American Red Cross

Enzymes and Chemicals: Reference Lab Case Studies

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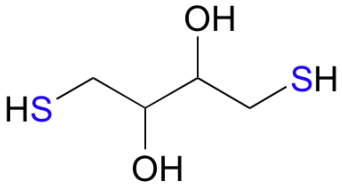
Enzymes and Chemicals: Reference Lab Case Studies

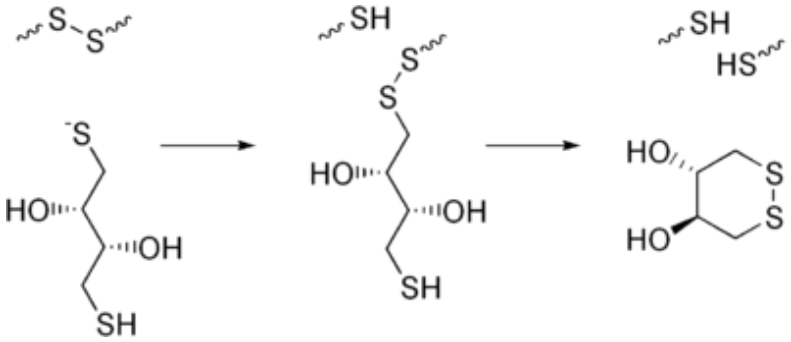
- What are the commonly used enzymes and chemicals in blood banking?
- What are their mechanisms of action?
- How do they help us identify antibodies?

Sulfhydryl Reagents

- Dithiothreitol (DTT), 2-aminoethylisothiouronium (AET), 2-mercapto-ethanol (2-ME)
  - Cleave disulfide bonds between cysteine amino acids
    - Destroy (or weaken) KEL, YT, LU, KN, LW, JMH, IN antigens, CD38
    - Disrupt IgM intermolecular disulfide bonds
      - Treated RBCs- useful in dispersing autoagglutination
      - Treated serum/plasma- used to reduce or eliminate IgM reacting antibodies



dithiothreitol
(DTT)



Ficin/Papain

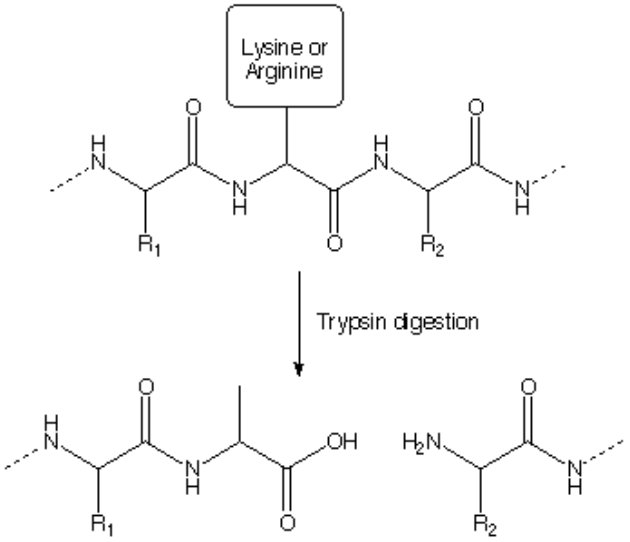
- Proteolytic enzymes derived from plant sources, are cysteine endoproteases
- Cleave glycoproteins of sialic acid on the red blood cell surface altering the surface charge on the cell, reducing the zeta potential
- Denatures some blood group antigens and makes others more accessible
- Enhance adsorptive capacity of RBCs

Depressed	Variable	Enhanced
M, N; Fy^a, Fy^b; Ch/Rg; XG; Bpa; IN; JMH;	S, s; YT	Rh system; Le^a, Le^b; P1; Jk^a, Jk^b; Vel; I

Trypsin

- Proteolytic enzyme
  - Serine protease that cleaves peptide chains, mainly at the carboxyl side of the amino acids lysine or arginine
  - Removes sialic acid from surface of red blood cell leading to altered surface charge
  - Causes denaturation of some antigens and increased accessibility of others

Depressed	Enhanced
M, N; LU; KN; Ch/Rg; JMH; DO; XG; Bp^a; IN; Ge2, Ge4	Rh system; Le^a, Le^b; P1; Jk^a, Jk^b; Vel; I



