- AB Positive
- History of Esophageal Varices
- Hgb 8.1
- Last transfused 3 RBC's 10 days prior
- Hospital reports history of cold nonspecific reactivity and hospital has been giving least incompatible
- Sent for antibody ID and units

Very strong reactivity at 22C

@ = agglutination after washing

[illegible]



# What could this be...

- Lewis, P1, and M are out
- High incidence Cold antibody?
  - Lets start there...



**T.J. maxx®**



@ = agglutination after washing

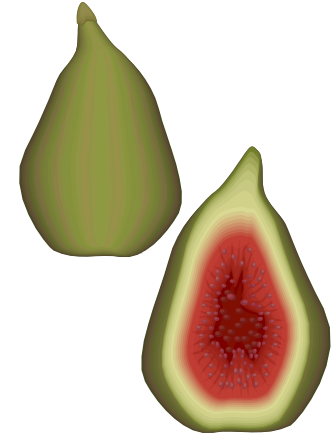
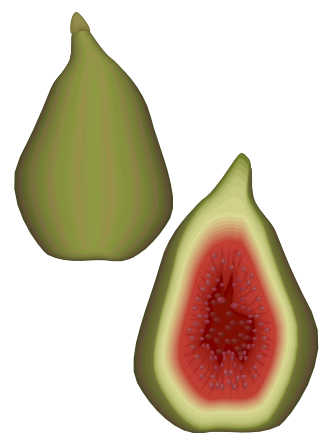
	Rare Factor	Rh						Kell		Duffy		Kidd		MNSs				Test Results	
		D	C	c	E	e	f	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	M	N	S	s	5 ' RT	Peg IAT
1	I-	0	0	+	0	+	+	0	+	0	+	0	+	+	0	0	+	3+ <sup>s</sup>	@
2	I-	0	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	3+	@
3	Ge -2 -3	0	0	+	0	+	+	0	+	0	+	+	+	+	0	+	0	3+	@
4	Ge -1 -2 -3	+	0	+	+	0	0	0	+	+	0	0	+	0	+	+	+	3+	@
5	Vel -	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	3+	@
6	Vel -	+	0	+	+	0	0	0	+	0	+	+	+	+	0	+	+	3+	@
7	Tj (a-) Group O	+	+	0	0	+	0	0	+	+	0	+	+	+	0	0	+	0	1+ <sup>w</sup>
8	Tj (a-) Group A	+	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	3+	@

# Could it really be? **Anti-Tj<sup>a</sup>**

@ = agglutination after washing

	Rare Factor	Rh						Kell		Duffy		Kidd		MNSs				Test Results	
		D	C	c	E	e	f	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	M	N	S	s	5 ' RT	PEG IAT
1	Tj(a-)	+	+	+	0	+		0	+	0	+	+	+	+	0	0	+	3+ <sup>s</sup>	@
2	Tj(a-)	+	+	+	0	+		0	+	+	+	+	0	0	+	0	+	3+	@
3	Tj(a-)	+	0	+	+	0		0	+	+	0	+	0	0	+	0	+	3+	@

Nope – Too good to be true – Keep hunting



# Time for some Ficin

Ficin will destroy M, N, S, s, Fy<sup>a</sup>, Fy<sup>b</sup>

	Rh					Kell		Duffy		Kidd		MNSs				Test Results	
	D	C	c	E	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	M	N	S	s	30 ' 37C	IAT
1	+	+	0	+	+	0	+	0	+	+	0	+	+	+	+	3+	@
2	+	+	+	0	+	+	+	+	0	+	0	+	+	+	+	3+	@
3	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	3+	@
4	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	3+	@
5	+	0	+	+	0	0	+	0	+	0	+	+	0	+	+	3+	@
6	+	0	+	+	0	0	+	0	+	+	+	+	0	+	+	3+	@
7	+	+	0	0	+	0	+	+	0	+	+	+	0	+	+	0	1+ <sup>w</sup>
8	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	3+	@

# 2 negative cells

What do our 2 negative cells have in common?

