

# **ABO Type Discrepancies**

# ABO Discrepancies

- Unexpected reactions in forward or reverse grouping
- Technical Errors
- Group 1- Reverse group
- Group 2- Front type
- Group 3- Rouleaux
- Group 4-Miscellaneous

# Technical Errors

- Incorrect identification- sample, reagents
- Failure to add reagent
- Failure to add sample
- Did not follow instructions
- Over/undercentrifugation, faulty equipment
- Contamination of reagents

# Group I Discrepancy

- Weak or missing antibodies in the backtype
- Most common discrepancy
- Depressed antibody production
- Inability to produce antibodies

	Forward Grouping			Reverse Grouping	
	Anti-A	Anti-B	Anti-D	A1 cells	B cells
Patient (Elderly)	0	0	4+	0	0
Normal O pos	0	0	4+	4+	4+

# Causes for Group I Discrepancies

- Newborns - antibodies not detectable until 3-6 months
- Elderly – antibody production depressed
- Leukemia
- Immunosuppressive drugs
- Immunodeficiency diseases
- Bone Marrow Transplant
- ABO subgroups

# Resolution of Group I

- Incubate back type at room temperature for 15-30 minutes
- Add two more drops of plasma
- Still no reaction:
  - Incubate 4°C for 15-30 minutes
  - Run auto control and O cell control
  - Low temps enhance cold agglutinins- need controls



# Group II Discrepancies

- Weak or missing antigens in front type
- Least frequent

	Forward Grouping			Reverse Grouping	
	Anti-A	Anti-B	Anti-D	A1 cells	B cells
Patient (acquired B)	4+	2+	4+	0	4+
Patient (Leukemia)	+mf	0	4+	0	3+
Normal A pos	4+	0	4+	0	4+

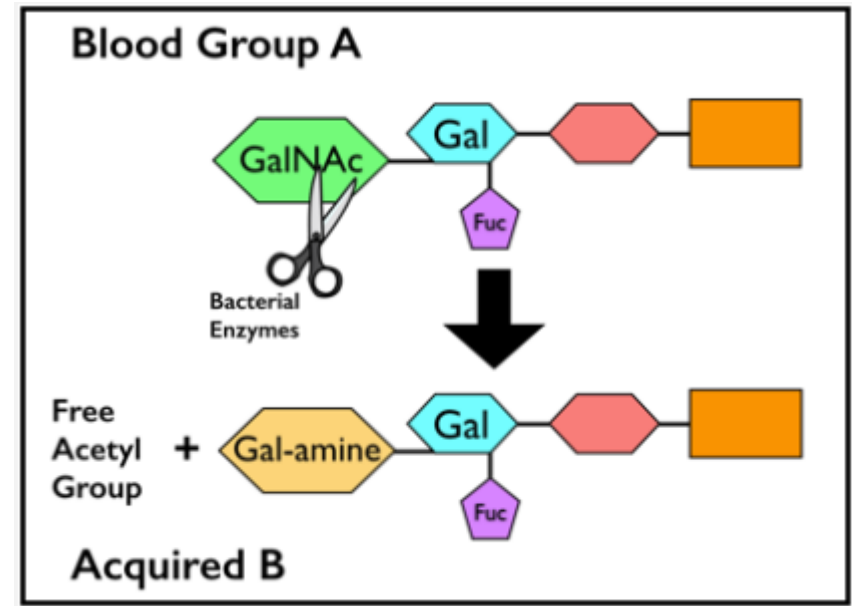
# Causes of Group II Discrepancies

- Subgroups of A or B
- Leukemia
- Hodgkin's Disease
- Acquired B phenomenon



# Acquired B Phenomenon

- Diseases of the digestive tract
- Usually only in type A individuals
- Blood comes in contact with bacterial enzymes that remove acetyl group from A
- Resulting sugar very similar to galactose
- B reagents may weakly react with galactosamine