Case Study 1

Previous anti-H in a para-Bombay autologous donor

- Donor currently pregnant, unit to be frozen in case transfusion needed during delivery
- ABO “discrepancy” and positive antibody screen need to be resolved so unit can be frozen

ABO/Rh

Antisera							Cells		
A	B	A, B	D (IS)	D Control (IS)	Weak D (IgG)	D Control (IgG)	A_1	A_2	B
3+ MF	0	4+	3+	0			3+	2+	4+

Antibody Screen

	Supplier Lot #	Additional Antigens	Patie Plasma Tes		
			IS	LISS 37	IgG
1 OOD	Imm-Pano 51146		3+	1+	3+
2 OOD	Imm-Pano 51146		3+	2+	3+
3 OOD	Imm-Pano 51146	Co(b+)	3+	2+	3+
4 OOD	Imm-20 51152		3+	2+	3+
		Auto ctrl	0	0	0 ✓

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Case Study 1 - Anti-H

Bombay O_h Phenotype

- Rare autosomal recessive phenotype
- Homozygous for non-functioning FUT1 and FUT2 genes
- Results in absence of H, A, and/or B antigens on RBCs and in secretions
- Type as Group O, Aby screen using O cells will be positive
- Clinically significant, usually IgM

Para-Bombay

- Red cells lack serologically detectable levels of H antigen
- Inherited at least one functional secretor gene, leading to small amount of H, A and/or B antigens in plasma/secretions
- Weak reactions with anti-A/anti-B
- Blood types reported as A_h, B_h, Ab_h
- Clinically significant but generally of lower titer

Case Study 1 – Anti-H

- Use allogeneic adsorbed plasma to resolve ABO
- Antibody screen is negative with addition of 0.01M DTT, but addition of DTT to plasma will denature any IgM reacting antibodies, so alternate methods may be needed if suspecting additional alloantibodies known to be IgM

ABO Serum Grouping

Cells		
A_1	A_2	B
3+	2+	4+

rr Adsorbed Plasma

Cells		
A_1	A_2	B
0	0	2+

Antibody Screen

	Supplier Lot #	Donor/ Vial#	Plasma + 0.01M DTT Patient's Plasma Test Results						
			IS	LISS 37	IgG				
1 OOD	Imm-Pano 51146	B7231 1	0	0	0✓				
2 OOD	Imm-Pano 51146	C586 2	0	0	0✓				
3 OOD	Imm-Pano 51146	H1638 3	0	0	0✓				
4 OOD	Imm-20 51152	B9607 3	0	0	0✓				

Case Study 1 – Anti-H

- H negative cells frozen in liquid nitrogen were thawed and tested
- Allogeneic adsorption performed to rule out anti-K
- Patient recently delivered and did not need any RBC transfusions

TECH: H neg frozen cells

	D	C	E	c	e	f	C^w	V	M	N	S	s	Lu^a	Lu^b	P^1	Le^a	Le^b	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	IS	LISS 37	SS IgG
615C	+	+	0	+	+		0		+	0	+	+	0	+	0	0	0	0	+	0		+	0	0	+	0	0	0
614C	+	+	0	0	+		0		+	+	0	+	0	+	+	+	0	0	+	0		+	+	+	0	0	0	0

	D	C	E	c	e	f	V	C^w	M	N	S	s	P1	Le^a	Le^b	Lu^a	Lu^b	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	IS	LISS 37	IgG
R_1R_1 W20111863178600G	+	+	0	0	+				0	+	+	0	+	0	+			0				+	+	0	+			
R_2R_2 W20111864061500I	+	0	+	+	0				+	+	+	+	+	0	+			0				+	0	+	0			
rr W20111864061300S	0	0	0	+	+				+	0	0	+	+	+	0			0				0	+	0	+	IS	37	IgG
rr																												
Imm-20 S1152 6520 6	+	0	+	+	0		0	0	0	+	0	+	+	0	+	0	+	0	+	0	0	0	+	0	+	0	0	0
Imm-20 S1152 6242 18	0	0	0	+	+		0	0	+	+.	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	0	0	0

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Case Study 2

- Patient FE is a 69 year old female, currently diagnosed with tracheal stenosis, hgb of 7.2, going to surgery STAT
- Multiple red cell transfusions, most recent was 13 days prior to current sample
- History of a cold autoantibody identified <2 weeks ago
  - no underlying alloantibodies found after allogeneic adsorption
  - 3 of 4 crossmatches were compatible using tube prewarm technique