5 min 22C

1) R1R1, K-, Fy(b-), N-, S-

Ficin 30' 37C

2) R1R1, K-, Fy(b-), N- S-



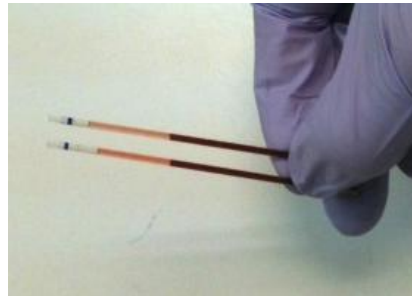
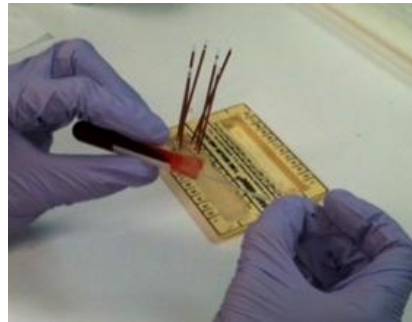
# Patient Phenotype

Microhematocrit cell separation

R1R1, K-, Fy(a+b+), Jk(a+b+) M+, N-, S-, s+

Patient can make:

- Anti-E
- Anti-c
- Anti-K
- Anti-N
- Anti-S



# Super Panel

	Rh					Kell		Duffy		Kidd		MNSs				Test Results	
	D	C	c	E	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	M	N	S	s	5 '22C	IAT
1	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	1+
2	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	0	1+
3	+	+	0	0	+	0	+	+	0	+	0	+	0	0	+	0	1+
4	+	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	1+
5	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	1+
6	+	0	0	0	0	0	+	0	+	+	+	+	0	+	0	2+	@
7	+	+	0	0	+	0	+	+	0	+	+	+	0	+	0	2+	@
8	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	3+	@
9	+	+	0	+	+	0	+	+	+	+	+	+	0	0	+	3+	@
10	+	0	+	0	+	0	+	+	0	+	0	+	0	0	+	2+	@
11	0	0	+	0	+	0	+	0	0	+	+	+	0	0	+	2+	@

- All reacting at 22C and all agglutinating after washing
  - Still something reacting at IAT 1+ even with antigen matched cells

# What we've identified at 22C

- Anti-E
  - Clinically significant
- Anti-c
  - Clinically significant
- Anti-S
  - Clinically significant



# What we have yet to identify...

- 1+ reactivity at IAT
  - Even with phenotypically matched cells
- Hospital has reported a history of Cold Reactive Autoantibody
  - The weak reactivity at IAT is consistent with a cold
  - Need to prove that's what it is

	Rh					Kell		Duffy		Kidd		MNSs				Test Results			
	D	C	c	E	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	M	N	S	s	5 '22C	IAT	30 min 4C	PEG Prewarm
1	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	1+	4+	0
2	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	0	1+	4+	0
3	+	+	0	0	+	0	+	+	0	+	0	+	0	0	+	0	1+	4+	0
4	+	+	0	0	+	+	+	+	0	0	+	0	+	0	+	0	1+	4+	0
5	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	1+	4+	0
6	+	0	0	0	0	0	+	0	+	+	+	+	0	+	0	2+	@		2+
7	+	+	0	0	+	0	+	+	0	+	+	+	0	+	0	2+	@		2+
8	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	3+	@		3+
9	+	+	0	+	+	0	+	+	+	+	+	+	0	0	+	3+	@		3+
10	+	0	+	0	+	0	+	+	0	+	0	+	0	0	+	2+	@		3+
11	0	0	+	0	+	0	+	0	0	+	+	+	0	0	+	2+	@		3+
Auto Control																		4+	0



# Conclusion

- Identified 3 clinically significant antibodies
  - Anti-E, anti-c, and anti-S
  - All reacted strongly at 22C
  - All are clinically significant
- Also identified a cold reactive auto antibody that was circumvented by prewarmed testing
- Patient requires E-, c- , S- units nonreactive with the prewarmed plasma

# Discussion

- Just because it reacts at 22C doesn't mean it can be ignored!!!
- Hospital reported history of cold antibody and had been giving least incompatible....!
- Possible that these are newly forming antibodies
  - Strongly reactive at 22C because they are still in IgM phase

# References

- Reid, M., & Lomas-Francis, C. (2004) *The Blood Group Antigen Facts Book* (2<sup>nd</sup> edition) London: Academic Press.
- Roback, J. (2011). *Technical Manual* (17<sup>th</sup> ed.). Bethesda, MD: AABB.
- Arndt, Pat., “Drug-Induced Hemolytic Anemia – The Last 30 years of Changes. AABB Annual Meeting. Boston, Massachusetts. 10-06-2012.



# Community Blood Center

Save a Life. **Right Here, Right Now.**

[savealifenow.org](http://savealifenow.org)