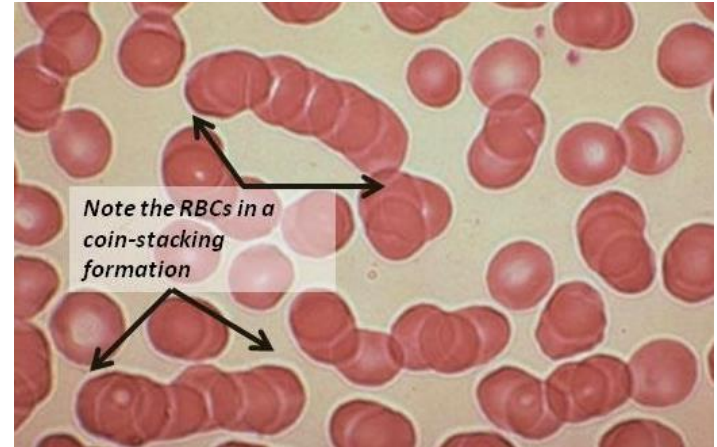


# Resolution of Group II

- Incubate front type at room temp for 15-30 min
- Still no reaction:
  - Incubate 4°C for 15-30 minutes
  - Run auto control, O cell control
- Wash patient RBCs and retest
- Acquired B:
  - Try different clones of anti-B
  - Incubate RBCs with acetic anhydride- re-acetylation of A

# Group III Discrepancies

- Discrepancies due to protein or plasma abnormalities resulting in rouleaux
- Rouleaux: Stacking of RBCs in coin-like fashion looking like agglutination



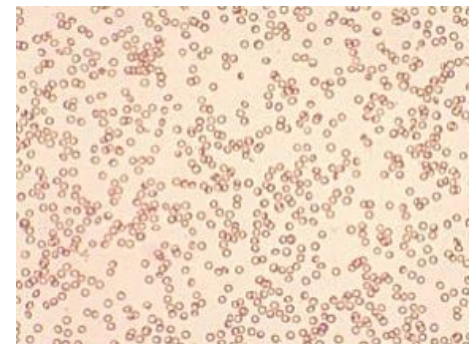
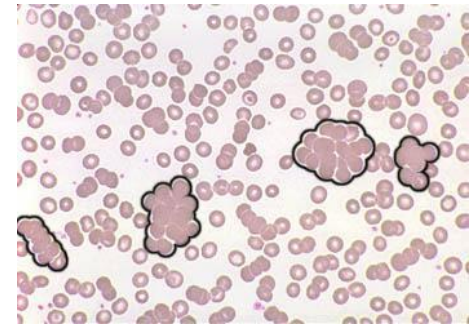
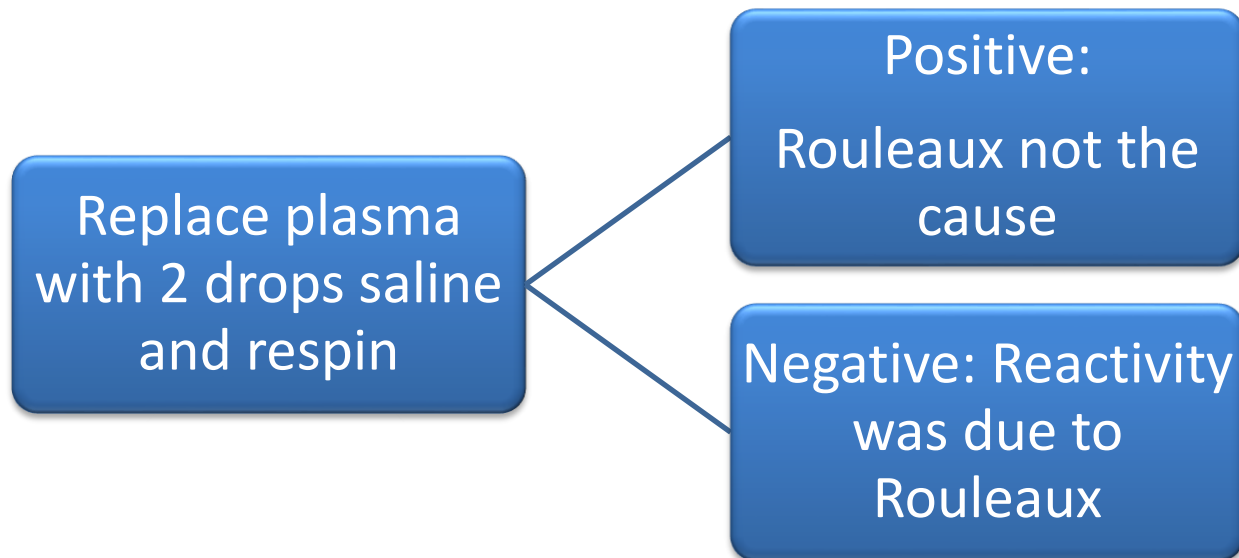
	Forward Grouping			Reverse Grouping	
	Anti-A	Anti-B	Anti-D	A1 cells	B cells
Patient (Rouleaux)	4+	0	4+	2+	3+
Normal A pos	4+	0	4+	0	4+