Case Study 2

- Current sample
  - Microscopic reactions using neat plasma at LISS/IAT with 7 of 8 cells tested, negative DAT, negative autocontrol
  - RESt adsorption fails to remove reactivity, prewarm tube crossmatches are all still microscopically positive

PANOSCREEN
Master List

412-11

IMMUCOR, INC. Norcross, GA 30071 USA
US LICENSE NO: 886
LOT NO: 51146
EXPIRES: 2018/02/23

VIAL	Donor	Rh - Hr							Kell						Duffy		Kidd		Lewis		P	MN				Luth-eran		X_g	IS	LISS 37	LISS IgG	RESt	LISS IgG		
		D	C	c	E	e	V*	Cʷ	K	k	Kp^a	Kp^b	Js^{a*}	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^{*a}							
I	R1R1 B7231	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	0	0	+	0	0	0	M+	M+			
II	R2R2 C586	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	0	+	+	0	0	M+	M+			
III	Co(b+) rr H1638	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	M+	M+			

Autoctrl 0 0 0✓

* Indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.
An antigen designated with a 'w' represents a weakened expression of the antigen that may or may not react with all examples of the corresponding antibody.

PANOSCREEN

Case Study 2

- Current sample
  - Allogeneic adsorption at 4° C appears successful, everything is ruled out except anti-S
  - BUT, patient types S positive serologically and autocontrol/DAT are negative ruling out the presence of autoanti-S

	D	C	E	c	e	f	V	C^w	M	N	S	s	P1	Le^a	Le^b	Lu^a	Lu^b	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	
R_1R_1 W201117544075005	+	+	0	0	+				+	0	+	0	+	+	0			+				+	0	0	+	
R_2R_2 W205517277592009	+	0	+	+	0				0	+	0	+	+	0	+			0				+	+	+	0	
rr W201118599054OOH	0	0	0	+	+				+	0	+	+	+	0	+			0				0	+	+	0	LISS IgG
R_1R_1																										
Bio 874 1911-00 6005449 2	+	0	+	+	0			0	0	+	0	+	0	+	0	0	+	+	+	0	0	0	+	+	0	0✓
Imm-Pure 47100 B7742 1	+	+	0	0	+		0	0	0	+	0	+	+	0	+	0	+	0	+	0	0	0	+	+	0	0✓
R_2R_2																										
Imm-Pure 51140 B7331 1	+	+	0	0	+		0	0	+	0	+	0	0	0	+	0	+	+	+	0	0	+	0	0	+	M+
Imm-10F 50135 C4474 3	+	0	+	+	0		0	0	0	+	0	+	+	0	0	0	+	+	+	0	0	0	+	+	+	0✓
OrMo B R8488 320105 17	+	0	+	+	0	0	0	0	+	0	0	+	S	0	+	0	+	0	+	0		+	0	+	+	0✓
Imm-Pure 49125 C4414 2	+	0	+	+	0		0	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	0	+	+	M+
Bio 874501-00 324120 2	+	0	+	+	0			0	+	0	+	0	S	+	0	+	+	0	+	0	0	+	0	+	0	M+
Imm-16 51140 B4561 2	+	+	0	0	+		0	+	+	+	0	+	0	+	0	0	+	0	+	0	0	0	+	+	0	0✓
rr																										
OrMed 2A975 314934 10	0	0	0	+	+	+	0	0	0	+	0	+	0	0	+	0	+	0	+	0		+	0	0	+	0✓
Imm-16 51140 B6936 9	+	+	0	0	+		0	0	+	+	+	+	0	+	0	0	+	+	0	0	0	+	+	+	0	0✓

DAT

	PS	IgG
IS	0	
RT	0✓	NA

S typing

N	S	s
I	I	A
2+	4+	3+

Case Study 2

- Treated and untreated allogeneic cells for adsorption were tried with no apparent difference in reactivity
- Now what?
  - Do titer
  - Test serum with treated reagent cells: DTT, trypsin and ficin/papain

Titration

Titer Section	Sample Date	Anti-	Phase	1	2	4	8	16	32	64	128	256	512	1024	~~2048~~ Sal Ctr	Titer	Score
	01262018		60' Sal IgG	M+	M+	M+	M+	M+	M+	M+	M+	M+	M+		0✓		

Case Study 2

- 0.2M DTT treatment failed to remove reactivity
- Cells treated with Ficin and Trypsin are all negative