# Causes of Group III Discrepancies

- Increase level of globulin due to disease states:
  - Multiple Myeloma
  - Waldenstrom's macroglobulinemia
  - Advanced cases of Hodgkin's lymphoma
  - Elevated fibrinogen
  - Plasma expanders
  - Wharton's Jelly- cord blood samples

# Resolution of Group III

- Forward type: wash RBCs several times with isotonic saline
- Back type: saline replacement technique



# Group IV Discrepancies

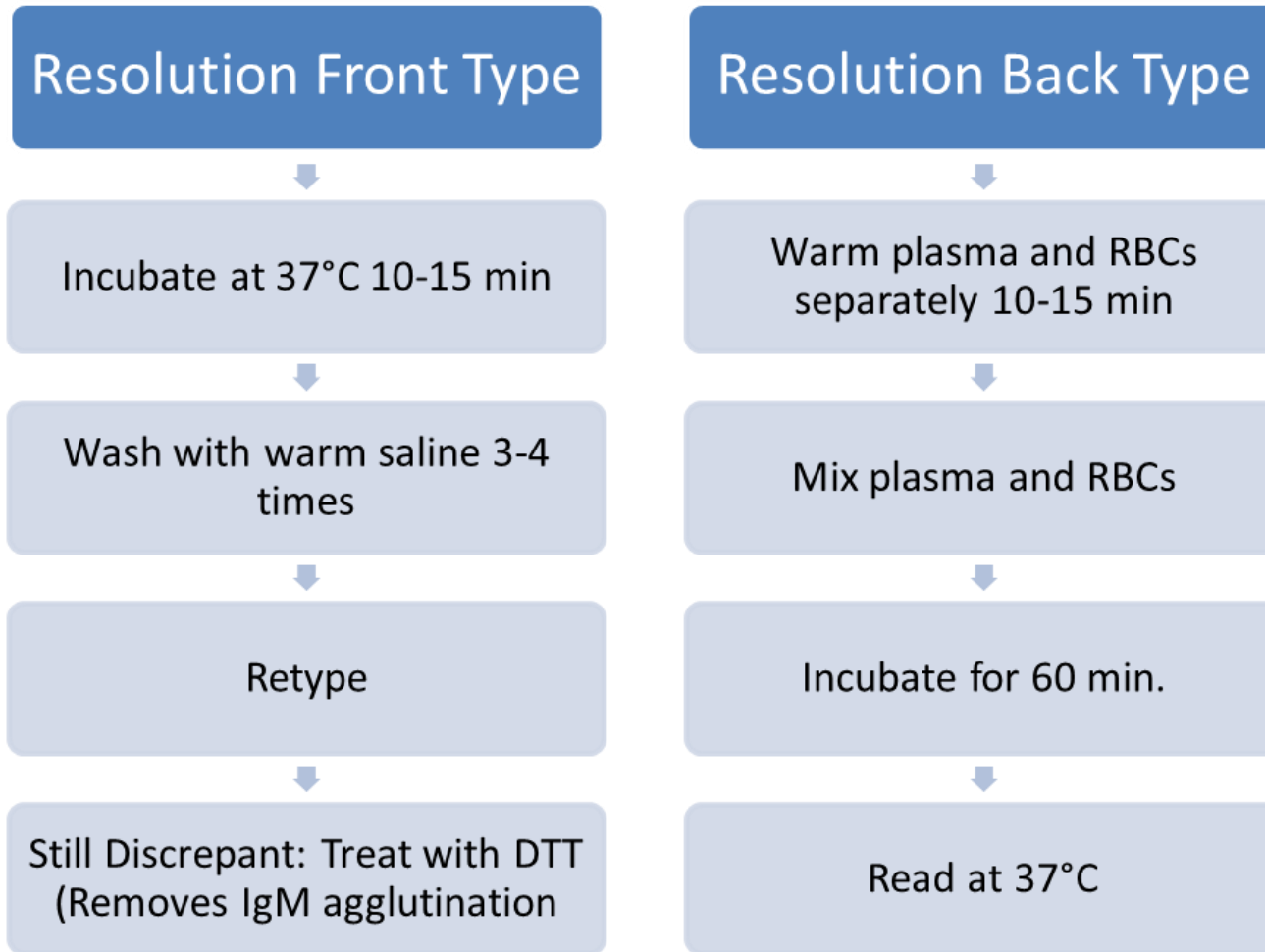
- Discrepancy between forward and reverse due to miscellaneous problems:
  - Cold autoantibody
  - RBCs of more than 1 blood type
    - RBC transfusion
    - Bone marrow transplant
  - Unexpected ABO isoagglutinins/alloantibodies

# Group IV: Cold Autoantibody

- Spontaneous agglutination with patient cells
- Extra reactivity in reverse grouping

	Forward Grouping			Reverse Grouping	
	Anti-A	Anti-B	Anti-D	A1 Cells	B Cells
Patient 1 (cold auto)	0	4+	4+	4+	2+
Patient 2 (cold auto)	2+	4+	4+	4+	3+
Normal B Pos	0	4+	4+	4+	0

# Cold Autoantibody Resolution





# Group IV: Unexpected ABO Isoagglutinins

- Usually in  $A_2$  and  $A_2B$  individuals
  - Produce Anti- $A_1$
- More rarely, in  $A_1$  and  $A_1B$  individuals
  - Produce Anti-H

	Forward Grouping			Reverse Grouping	
	Anti-A	Anti-B	Anti-D	A1 cell	B cells
Patient (Anti-A1)	4+	0	4+	1+	4+
Normal A pos	4+	0	4+	0	4+

# Anti-A<sub>1</sub> Resolution

- Run against anti-A<sub>1</sub> lectin (*Dolichos biflorus*)
  - Reacts with A<sub>1</sub> cells but not A<sub>2</sub> cells
- Repeat serum grouping with:
  - A<sub>1</sub> cells (minimum 3)
  - A<sub>2</sub> cells (minimum 3)
  - B cells
  - O cells
  - Autocontrol

Anti-A1	Interpretation
4+	A <sub>1</sub> or A <sub>1</sub> B
0	A <sub>2</sub> or A <sub>2</sub> B

	A1 cell	A2 cell	B cell	O cell	Auto
Patient (Anti-A1)	1+	0	4+	0	0
Normal A pos	0	0	4+	0	0

# Group IV: Unexpected non-ABO Isoagglutinins

- Most common Anti-M
- Antibodies reacting at room temperature
- Discrepancies in reverse grouping

	Forward Grouping			Reverse Grouping	
	Anti-A	Anti-B	Anti-D	A1 cells	B cells
Patient (anti-M)	4+	4+	0	1+	1+
Normal AB neg	4+	4+	0	0	0

# Alloantibody in Reverse Group Resolution

Two choices:

## Identify antibody

- Find A1 and B cells negative for the antibody

## Follow steps for cold autoantibody

- Warming and reading back type at 37°C

# RBCs of More Than One Blood Type

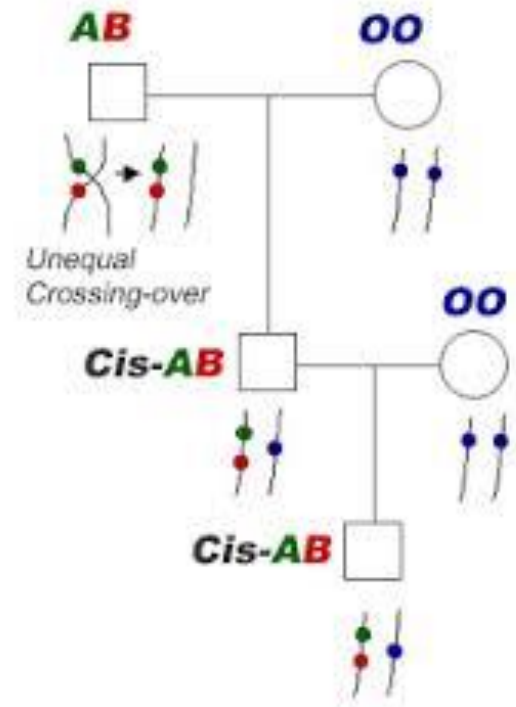
- Mixed field reactions
- No Resolution other than confirming history
- Bone Marrow Transplant
- Transfusion: Patient A pos, transfused O pos
  - Anti-A will be mixed field due to A and O RBCs present
- Chimera