DTT Treated Cells

	Supplier Lot #	Donor/ Vial#	RhHr								MN				P	Lew		Lut		Kell						Duf		Kid		X	Plas
			D	C	E	c	e	f	V	Cw	M	N	S	s	P1	Lea	Leb	Lua	Lub	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Xga	DTT LISS IAT
1	A DIF-18-2	63178600G 1	+	+	0	0	+				0	+	+	0	+	0	+			0						+	+	0	+		M+
2	A DIF-18-2	640615001 2	+	0	+	+	0				+	+	+	+	+	0	+			0						+	0	+	0		M+
3	A DIF-18-2	640613005 3	0	0	0	+	+				+	0	0	+	+	+	0			0						0	+	0	+		M+

Ficin/Trypsin Treated Cells

IMMUCOR, INC. Norcross, GA 30071 USA
US LICENSE NO: 886
LOT NO: 50135
EXPIRES: 2018/02/16

VIAL	Special Type	Donor	Rh - Hr							Kell						Duffy		Kidd		Lewis		P	MN				Luth-eran		Xg	PATIENT'S SERUM TEST RESULTS TEST METHODS					
			D	C	c	E	e	V	Cw	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga		IS	4 37	SS IAT		FICIN IAT	TRYPSIN IAT
1		R1R1 B9454	+	+	0	0	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	0	+	0	+	+	1	0	0	M+		0	0
2		R1wR1 B8854	+	+	0	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	+	+	+	+	+	0	+	0	2						
3		R2R2 C4676	+	0	+	+	0	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	0	3	0	0	M+		0	0
4	He+	Ror D563	+	0	+	0	+	0	0	0	+	0	+	+	+	0	0	+	0	0	0	+	0	+	+	+	0	+	0	4						
5		r'r E1046	0	+	+	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	+	+	+	+	0	+	0	+	+	5						
6		r"r F933	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	0	0	+	+	6						
7		rr G1729	0	0	+	0	+	0	0	+	0	0	+	0	+	0	+	0	+	0	0	0	0	+	+	+	0	+	0	7						
8		rr H857	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	0	+	0	+	+	8	0	0	M+		0	0

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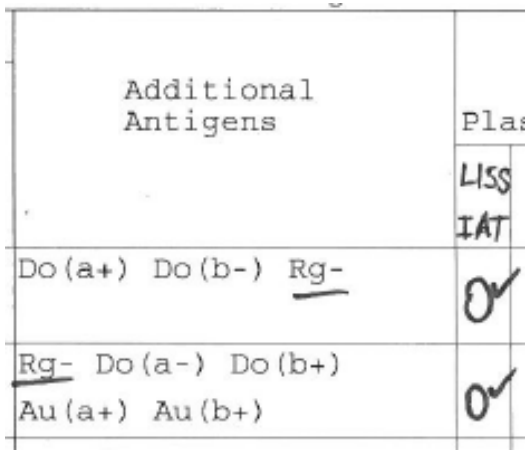
Case Study 2

Ficin/Papain	Trypsin	200mM DTT	Possible specificity
Negative	**Negative**	**Positive**	Bp^a; **Ch/Rg**; XG; M, N, En^{a}TS; Ge2,Ge4

- Chido/Rodgers Blood Group System
  - Not true blood group antigens. Located on the fourth component of complement (C4d)
  - Adsorbed onto RBC membrane from plasma
  - Clinically insignificant antibodies but have broad reactivity that can cause confusing serological results

Case Study 2

- Identifying and characterizing Ch/Rg antibodies
  - Test with Ch/Rg negative cells
  - Test with C4 coated RBCs
  - Type for Ch/Rg antigens using hemagglutination inhibition



Additional Antigens	Pla LISS IAT
Do(a+) Do(b-) Rg-	0
Rg- Do(a-) Do(b+) Au(a+) Au(b+)	0

Case Study 2

C4 coated adsorptions

- Trace amounts of C4 normally found on RBCs, leading to weak reactions with reagent cells
- Reagent red cells are coated with excess C4b or C4d by incubating with pooled normal serum (source of complement) in a sucrose solution
- Direct agglutination can be observed with Ch/Rg antibodies when incubated with coated cells
- Coated cells can also be used to adsorb anti-Ch or anti-Rg from plasma/serum
- Patient sample reacted strongly with C4 coated allogeneic cells