	Forward Grouping			Reverse Grouping	
	Anti-A	Anti-B	Anti-D	A1 cells	B cells
Patient (Transfused O pos)	4+ mf	0	4+	0	4+
Normal A pos	4+	0	4+	0	4+



# Cis-AB

- Inherit both A and B genes from one parent and O from another parent
- Due to unequal crossing over (rare)
- Weakly reactive A and B antigen
- Many times make weak Anti-B



# A Subgroups

- A Subgroups other than A<sub>1</sub> and A<sub>2</sub>:
- 1% of individuals
- Characteristics:
  - Decreased number of A antigen sites (weak/no agglutination with anti-A)
  - Varying agglutination with anti-A,B
  - Increased detectability of H antigen (strong anti-H reactions)
  - Can have anti-A<sub>1</sub> in serum

# A3 Subgroup

- **Mixed-field** with anti-A and anti-A,B- some cells react with anti-A others don't
- Decrease antigen sites to 35,000 per red cell

	Reagents				Antibodies in Serum			Other		
Pheno type	Anti-A	Anti-B	Anti-A,B	Anti-H	Anti-A	Anti-B	Anti-A1	Substance in secretions	Presence of A transferase	Number antigen sites RBC x 10 <sup>3</sup>
A <sub>3</sub>	+mf	0	+mf	3+	no	Yes	Some	A, H	Sometimes	35



# Ax Subgroup

- Many times **no or weak anti-A agglutination**
- Only A subgroup that **agglutinates anti-A,B very well**
- Decreased to 4,000 antigen sites
- **Usually produce anti-A<sub>1</sub>**
- **Can be confused with Group O** (If negative with anti-A and weak reactivity with A1 cells in backtype it will type like an O)

	Reagents				Antibodies in Serum			Other		
Phenotype	Anti-A	Anti-B	Anti-A,B	Anti-H	Anti-A	Anti-B	Anti-A <sub>1</sub>	Substance in secretions	Presence of A transferase	Number antigen sites RBC x 10 <sup>3</sup>
A <sub>x</sub>	wk/0	0	2+	4+	no/wk	Yes	Almost always	H	Rarely	4

# A<sub>end</sub> Subgroup

- Weak mixed field with anti-A and anti-A,B
- Even fewer antigen sites (3,500)

	Reagents				Antibodies in Serum			Other		
Phenotype	Anti-A	Anti-B	Anti-A,B	Anti-H	Anti-A	Anti-B	Anti-A1	Substance in secretions	Presence of A transferase	Number antigen sites RBC x 10 <sup>3</sup>
A <sub>end</sub>	wk mf	0	wk mf	4+	no	Yes	Some	H	No	3.5

# A<sub>M</sub> SUBGROUP

- No or weak anti-A and anti-A,B reactions
- Usually no anti-A1
- Barely 1,000 antigen sites

	Reagents				Antibodies in Serum			Other		
Phenotype	Anti-A	Anti-B	Anti-A,B	Anti-H	Anti-A	Anti-B	Anti-A1	Substance in secretions	Presence of A transferase	Number antigen sites RBC x 10 <sup>3</sup>
A <sub>m</sub>	0/wk	0	0/wk	4+	no	Yes	No	A, H	Yes	1

# A<sub>y</sub> Subgroup

- No anti-A or anti-A,B reactions/agglutination
- No anti-A1

	Reagents				Antibodies in Serum			Other		
Phenotype	Anti-A	Anti-B	Anti-A,B	Anti-H	Anti-A	Anti-B	Anti-A1	Substance in secretions	Presence of A transferase	Number antigen sites RBC x 10 <sup>3</sup>
A <sub>y</sub>	0	0	0	4+	no	Yes	No	A, H	Trace	1

# A<sub>el</sub> Subgroup

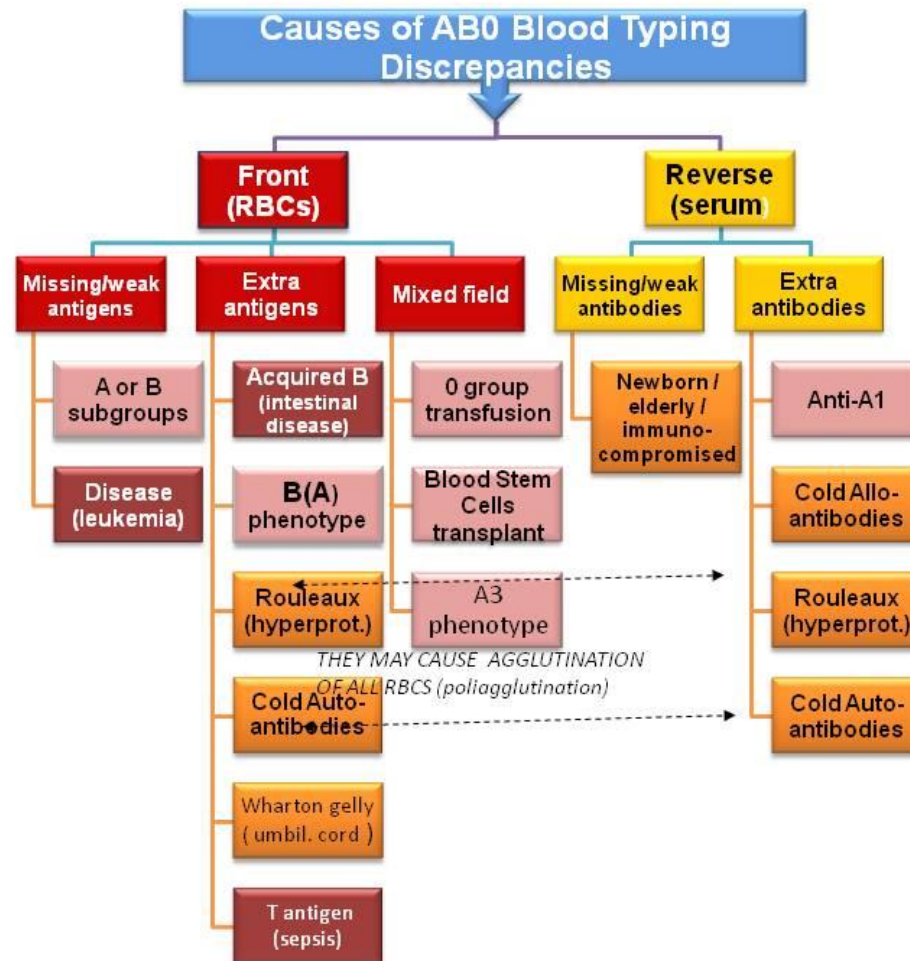
- No anti-A or anti-A,B reactions/agglutination
- Produces anti-A1

	Reagents				Antibodies in Serum			Other		
Phenotype	Anti-A	Anti-B	Anti-A,B	Anti-H	Anti-A	Anti-B	Anti-A1	Substance in secretions	Presence of A transferase	Number antigen sites RBC x 10 <sup>3</sup>
A <sub>el</sub>	0	0	0	4+	some	Yes	Yes	H	No	.7

# A Subgroup Summary

	Reagents				Antibodies in Serum			Other		
Phenotype	Anti-A	Anti-B	Anti-A,B	Anti-H	Anti-A	Anti-B	Anti-A1	Substance in secretions	Presence of A transferase	Number antigen sites RBC x 10 <sup>3</sup>
A <sub>3</sub>	+mf	0	+mf	3+	no	Yes	Some	A, H	Sometimes	35
A <sub>x</sub>	wk/0	0	2+	4+	no/wk	Yes	Almost always	H	Rarely	4
A <sub>end</sub>	wk mf	0	wk mf	4+	no	Yes	Some	H	No	3.5
A <sub>m</sub>	0/wk	0	0/wk	4+	no	Yes	No	A, H	Yes	1
A <sub>y</sub>	0	0	0	4+	no	Yes	No	A, H	Trace	1
A <sub>el</sub>	0	0	0	4+	some	Yes	Yes	H	No	.7

# Summary of Type Discrepancies



Also on p.145 Figure 6-18



**Every life deserves world class care.**