	Patient	Ctrl
Untreated Control	5' RT	5' RT
R_1R_1	0	0
R_2R_2	0	0
$\overline{rr}$	0	0
	Patient	Ctrl
C4 coated cells	5' RT	5' RT
R_1R_1	1+	M+
R_2R_2	2+	M+
$\overline{rr}$	2+	M+

Case Study 2

Typing for Ch/Rg by Hemagglutination Inhibition

- Direct cell typing for Ch/Rg antigens is not reliable, antigen strength is variable and may lead to false negatives
- To determine patient's phenotype, necessary to demonstrate presence or absence of the antigen in the serum

Patient	Positive control	Negative control	Saline control	Interpretation
Negative	Negative	Positive	Positive	Rodgers Positive
Positive	Negative	Positive	Positive	Rodgers Negative
Any	Positive	Any	Any	Invalid
Any	Any	Negative	Any	Invalid
Any	Any	Any	Negative	Invalid

Results:

Patient	Positive control	Negative control	Saline control	Interpretation
Positive	Negative	Positive	Positive	Rodgers Negative

Case Study 2 - HTLA-like Antibody

- Patient was discharged before antibody ID was completed
- All common clinically significant antibodies were ruled out using a combination of trypsin treated RBCs, ficin treated RBCs, Rg- neat cells, and 1 non-reactive neat reagent cell at LISS/IAT
- Previous cold autoantibody not detected
- Incompatible units recommended for transfusion
- HTLAs = ☹

Case Study 3

- Patient ST is a 73 year old caucasian female diagnosed with UTI/flu
- 9.3 Hgb, no transfusion required
- No known RBC antibodies
- Transfused within the past 3 months, unknown pregnancy history
- Hospital reports that all cells are positive in PEG and Gel except auto control.

Case Study 3

- Initial results: Negative DAT, panel and screen all positive at PEG and LISS IAT with a negative autocontrol
- Cold screen negative

LOT NO: 51146
EXPIRES: 2018/02/23

VIAL	Donor	Rh - Hr							Kell						Duffy		Kidd		Lewis		P	MN				Luth-eran		X_g	15° 4°	15° AT	IS	LISS 37	LISS IAT
		D	C	c	E	e	V*	C^w	K	k	Kp^a	Kp^b	Js^a*	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^{*a}					
I	R1R1 B7231	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	0	1+
II	R2R2 C586	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	0	+	+	0	0	0	0	1+
III	Co(b+) rr H1638	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	0	0	1+
	Auto control																											0	0	0	0	0✓

* Indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.
An antigen designated with a 'w' represents a weakened expression of the antigen that may or may not react with all examples of the corresponding antibody.

Cell #	IS	PEG IAT
1	0	1+
2	0	1+
3	0	1+
4	0	1+
5	0	1+
6	0	1+
7	0	1+
8	0	1+
9	0	1+
10	0	1+
11		
AC	0	0✓

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Case Study 3

- Next steps with this pattern of reactivity
  - Run phenotypically similar cell to determine if there are multiple alloantibodies or an antibody to high frequency antigen

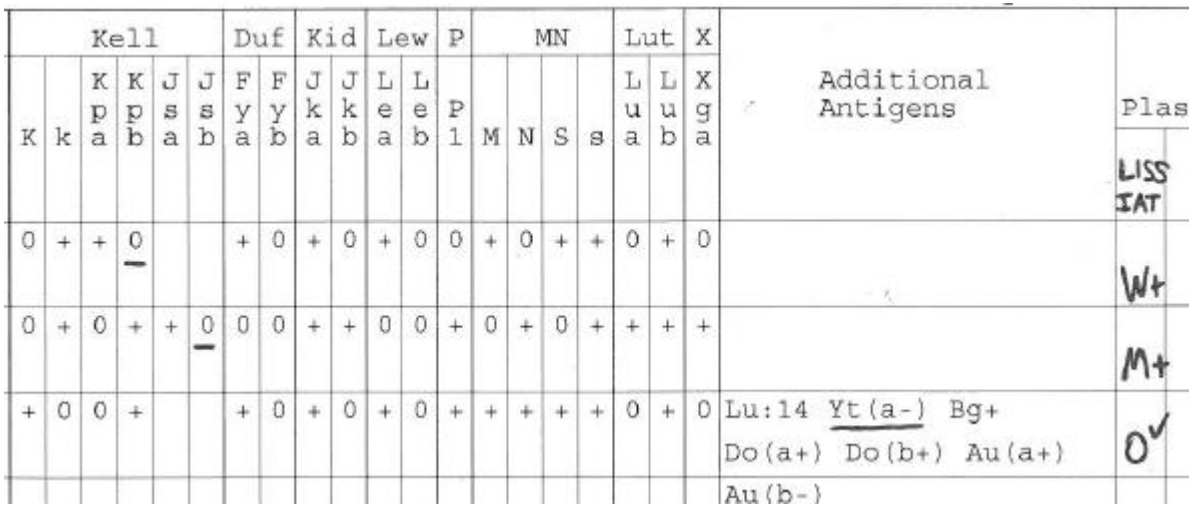
C	E	c	e	Sera-clone control ABO + Rh	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	Le^a	Le^b	P1
0	0	4+	4+	Mfr: B Lot:	0✓		2+	2+	3+	3+	3+	3+	3+	3+	3+	0	4+

  - Test serum with treated reagent cells: DTT, trypsin and ficin

Rh - Hr							Kell						Duffy		Kidd		Lewis		P	MN				Luth-eran		Xg	PATIENT'S SERUM TEST RESULTS TEST METHODS						
D	C	c	E	e	V*	C^w	K	k	Kp^a	Kp^b	Js^{a*}	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P$_1$	M	N	S	s	Lu^a	Lu^b	Xg^{*a}		LISS IAT		Ficin IAT	Trypsin IAT	DTT LISS IAT	
+	+	0	0	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	0	+	0	+	+	1	1+		1+	1+	0✓	
+	+	0	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	+	+	+	+	+	0	+	0	2						
+	0	+	+	0	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	0	3	1+		1+	1+	0✓	
+	0	+	0	+	0	0	0	+	0	+	+	+	0	0	+	0	0	0	+	0	+	+	+	0	+	0	4						
0	+	+	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	+	+	+	+	0	+	0	+	+	5						
0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	0	0	+	+	6						
0	0	+	0	+	0	0	+	0	0	+	0	+	0	+	0	+	0	0	0	0	+	+	+	0	+	0	7	1+		1+	1+	0✓	
0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	0	+	0	+	+	8						
0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	9	1+		1+	1+	0✓	
0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	10						

Case Study 3

Ficin	Trypsin	200mM DTT	Possible specificity
Positive	**Positive**	**Negative**	LW, KEL
Positive	**Positive**	**Weak**	CROM
Variable	**Positive**	**Weak or Negative**	YT



Kell						Duf		Kid		Lew		P	MN				Lut		X	Additional Antigens	Plas
K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga		LISS IAT
0	+	+	0			+	0	+	0	+	0	0	+	0	+	+	0	+	0		W+
0	+	0	+	+	0	0	0	+	+	0	0	+	0	+	0	+	+	+	+		M+
+	0	0	+			+	0	+	0	+	0	+	+	+	+	+	0	+	0	Lu:14 Yt(a-) Bg+ Do(a+) Do(b+) Au(a+) Au(b-)	0

Case Study 3 – Anti-Yt^a

- The YT blood group system
  - Two allele system, no inherited Yt(a-b-) individuals have been identified.
  - YT antigens are found within the enzyme acetylcholinesterase. The function of this enzyme on RBC membranes is unknown.
  - Occurrence of Yt^a antigen is >99.8% in most populations
  - Clinical significance of anti-Yt^a is variable, antigen negative units may not be required

Yta
0

	D	C	E	c	e	f	C^w	V	M	N	S	s	Lu^a	Lu^b	P^1	Le^a	Le^b	K	k	Kp^a	Js^a	Fy^a	Fy^b	Jk^a	Jk^b	Yta		LISS IAT
540c	+	+	0	+	+				+	0	0	+						+	+			+	0	+	+	0		0✓
569c	0	0	0	+	+		0		+	0	+	0	0	+	+	0	+	0	+	0		+	+	+	+	0		0✓
522c	+	+	0	+	+			0	+	+	+	0						+	+			0	+	+	0	0		0✓

Conclusions

Enzymes and chemicals are invaluable in a reference blood bank

- Remove strong IgM reactivity to look for underlying IgG alloantibodies
- Narrow down suspects in suspected high frequency antibody identifications
- Identify and characterize HTLA-like antibodies
- Rule out alloantibodies in complex antibody identifications

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